DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993 0002

BIO-RAD LABORATORIES, INC.
JACKIE BUCKLEY
REGULATORY AFFAIRS REP IV
4000 ALFRED NOBEL DR.
HERCULES CA 94547

December 9, 2015

Re: K151321
Trade/Device Name: D-100™ HbA1c
D-100™ HbA1c Calibrator Pack
Regulation Number: 21 CFR 862.1373
Regulation Name: Glycosylated hemoglobin assay
Regulatory Class: II
Product Code: PDJ, LCP, JIT
Dated: November 06, 2015
Received: November 09, 2015

Dear Jackie Buckley:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151321

Device Name
D-100™ HbA1c
D-100™ HbA1c Calibrator Pack

Indications for Use *(Describe)*
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100™ HbA1c test is intended for Professional Use Only.

Calibrators:
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c(HbA1c) in human whole blood.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
   Bio-Rad Laboratories, Inc.
   Clinical Diagnostics Group
   4000 Alfred Nobel Drive
   Hercules, California 94547

2. **Contact Person(s):**
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3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 and 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack** (K)151321	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) 070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown in Tables 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method are summarized in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

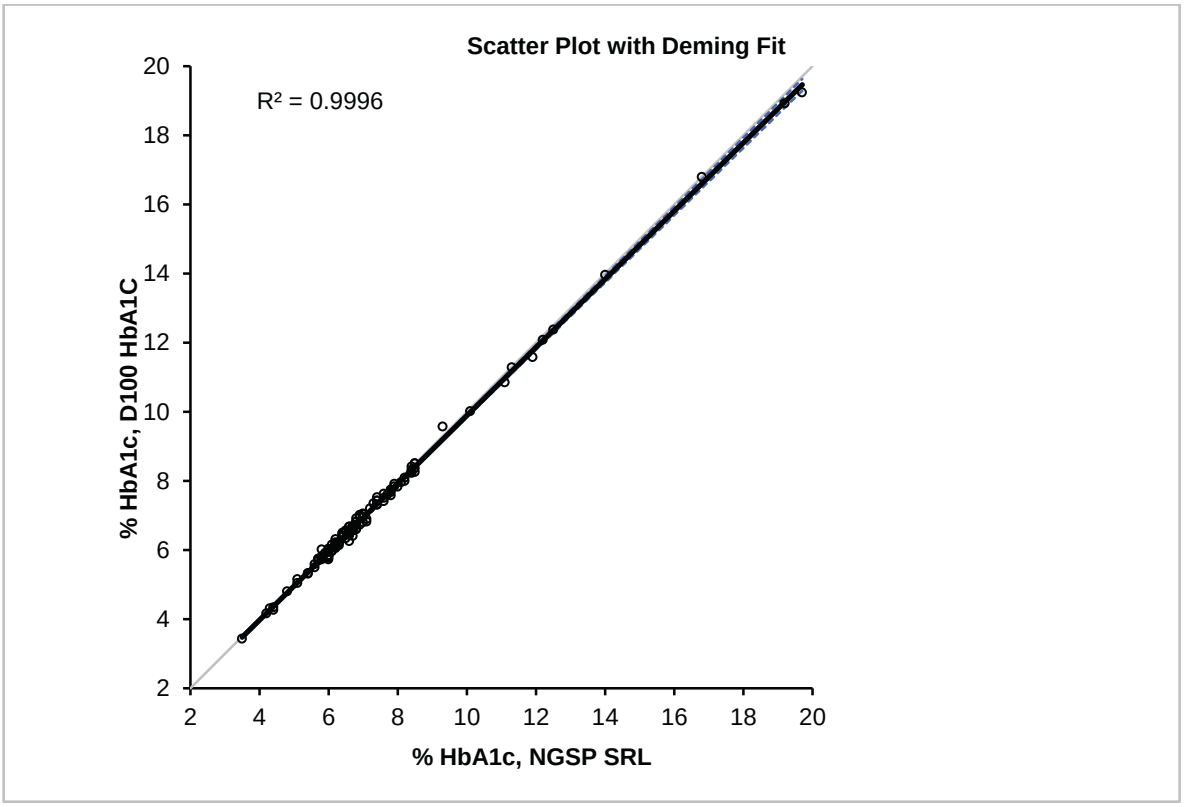

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85

6.5	-0.066	-0.98
8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure

form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18 on the following page.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	SI units
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of

~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 1200 mg/dL of glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative %Bias (Range of %Bias) for HbA1c	Relative %Bias (Range of %Bias) for HbA1c
	~6.5%	~9.0%
HbS	-0.6 (-5.8 to 5.5)	-1.5 (-3.3 to -0.1)
HbC	-1.3 (-4.0 to 1.3)	-3.9 (-5.5 to -2.4)
HbD	-4.7 (-6.7 to -1.1)	-4.4 (-6.3 to -.2.4)
HbE	-2.7 (-6.7 to 1.6)	-1.3 (-2.0 to -0.6)
HbA2	-1.3 (-5.1 to 0.5)	3.4 (2.8 to 4.1)
HbF	-2.3 (-4.1 to -0.7)	-3.5 (-4.2 to -2.8)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3.EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes are presented in Table 22.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
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2. **Contact Person(s):**
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3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ HbA_{1c} Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
The following table provides the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack**	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) K070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown on Table 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

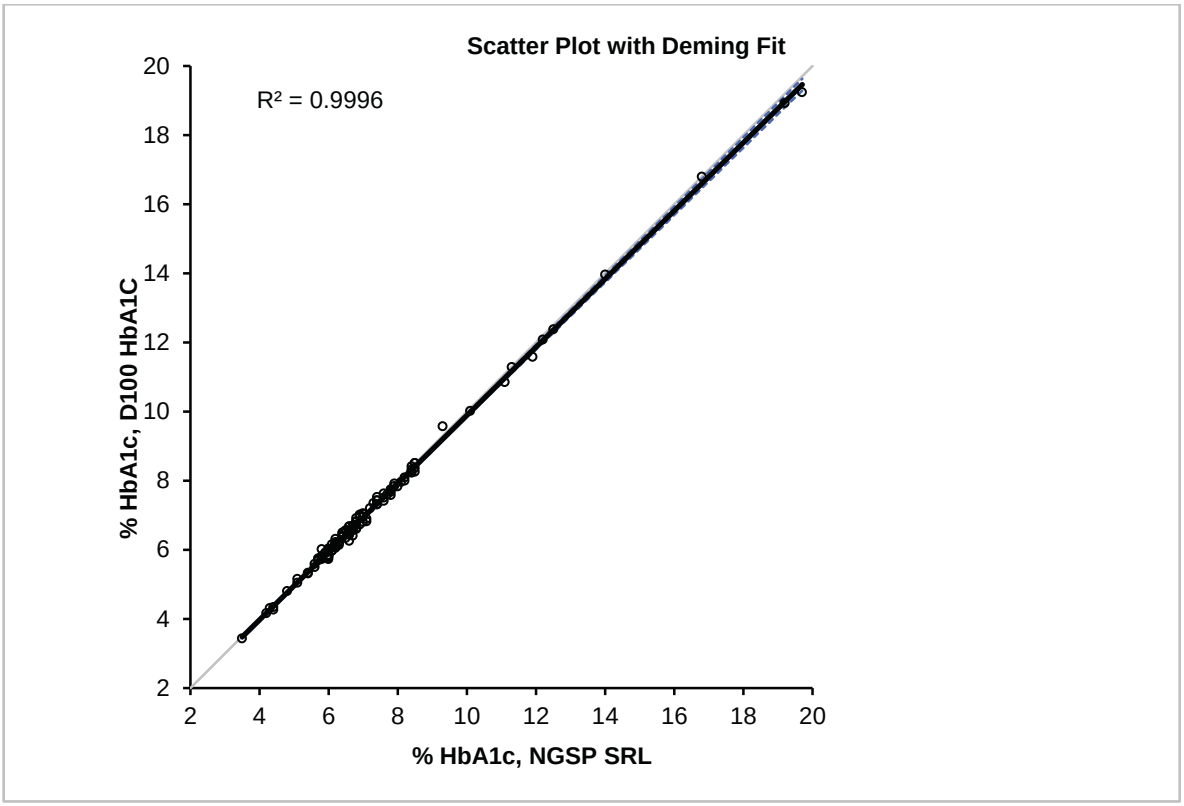

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85
6.5	-0.066	-0.98

8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in the Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure form, were obtained and stock solutions prepared at 10x the intended test

concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18.

Table 18: Drug Interference Study Results

Potential Drug Interferent	**Highest Level Tested showing no Significant Interference**	
	Conventional (US) units	**SI units**

Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of ~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 7% glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Percent Relative Bias to Comparative Method	
	HbA1c Concentration	
	~6.5%	~9.0%
HbS	-0.6 (±4.1)	-1.5 (±1.2)
HbC	-1.3 (±1.9)	-3.9 (±2.2)
HbD	-4.7 (±1.6)	-4.4 (±2.7)
HbE	-2.7 (±2.2)	-1.3 (±1.0)
HbA2	-1.3 (±0.6)	3.4 (±0.9)
HbF	-2.3 (±0.9)	-3.5 (±0.6)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3-EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association

Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
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   Clinical Diagnostics Group
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   Hercules, California 94547

2. **Contact Person(s):**
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3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 and 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack** (K)151321	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) 070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown in Tables 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method are summarized in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

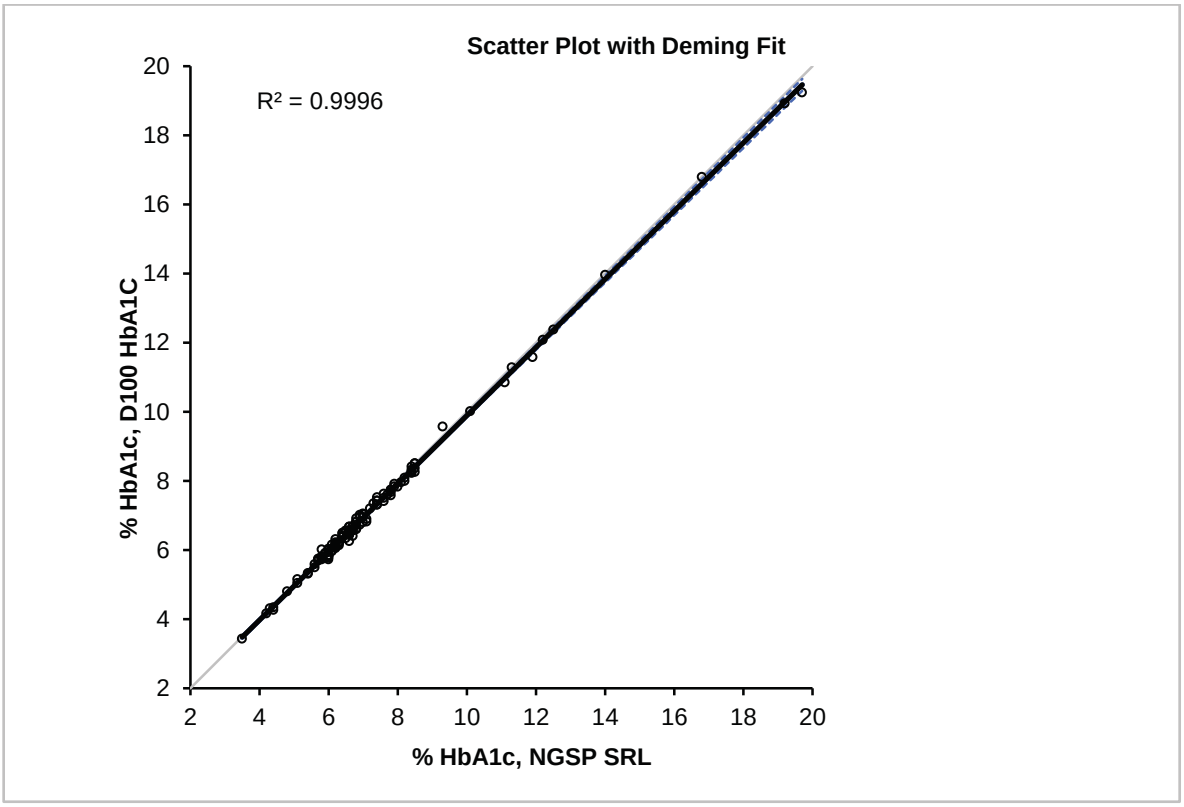

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85

6.5	-0.066	-0.98
8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure

form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18 on the following page.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	SI units
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of

~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 1200 mg/dL of glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative %Bias (Range of %Bias) for HbA1c	Relative %Bias (Range of %Bias) for HbA1c
	~6.5%	~9.0%
HbS	-0.6 (-5.8 to 5.5)	-1.5 (-3.3 to -0.1)
HbC	-1.3 (-4.0 to 1.3)	-3.9 (-5.5 to -2.4)
HbD	-4.7 (-6.7 to -1.1)	-4.4 (-6.3 to -.2.4)
HbE	-2.7 (-6.7 to 1.6)	-1.3 (-2.0 to -0.6)
HbA2	-1.3 (-5.1 to 0.5)	3.4 (2.8 to 4.1)
HbF	-2.3 (-4.1 to -0.7)	-3.5 (-4.2 to -2.8)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3.EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes are presented in Table 22.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151321

Device Name
D-100™ HbA1c
D-100™ HbA1c Calibrator Pack

Indications for Use *(Describe)*
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100™ HbA1c test is intended for Professional Use Only.

Calibrators:
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c(HbA1c) in human whole blood.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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BIO-RAD

D-100™ HbA_{1c}

Instructions For Use

USE REF

290-1004
290-1006
290-1007
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290-1011
290-1012

Σ 10000

CE

IVD

US: Rx Only

December 2015
16000328revB

UNITED STATES, *Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000*

EC REP **FRANCE**, *Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1), Ethoxylated Alcohol surfactant **Danger**	
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P311	If exposed or concerned: Call a poison center/doctor.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A_{1c} measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood.

Specimen Additives, Preservatives

The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ~9.0%
HbS	–0.6 (± 4.1)	–1.5 (± 1.2)
HbC	–1.3 (± 1.9)	–3.9 (± 2.2)
HbD	–4.7 (± 1.6)	–4.4 (± 2.7)
HbE	–2.7 (± 2.2)	–1.3 (± 1.0)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ~9.0%
HbF	–2.3 (± 0.9)	–3.5 (± 0.6)
HbA_2	–1.3 (± 0.6)	3.4 (± 0.9)

- At physiologically occurring concentrations, there is no interference from labile A_{1c}, carbamylated hemoglobin, or acetylated hemoglobin.[25]
- Common drugs at therapeutic concentrations do not interfere with the test.[25]

- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

The expected HbA_{1c} range for non-diabetic adults is 4–6%.[26]

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

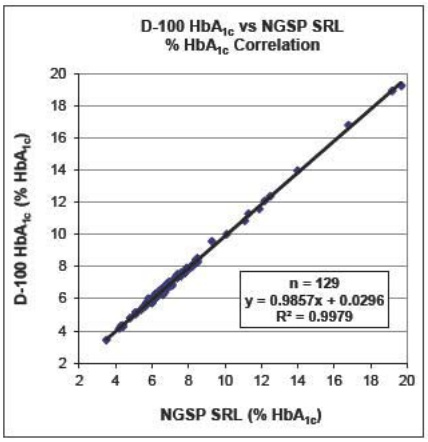


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

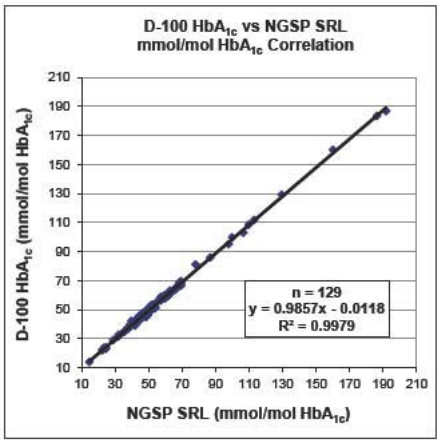


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.05	–0.85
6.5	–0.07	–0.98
8.0	–0.09	–1.11
12.0	–0.19	–1.57

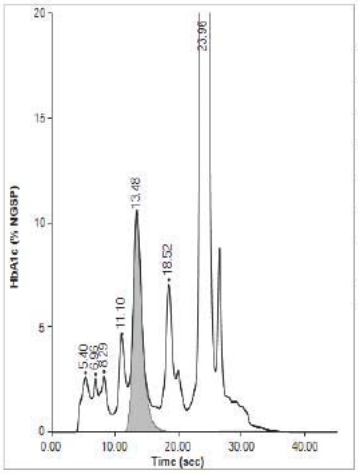
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



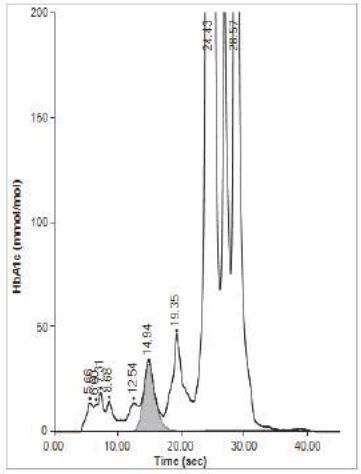
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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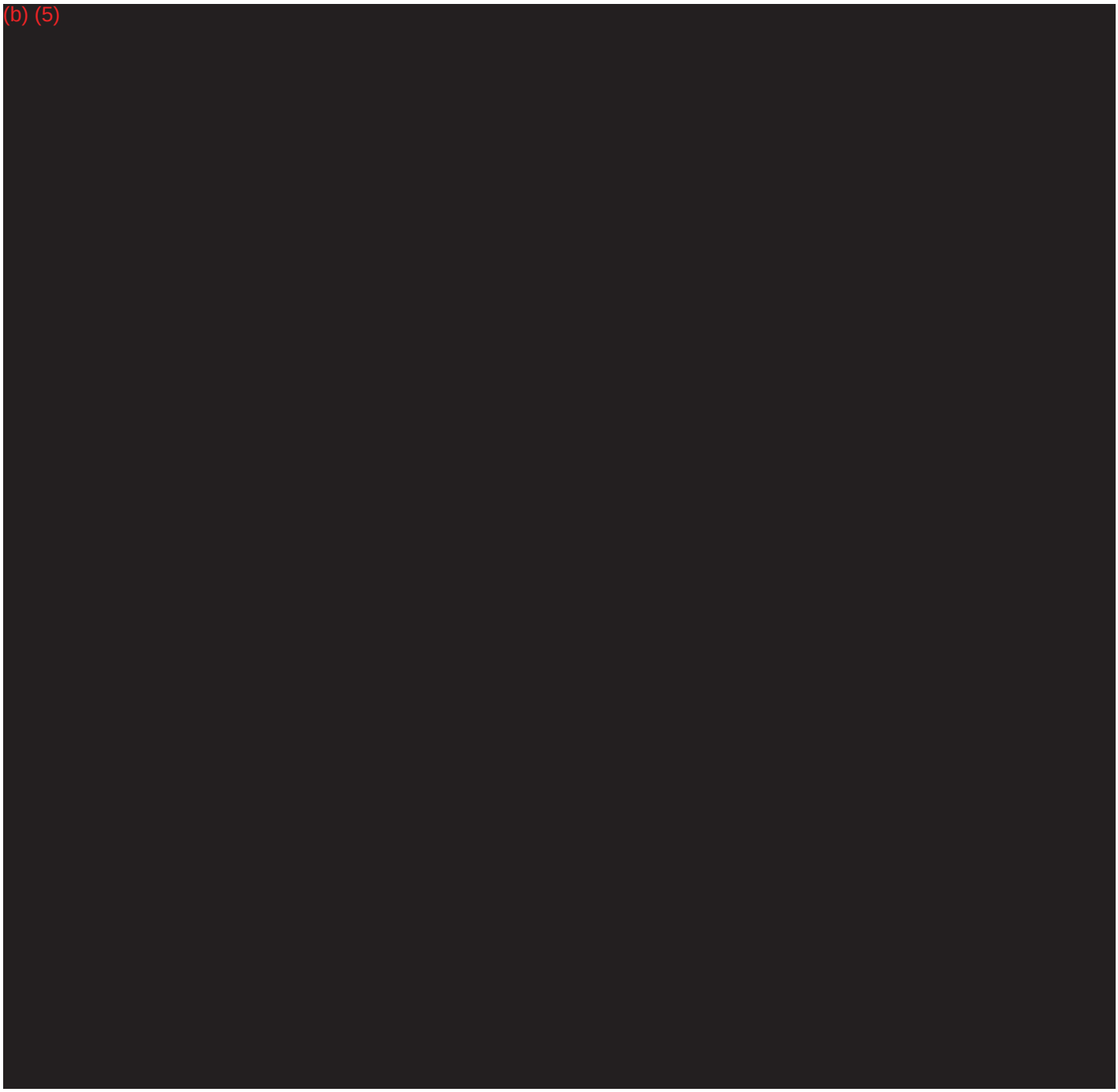

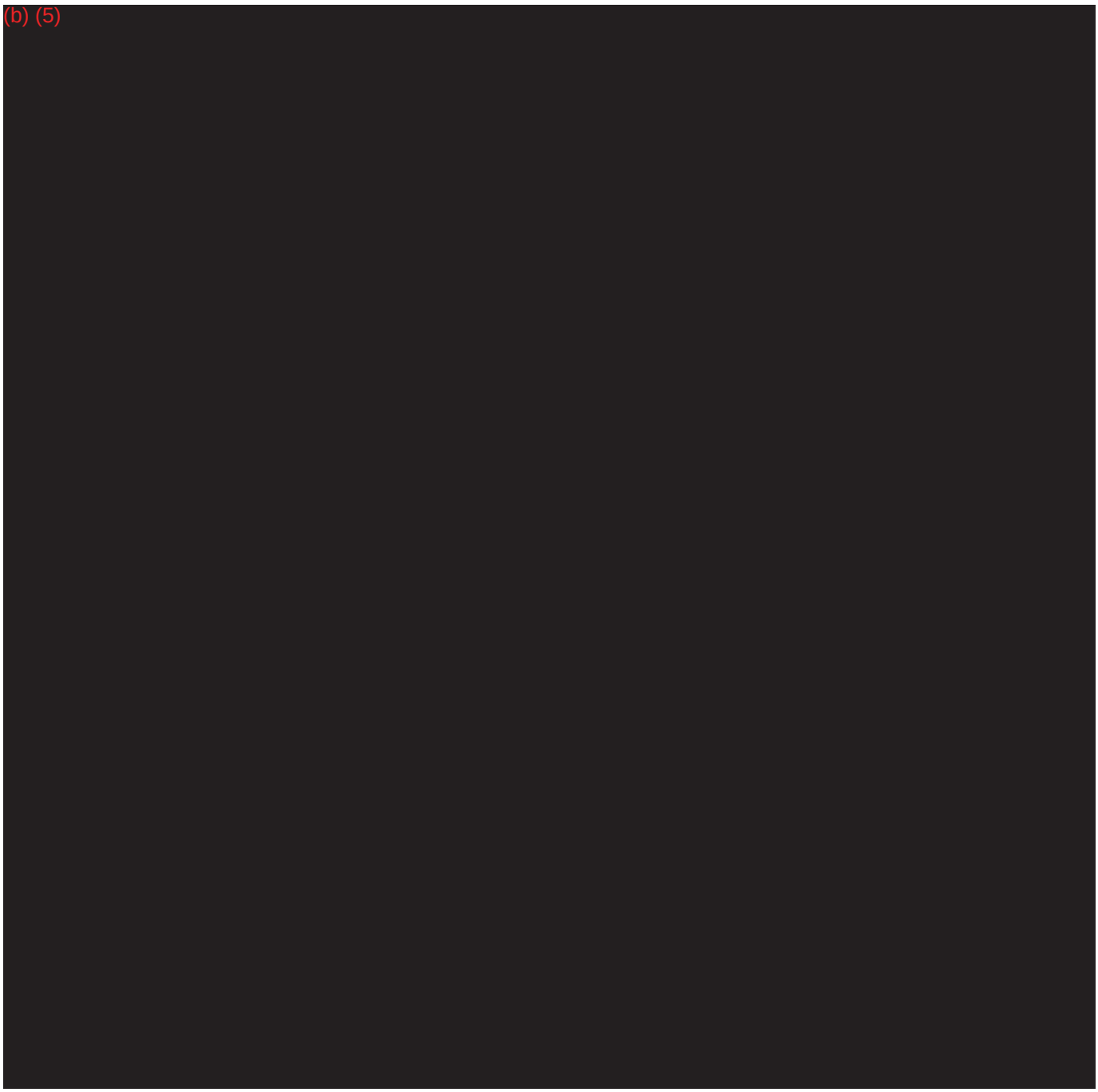

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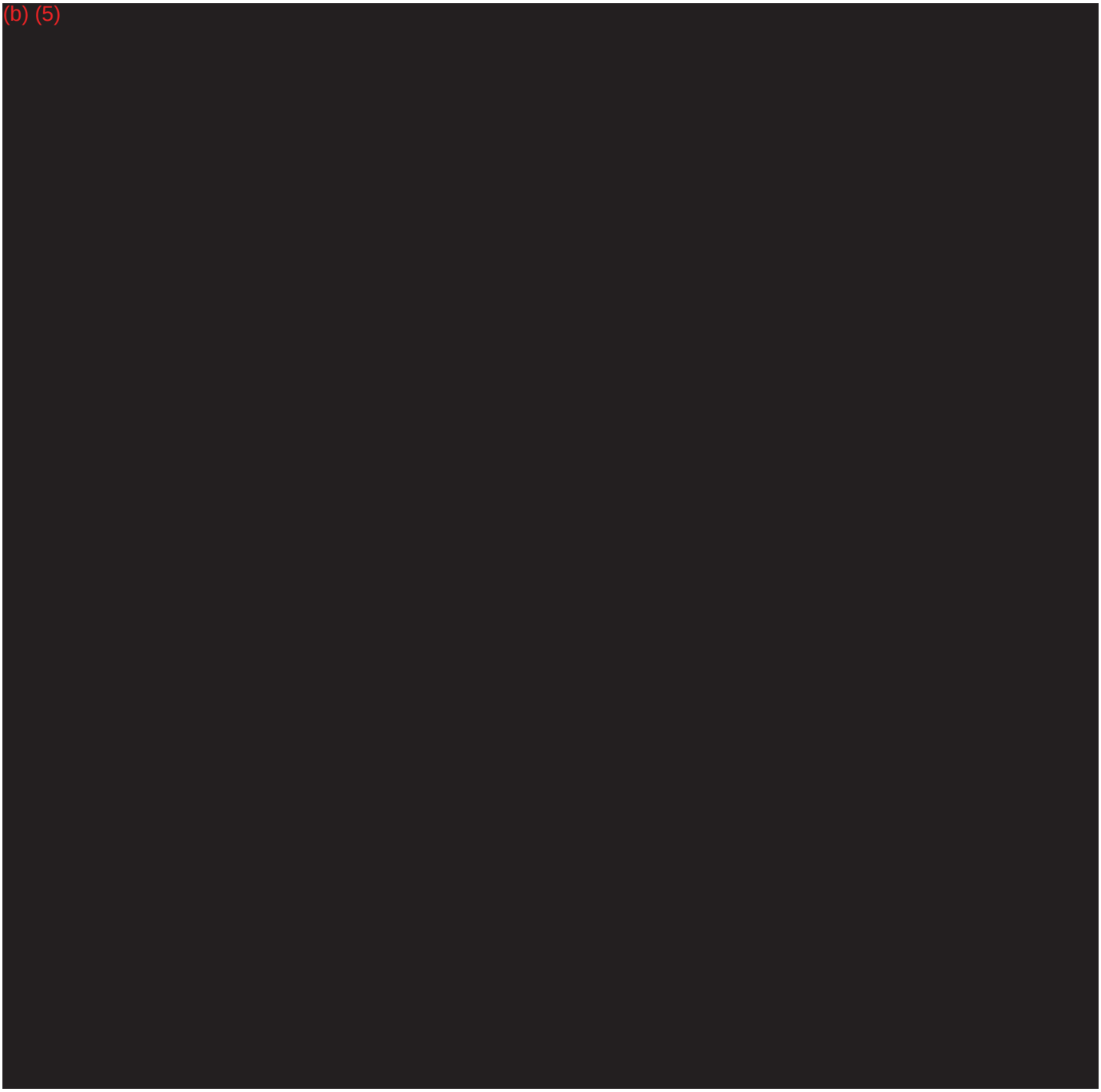

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Table 6: Mean HbA1c Retention Time in seconds for Group 1 – Calibrator Pack sets in MP1, MP2 and MP3 lots

Sample ID	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Test Point	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
t_o	13.58	13.52	13.51	13.57	13.49	13.49	13.57	13.48	13.51	13.53	13.48	13.46
t_2	13.57	13.47	13.55	13.55	13.47	13.53	13.56	13.48	13.54	13.52	13.44	13.52
$t_{o(6month)}$	13.89	13.74	13.78	13.86	13.79	13.75	13.85	13.83	13.80	13.83	13.79	13.85
t_6	13.85	13.80	13.84	13.83	13.75	13.82	13.84	13.79	13.82	13.82	13.74	13.80
$t_{o(12month)}$	13.39	13.39	13.38	13.34	13.41	13.37	13.36	13.39	13.34	13.33	13.34	13.33
t_{12}	13.47	13.45	13.40	13.37	13.33	13.38	13.41	13.37	13.37	13.41	13.37	13.36
$t_{o(18month)}$	13.97	13.99	14.04	13.96	13.97	14.00	13.97	13.96	13.99	13.96	13.97	13.93
t_{18}	13.98	13.95	13.40	13.95	13.94	14.04	13.97	13.94	14.01	13.95	13.93	13.97
$t_{o(24month)}$	13.83	13.82	13.98	13.83	13.82	13.95	14.10	13.82	13.97	13.80	13.80	13.93
t_{24}	13.86	13.84	13.90	13.85	13.85	13.91	13.86	13.85	13.91	13.82	13.83	13.88
$t_{o(28month)}$	13.97	13.94	14.08	13.95	13.94	14.34	13.96	13.96	14.36	13.91	13.90	14.35
t_{28}	13.95	13.95	14.28	13.93	13.93	14.24	13.95	13.95	14.25	13.89	13.90	14.24

Table 7: Mean %HbA1c Concentrations for Group 1 – Calibrator Pack sets in MP1, MP2 and MP3 lots

Sample ID	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Test Point	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
t_o	5.18	5.20	5.28	9.80	9.63	9.64	5.85	5.86	5.97	7.89	7.81	7.85
t_2	5.20	5.37	5.21	9.73	9.89	9.60	5.87	5.85	5.87	7.90	7.84	7.82
$t_{o(6month)}$	5.39	5.36	5.09	9.42	9.59	9.73	5.99	6.01	5.85	7.76	7.88	7.89
t_6	5.21	5.25	5.30	9.63	9.68	9.83	5.87	5.89	5.97	7.78	7.86	7.90
$t_{o(12month)}$	5.38	5.47	5.41	9.79	9.76	9.94	5.83	5.90	5.88	7.79	7.82	7.88
t_{12}	5.39	5.38	5.37	9.79	9.86	9.89	5.83	5.82	5.85	7.78	7.83	7.84
$t_{o(18month)}$	5.23	5.24	5.24	9.54	9.59	9.59	5.75	5.75	5.75	7.67	7.60	7.85
t_{18}	5.16	5.22	5.23	9.56	9.60	9.60	5.74	5.70	5.70	7.66	7.58	7.87
$t_{o(24month)}$	5.18	5.33	5.60	9.60	9.60	9.60	5.84	5.94	5.96	7.76	7.84	7.66
t_{24}	5.16	5.16	5.39	9.53	9.58	9.68	5.79	5.79	5.78	7.73	7.79	7.54
$t_{o(28month)}$	5.21	5.26	5.54	9.50	9.55	9.74	5.87	5.92	6.07	7.80	7.82	7.83
t_{28}	5.18	5.15	5.34	9.54	9.49	9.68	5.85	5.82	5.82	7.79	7.77	7.77

Table 8: %HbA1c Bias from Control for Group 1 – Calibrator Pack sets in MP1, MP2 and MP3 lots

Sample ID	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Test Point	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
t_o												
t_2	-0.39	-3.18	1.51	0.68	-2.70	0.46	-0.23	0.19	1.64	-0.12	-0.42	0.45
$t_{o(6month)}$												
t_6	3.45	2.06	-4.20	-2.31	-1.00	-1.09	1.98	1.95	-2.06	-0.21	0.19	-0.09
$t_{o(12month)}$												
t_{12}	-0.12	1.66	0.78	-0.03	-1.08	0.42	0.00	1.28	0.59	0.21	-0.13	0.56
$t_{o(18month)}$												
t_{18}	1.40	0.47	0.21	-0.19	-0.06	-1.21	0.21	0.83	0.25	0.19	0.32	-0.17
$t_{o(24month)}$												
t_{24}	0.32	3.17	3.71	0.72	0.22	-0.82	0.78	2.40	2.95	0.33	0.61	1.47
$t_{o(28month)}$												
t_{28}	0.43	2.11	3.63	-0.44	0.59	0.67	0.40	1.82	4.05	0.13	0.67	0.70
% Bias from Control ≤6%	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 10: Mean %HbA1c Concentrations for Cartridge/Prefilter sets in MP1, MP2 and MP3 lots

Sample ID	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Test Point	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
t_o	5.55	5.51	5.19	9.50	9.61	9.82	6.15	6.13	5.89	7.86	7.96	7.87
t_2	5.55	5.59	5.31	9.61	9.60	9.76	6.05	6.19	6.02	7.88	8.00	8.00
t_6	5.66	5.46	5.40	9.67	9.69	9.80	6.24	6.14	6.07	8.11	7.99	8.01
t_{12}	5.35	5.37	5.37	9.87	9.84	9.84	5.84	5.87	5.89	7.87	7.84	7.81
t_{18}	5.26	5.27	5.25	9.61	9.62	9.70	5.91	5.94	5.92	7.84	7.89	7.90
t_{24}	5.29	5.40	5.22	9.74	9.78	9.72	5.85	5.93	5.75	7.69	7.83	7.59
t_{28}	5.24	5.31	5.28	9.77	9.83	9.76	5.87	5.90	5.83	7.83	7.90	7.73

Table 9: Mean HbA1c Retention Time in seconds for Group 1 - Cartridge/Prefilter sets in MP1, MP2 and

Sample ID	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Test Point	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
t_o	14.43	13.74	13.47	14.39	13.72	13.44	14.43	13.70	13.46	14.36	13.67	13.43
t_2	14.54	13.81	13.63	14.50	13.79	13.62	14.58	13.77	13.61	14.56	13.75	13.59
t_6	14.52	14.19	14.30	14.41	14.17	14.30	14.44	14.07	14.30	14.37	14.19	14.32
t_{12}	13.70	13.59	13.57	13.72	13.61	13.62	13.67	13.57	13.59	13.67	13.56	13.58
t_{18}	14.33	14.12	14.26	14.32	14.07	14.18	14.31	14.04	14.21	14.28	14.03	14.14
t_{24}	14.52	14.21	14.32	14.52	14.18	14.30	14.57	14.20	14.32	14.51	14.16	14.28
t_{28}	14.29	14.12	14.11	14.30	14.11	14.09	14.27	14.09	14.12	14.25	14.06	14.11

	QSD Control Level 1			QSD Control Level 2			Normal Patient			Diabetic Patient		
Table 11: %H	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3	MP1	MP2	MP3
Sample ID												
Test Point												
t_o												
t_2	0.07	1.42	2.38	1.14	-0.06	-0.62	-1.62	0.91	2.30	0.18	0.53	1.65
t_6	2.13	-0.92	4.06	1.80	0.88	-0.29	1.57	0.07	3.04	3.15	0.34	1.87
t_{12}	-3.46	-2.50	3.60	3.88	2.46	0.19	-5.01	-4.20	-0.02	0.09	-1.48	-0.66
t_{18}	-5.17	-4.38	1.16	1.17	0.14	-1.28	-3.79	-3.19	0.49	-0.27	-0.85	0.44
t_{24}	-4.71	-1.92	0.54	2.47	1.81	-1.08	-4.87	-3.26	-2.38	-2.27	-1.70	-3.53
t_{28}	-5.51	-3.67	1.84	2.81	2.34	-0.64	-4.44	-3.84	-0.92	-0.47	-0.75	-1.75
% Bias from Control ≤ 6%	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BIO-RAD

D-100™ HbA_{1c}

Instructions For Use

USE REF

290-1004
290-1006
290-1007
290-1008
290-1009
290-1010
290-1011
290-1012

Σ 10000

CE

IVD

US: Rx Only

December 2015
16000328revB

UNITED STATES, *Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000*

EC REP **FRANCE**, *Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1), Ethoxylated Alcohol surfactant **Danger**	
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P311	If exposed or concerned: Call a POISON CENTER/doctor.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A_{1c} measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood.

Specimen Additives, Preservatives

The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbS	–0.6 (–5.8 to 5.5)	–1.5 (–3.3 to –0.1)
HbC	–1.3 (–4.0 to 1.3)	–3.9 (–5.5 to –2.4)
HbD	–4.7 (–6.7 to –1.1)	–4.4 (–6.3 to –2.4)
HbE	–2.7 (–6.7 to 1.6)	–1.3 (–2.0 to –0.6)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbF	–2.3 (–4.1 to –0.7)	–3.5 (–4.2 to –2.8)
HbA_2	–1.3 (–5.1 to 0.5)	3.4 (2.8 to 4.1)

- Labile A_{1c}, as indicated by glucose concentrations up to 1200 mg/dL, does not interfere with the assay.
- At physiologically occurring concentrations, there is no interference from carbamylated hemoglobin or acetylated hemoglobin.[25]

- Common drugs at therapeutic concentrations do not interfere with the test.[25]
- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

The expected HbA_{1c} range for non-diabetic adults is 4–6%.[26]

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

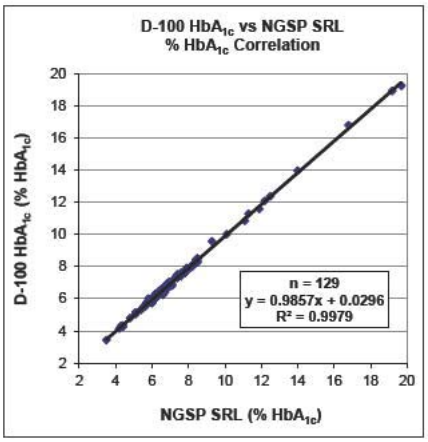


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

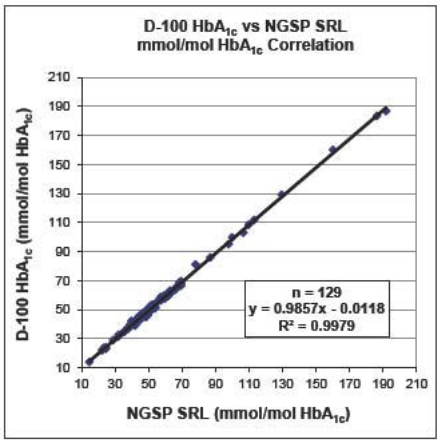


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.05	–0.85
6.5	–0.07	–0.98
8.0	–0.09	–1.11
12.0	–0.19	–1.57

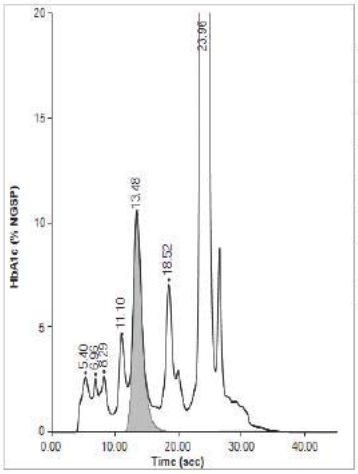
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



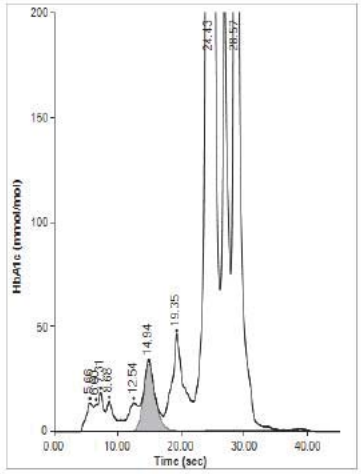
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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D-100™ HbA_{1c}

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Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1), Ethoxylated Alcohol surfactant **Danger**	
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P311	If exposed or concerned: Call a POISON CENTER/doctor.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A_{1c} measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood.

Specimen Additives, Preservatives

The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbS	–0.6 (–5.8 to 5.5)	–1.5 (–3.3 to –0.1)
HbC	–1.3 (–4.0 to 1.3)	–3.9 (–5.5 to –2.4)
HbD	–4.7 (–6.7 to –1.1)	–4.4 (–6.3 to –2.4)
HbE	–2.7 (–6.7 to 1.6)	–1.3 (–2.0 to –0.6)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbF	–2.3 (–4.1 to –0.7)	–3.5 (–4.2 to –2.8)
HbA_2	–1.3 (–5.1 to 0.5)	3.4 (2.8 to 4.1)

- Labile A_{1c}, as indicated by glucose concentrations up to 1200 mg/dL, does not interfere with the assay.
- At physiologically occurring concentrations, there is no interference from carbamylated hemoglobin or acetylated hemoglobin.[25]

- Common drugs at therapeutic concentrations do not interfere with the test.[25]
- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

The expected HbA_{1c} range for non-diabetic adults is 4–6%.[26]

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

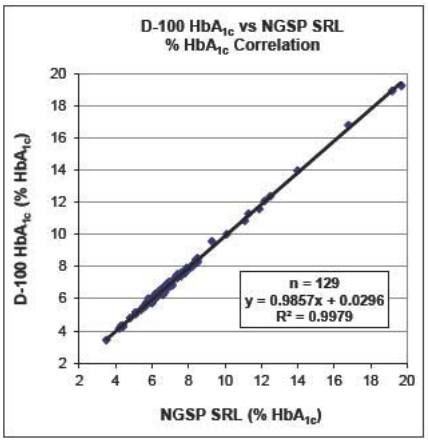


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

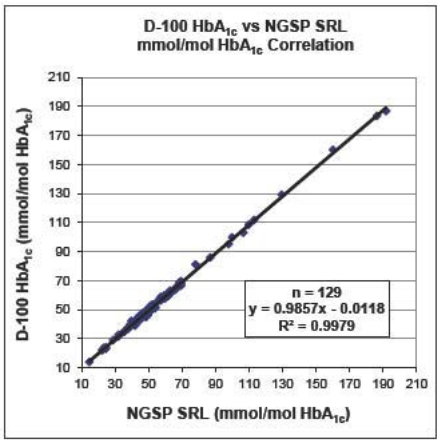


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.05	–0.85
6.5	–0.07	–0.98
8.0	–0.09	–1.11
12.0	–0.19	–1.57

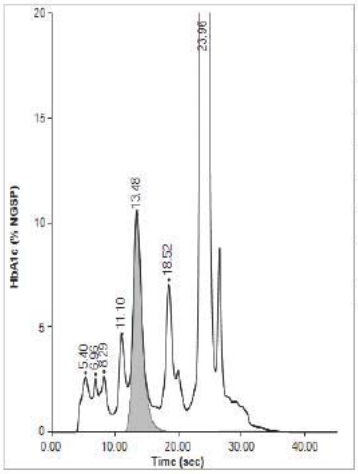
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



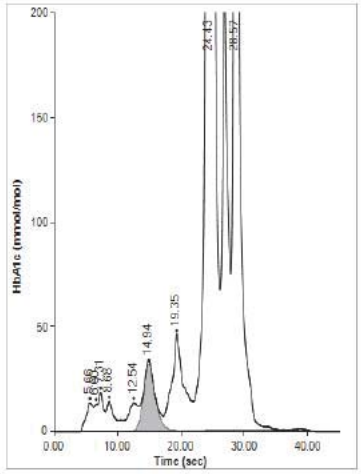
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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NOTES:

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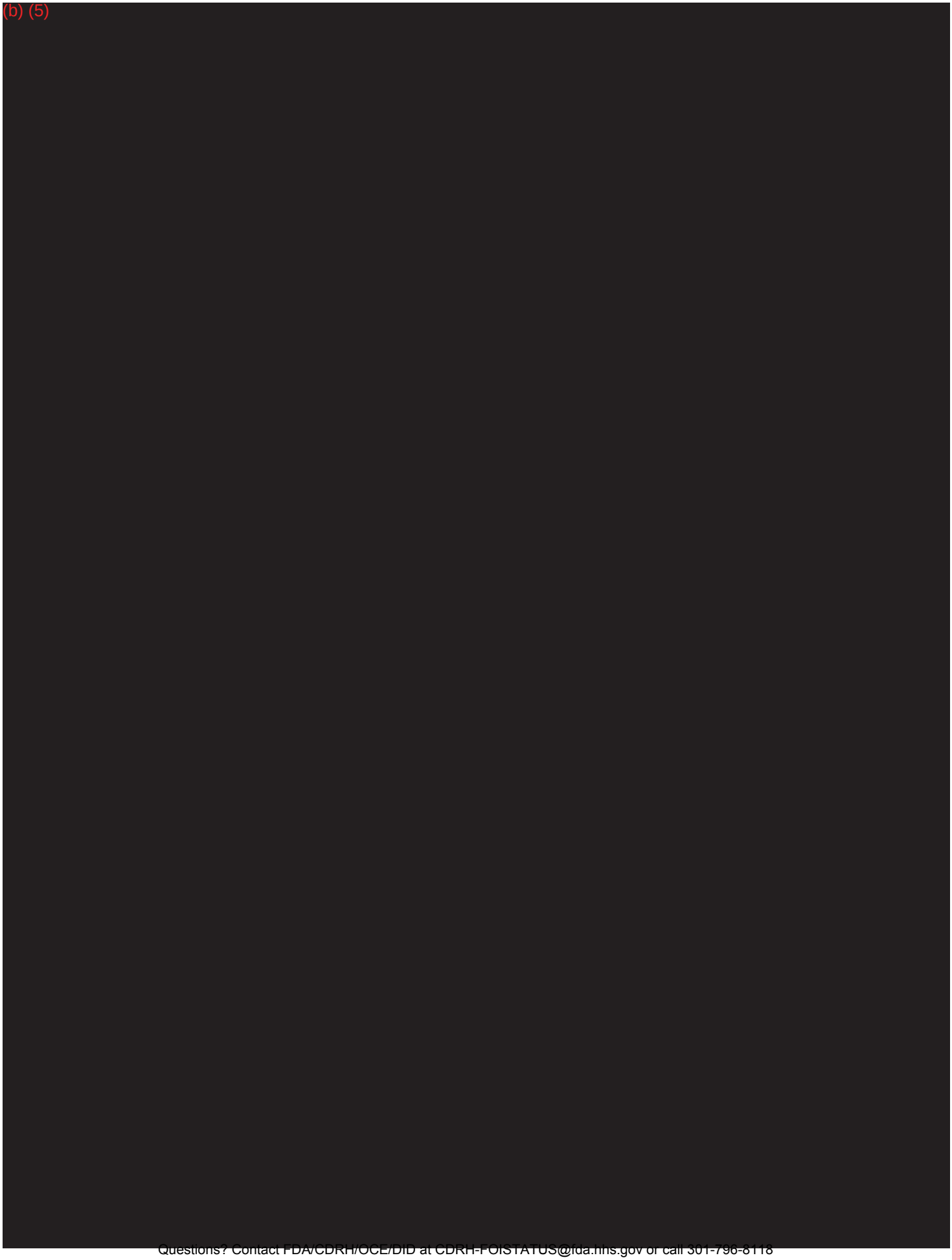

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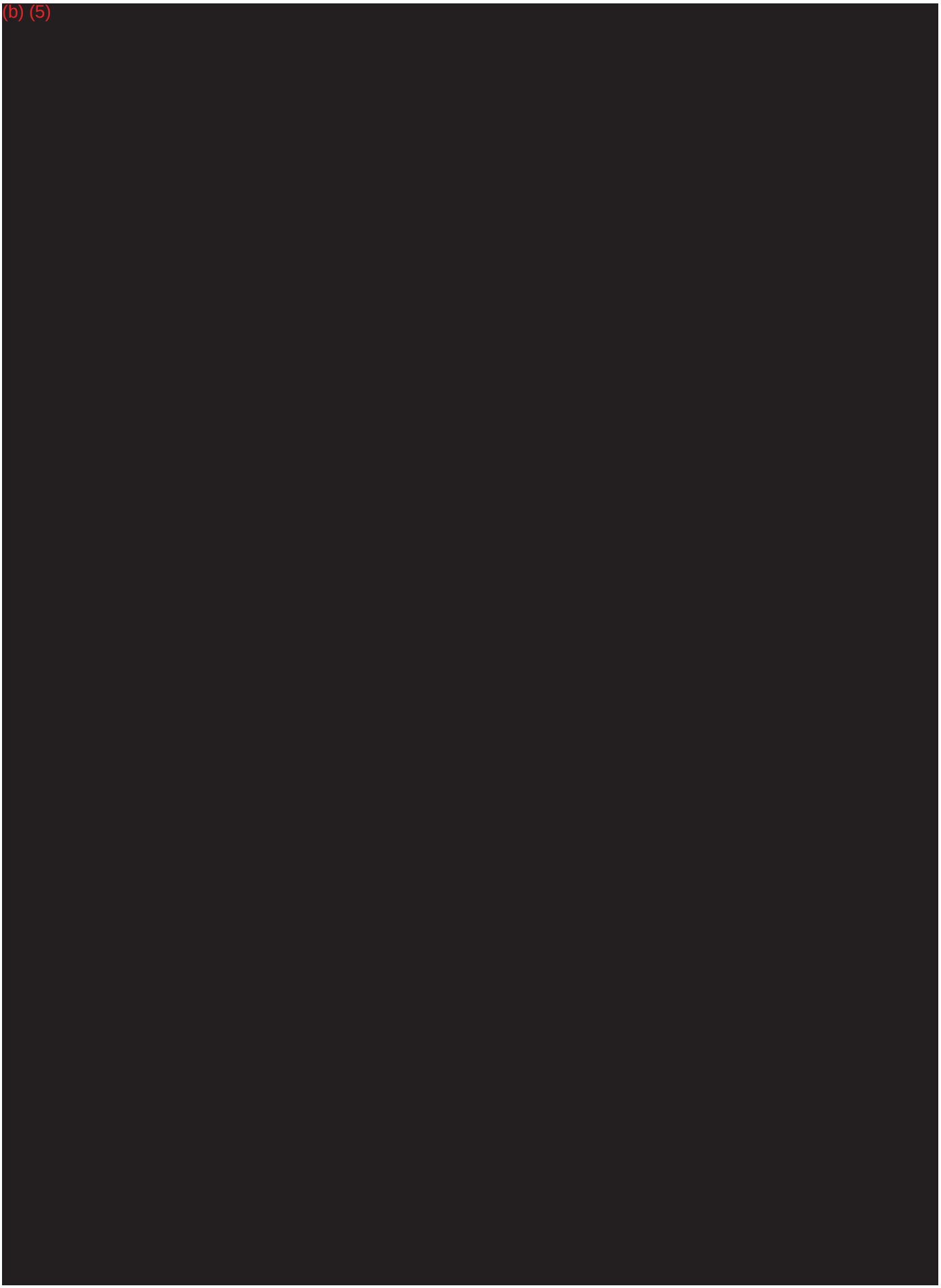

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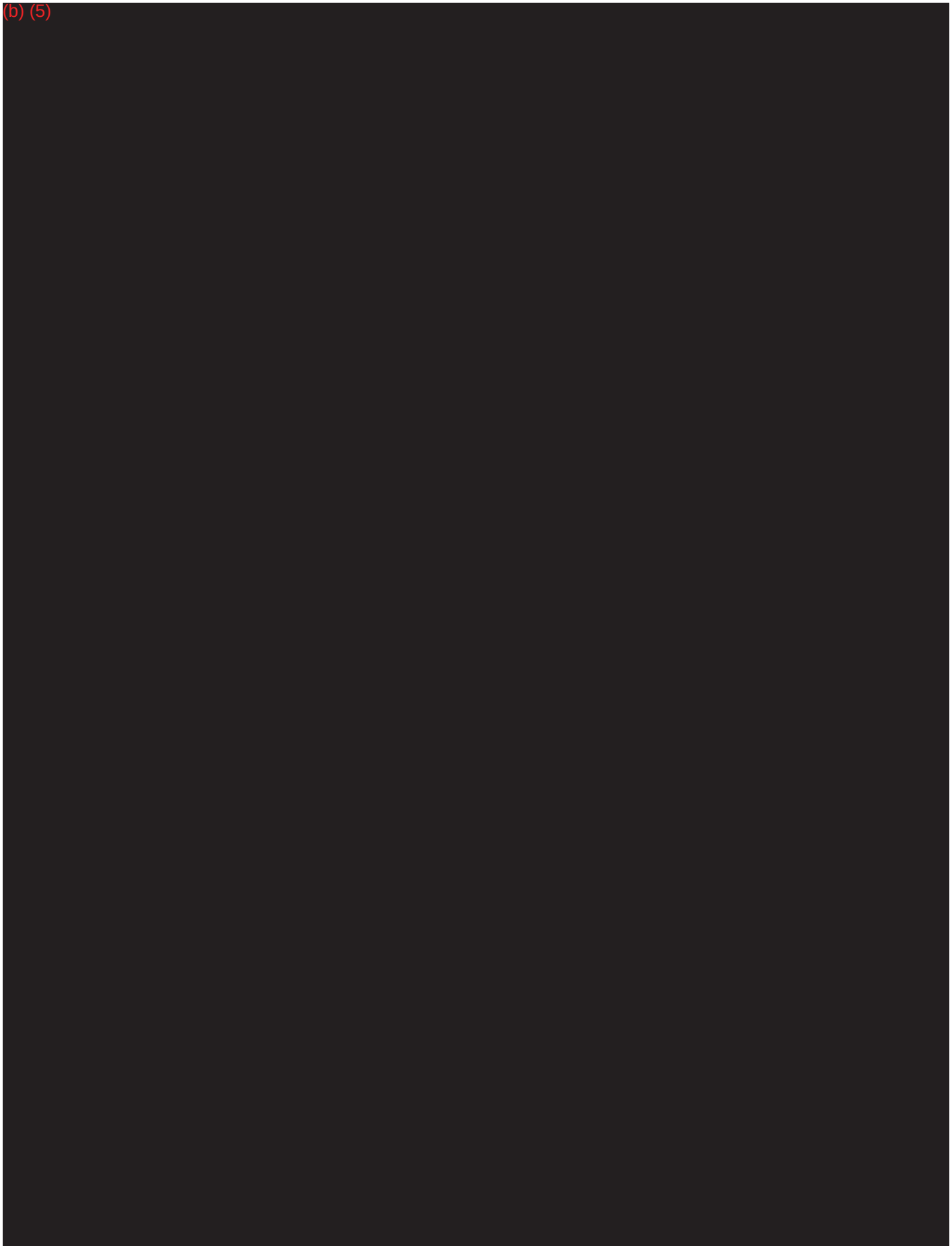

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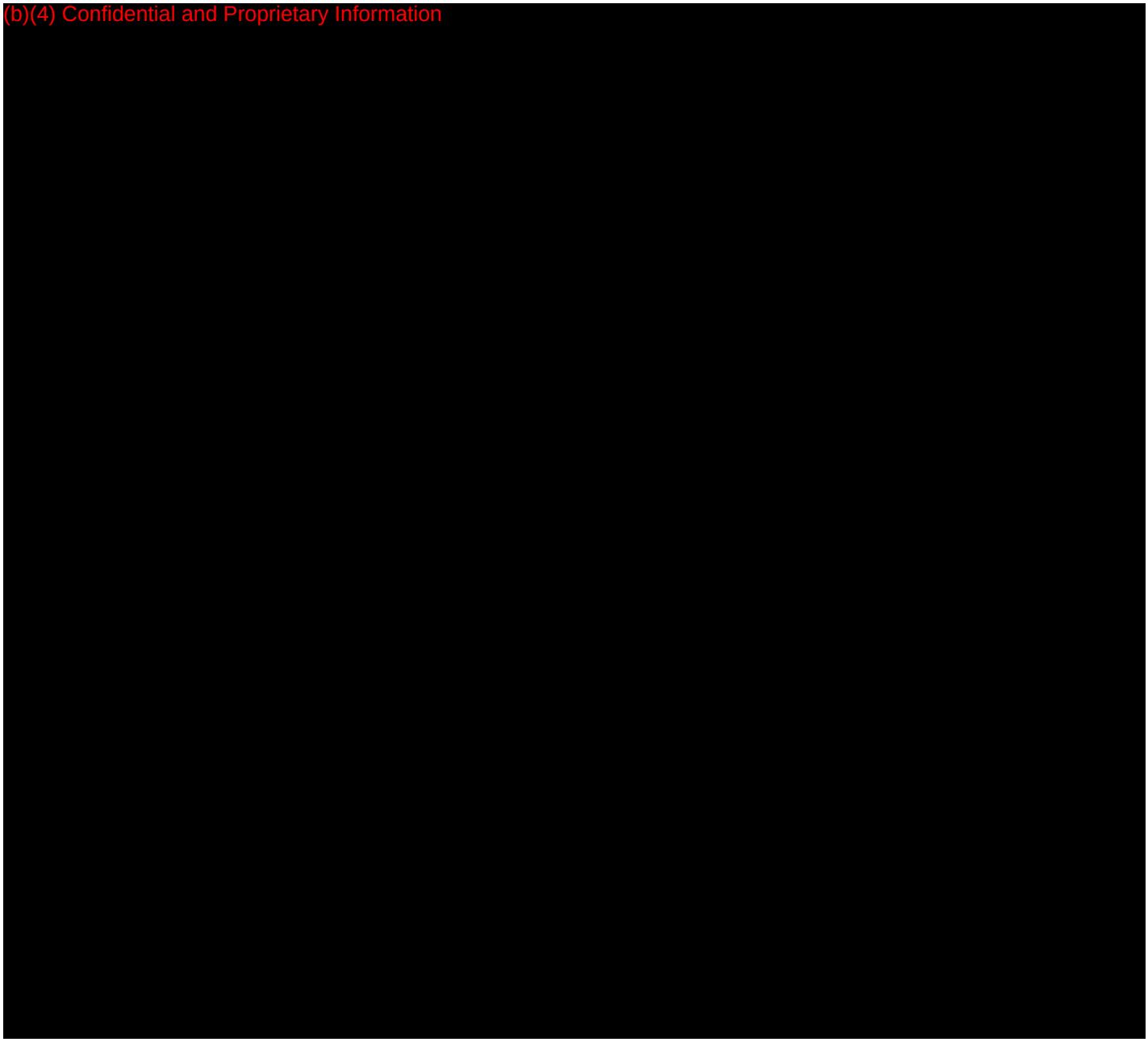

Dear Ms. Buckley,

My name is Alain Silk, I am a scientific/regulatory reviewer at the US FDA in the Office of In Vitro Diagnostics and Radiological Health. I have been assigned to review your Traditional 510(k) Submission for the D-100 HbA1c test. Your submission has been received by the Agency and the number assigned to your submission is K151321. We will contact you as soon as possible regarding any review decision or to request additional information as necessary. Please feel free to contact me via email or phone if you have any comments or questions.

Best Regards,

Alain

Alain Silk, Ph.D.
Scientific/Regulatory Reviewer
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics and Radiological Health
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10903 New Hampshire Avenue
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tel: 301-796-2129 |email: alain.silk@fda.hhs.gov

From: Silk, Alain
To: jackie_buckley@bio-rad.com
Cc: K151321@docs.fda.gov
Subject: K151321 received
Date: Monday, June 01, 2015 12:26:00 PM

Dear Ms. Buckley,

My name is Alain Silk, I am a scientific/regulatory reviewer at the US FDA in the Office of In Vitro Diagnostics and Radiological Health. I have been assigned to review your Traditional 510(k) Submission for the D-100 HbA1c test. Your submission has been received by the Agency and the number assigned to your submission is K151321. We will contact you as soon as possible regarding any review decision or to request additional information as necessary. Please feel free to contact me via email or phone if you have any comments or questions.

Best Regards,

Alain

Alain Silk, Ph.D.
Scientific/Regulatory Reviewer
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

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10903 New Hampshire Avenue
WO 66, Room 5623
Silver Spring, MD 20993-0002
tel: 301-796-2129 |email: alain.silk@fda.hhs.gov

From: Alain Silk
To: jackie_buckley@bio-rad.com
Cc: Silk, Alain
Subject: K151321 was Accepted
Date: Monday, June 01, 2015 4:32:16 PM

June 1, 2015

Acceptance Review Notification - Accepted

An administrative acceptance review was conducted on your premarket notification (510(k)) K151321, and it was found to contain all of the necessary elements and information needed to proceed with the substantive review. We will contact you should we require any additional information during the course of the substantive review. The lead reviewer assigned to your submission is Alain Silk.

*** This is a system-generated email notification ***

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
   Bio-Rad Laboratories, Inc.
   Clinical Diagnostics Group
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   Hercules, California 94547

2. **Contact Person(s):**
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   FAX: (510) 741-6471
   E-Mail: jackie_buckley@bio-rad.com

   Alfred Evans, RA/QA Director
   Telephone Number: (510) 741-4579
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   E-Mail:al_evans@bio-rad.com

3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 and 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack** (K)151321	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) 070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown in Tables 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method are summarized in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

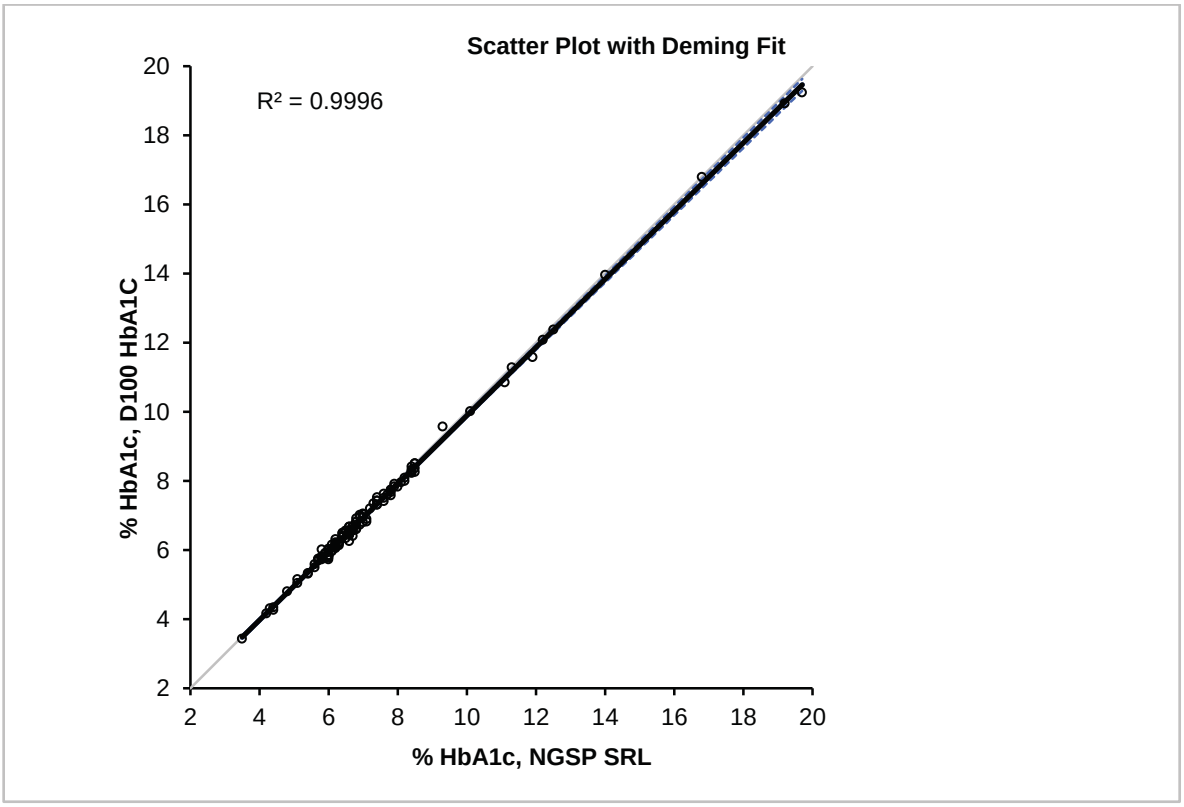

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85

6.5	-0.066	-0.98
8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure

form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18 on the following page.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	SI units
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of

~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 1200 mg/dL of glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative %Bias (Range of %Bias) for HbA1c	Relative %Bias (Range of %Bias) for HbA1c
	~6.5%	~9.0%
HbS	-0.6 (-5.8 to 5.5)	-1.5 (-3.3 to -0.1)
HbC	-1.3 (-4.0 to 1.3)	-3.9 (-5.5 to -2.4)
HbD	-4.7 (-6.7 to -1.1)	-4.4 (-6.3 to -.2.4)
HbE	-2.7 (-6.7 to 1.6)	-1.3 (-2.0 to -0.6)
HbA2	-1.3 (-5.1 to 0.5)	3.4 (2.8 to 4.1)
HbF	-2.3 (-4.1 to -0.7)	-3.5 (-4.2 to -2.8)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3.EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes are presented in Table 22.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
   Bio-Rad Laboratories, Inc.
   Clinical Diagnostics Group
   4000 Alfred Nobel Drive
   Hercules, California 94547

2. **Contact Person(s):**
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3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 and 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack** (K)151321	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) 070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown in Tables 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method are summarized in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

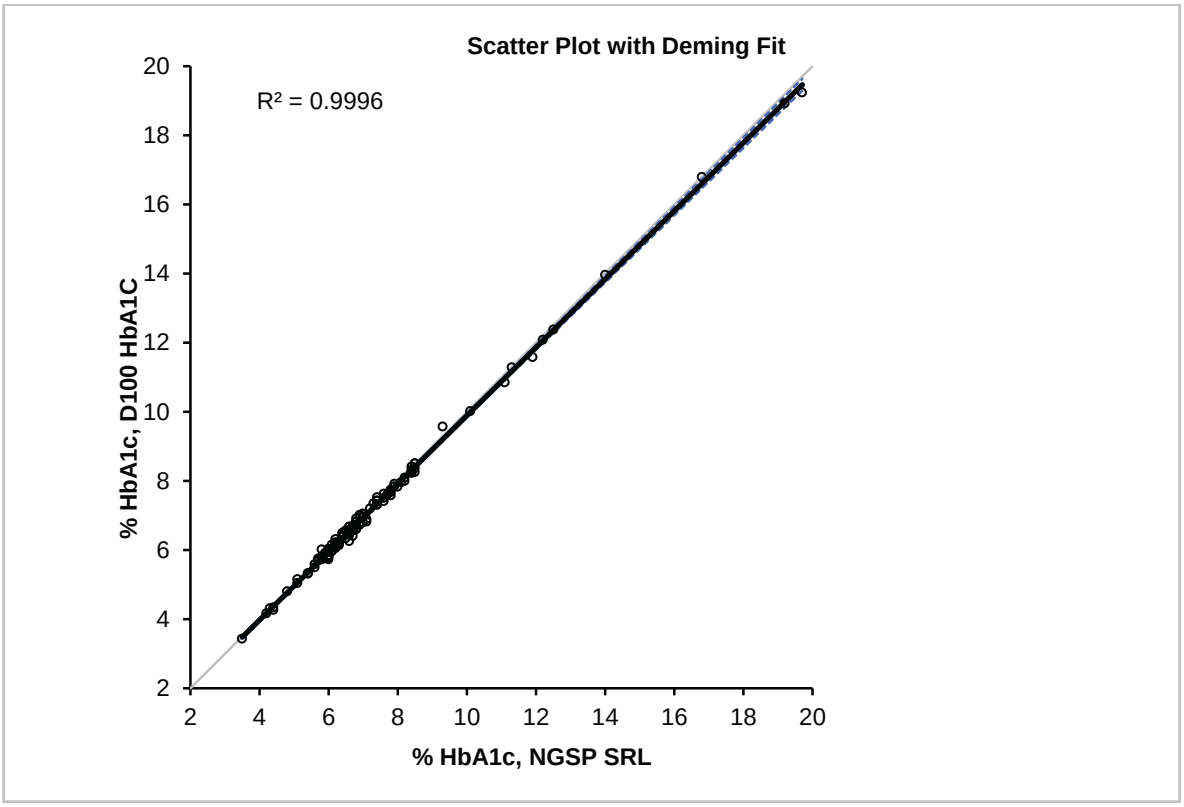

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85

6.5	-0.066	-0.98
8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure

form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18 on the following page.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	SI units
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of

~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 1200 mg/dL of glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative %Bias (Range of %Bias) for HbA1c	Relative %Bias (Range of %Bias) for HbA1c
	~6.5%	~9.0%
HbS	-0.6 (-5.8 to 5.5)	-1.5 (-3.3 to -0.1)
HbC	-1.3 (-4.0 to 1.3)	-3.9 (-5.5 to -2.4)
HbD	-4.7 (-6.7 to -1.1)	-4.4 (-6.3 to -.2.4)
HbE	-2.7 (-6.7 to 1.6)	-1.3 (-2.0 to -0.6)
HbA2	-1.3 (-5.1 to 0.5)	3.4 (2.8 to 4.1)
HbF	-2.3 (-4.1 to -0.7)	-3.5 (-4.2 to -2.8)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3.EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes are presented in Table 22.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

Dear Ms. Buckley,

Thank you for submitting k151321 for the Bio-Rad D-100 Hemoglobin Testing System. We have reviewed your submission and we cannot determine if the device is substantially equivalent to a legally marketed predicate device based solely on the information you provided. The following deficiencies will need to be addressed before the review of this submission can be completed. To complete the review of your submission, we require responses to the following deficiencies:

(b)(4) Confidential and Proprietary Information - Deficiencies

[redacted]

(b)(4) Confidential and Proprietary Information - Deficiencies

(b)(4) Confidential and Proprietary Information - Deficiencies

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(b)(4) Confidential and Proprietary Information - Deficiencies

(b)(4) Confidential and Proprietary Information - Deficiencies

(b)(4) Confidential and Proprietary Information - Deficiencies

[redacted]

The deficiencies identified above represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act (Act) for determining substantial equivalence of your device.

Once we receive your responses to the outlined deficiencies regarding your 510(k) submission, please be advised that there may be additional issues that arise after further review of your submission.

If you have any questions regarding the contents of this letter, please contact Alain Silk at 301-796-2129 or alain.silk@fda.hhs.gov.

Best Regards,

Alain D. Silk -S
2015.07.20 13:00:13 -04'00'

Alain Silk, Ph.D.
Scientific Reviewer
FDA/CDRH/OIR/DCTD

Through:

Stayce Beck, Ph.D., M.P.H.
Chief, Diabetes Diagnostic Devices Branch
FDA/CDRH/OIR/DCTD

Dear Ms. Buckley,

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Best Regards,

Alain D. Silk -S
2015.07.20 13:00:13 -04'00'

Alain Silk, Ph.D.
Scientific Reviewer
FDA/CDRH/OIR/DCTD

Through:

Jacquline A. Yancy -S
2015.07.20 13:12:39 -04'00'

For
Stayce Beck, Ph.D., M.P.H.
Chief, Diabetes Diagnostic Devices Branch
FDA/CDRH/OIR/DCTD

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151321

Device Name
D-100™ HbA1c
D-100™ HbA1c Calibrator Pack

Indications for Use *(Describe)*
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100™ HbA1c test is intended for Professional Use Only.

Calibrators:
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c(HbA1c) in human whole blood.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

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BIO-RAD

D-100™ HbA_{1c}

Instructions For Use

USE REF

290-1004
290-1006
290-1007
290-1008
290-1009
290-1010
290-1011
290-1012

Σ 10000

CE

IVD

US: Rx Only

December 2015
16000328revB

UNITED STATES, *Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000*

EC REP **FRANCE**, *Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1), Ethoxylated Alcohol surfactant **Danger**	
H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P311	If exposed or concerned: Call a POISON CENTER/doctor.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A_{1c} measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood.

Specimen Additives, Preservatives

The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbS	–0.6 (–5.8 to 5.5)	–1.5 (–3.3 to –0.1)
HbC	–1.3 (–4.0 to 1.3)	–3.9 (–5.5 to –2.4)
HbD	–4.7 (–6.7 to –1.1)	–4.4 (–6.3 to –2.4)
HbE	–2.7 (–6.7 to 1.6)	–1.3 (–2.0 to –0.6)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	Relative % Bias (Range of % Bias) for HbA_{1c} ~6.5%	Relative % Bias (Range of % Bias) for HbA_{1c} ~9.0%
HbF	–2.3 (–4.1 to –0.7)	–3.5 (–4.2 to –2.8)
HbA_2	–1.3 (–5.1 to 0.5)	3.4 (2.8 to 4.1)

- Labile A_{1c}, as indicated by glucose concentrations up to 1200 mg/dL, does not interfere with the assay.
- At physiologically occurring concentrations, there is no interference from carbamylated hemoglobin or acetylated hemoglobin.[25]

- Common drugs at therapeutic concentrations do not interfere with the test.[25]
- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

The expected HbA_{1c} range for non-diabetic adults is 4–6%.[26]

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

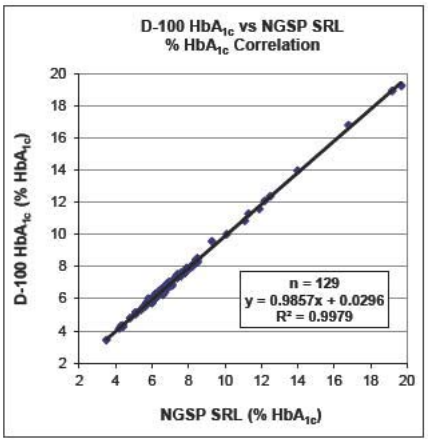


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

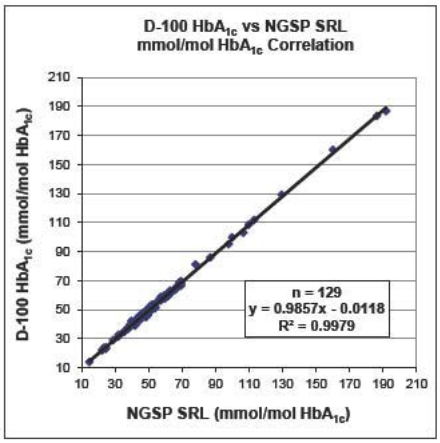


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.05	–0.85
6.5	–0.07	–0.98
8.0	–0.09	–1.11
12.0	–0.19	–1.57

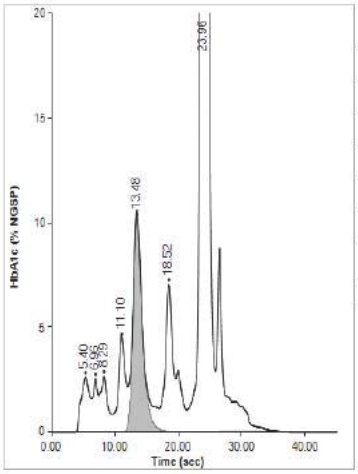
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



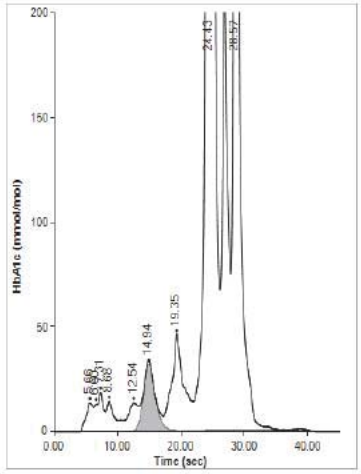
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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NOTES:

TECHNICAL ASSISTANCE

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

Outside the USA: Contact your regional Bio-Rad office. Go to www.bio-rad.com for contact information.

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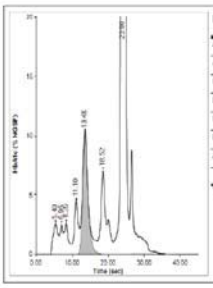
Result Examples

Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: 10.2 %



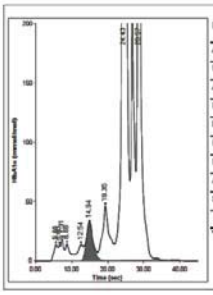
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1.88	---
A1b	6.06	1573.43	1.05	---
F	8.29	2612.14	1.74	---
LA1c	11.10	4492.47	2.99	---
HbA1c	13.48	12431.12	---	10.2
P3	18.52	9123.63	6.06	---
A0	23.95	117434.53	78.03	---

Total Area: 150490

Status: Held

Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

HbA1c: 32 mmol/mol 5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	---
Unknown	6.60	391.10	0.23	---
A1b	7.31	994.53	0.59	---
F	8.66	1222.50	0.73	---
LA1c	12.54	1345.98	0.80	---
HbA1c	14.94	3705.06	---	32
P3	19.36	6726.02	3.42	---
A0	24.43	83432.11	49.81	---
S-Window	28.57	69897.36	41.61	---

Total Area: 167509

Status: Held

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LB0003610revA
December 2014

Result Review

Any results that do not meet the following criteria should not be reported.

Item	Criteria
Total Area range	50,000–350,000
Quality Control	Values should be in range
HbA_{1c} reportable range	• NGSP: 3.5–20.0% • IFCC: 15–195 mmol/mol • Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.
HbF	≤30%
Labile A_{1c} (LA1c)	No interference
Carbamylated hemoglobin (CHb)	• No interference • CHb elutes in the LA1c window
P3 peak	≤10%
Heterozygous hemoglobins E, D, S, and C	No interference
E, D, S, and/or C windows	Combined area <50%
"Unknown" peaks	"Unknown" peak ≤10%

Test Components

USE	REF	Symbol	Description
290-1004		ANLT CRTR CAL PACK	Analytical Cartridge/Calibrator Pack
290-1006		CAL PACK	Calibrator Pack
290-1007		PRE FIL	Prefilters (5 per package)
290-1008		CLN TUBE	Cleaning Tube
290-1009		SAMP DIL	Sample Diluent
290-1010		BUF A	Elution Buffer A
290-1011		BUF B	Elution Buffer B
290-1012		WSH SOLN	Wash Solution

D-100™ HbA_{1c} Quick Guide

CE IVD

- *This Quick Guide is for reference use only; for detailed information, see the Instructions For Use and Operation Manual.*
- *Consider any materials of human origin as infectious and handle them using appropriate biosafety procedures.*
- *Wear personal protective equipment while handling all reagents and samples and while operating the D-100 system.*

Sample Preparation

HbA_{1c} Calibrator Pack:

- Contains Conditioner, Calibrator Level 1, and Calibrator Level 2.
- No manual preparation required. Can be used immediately after removing from refrigerator.
- Once reconstituted by system, stable for 24 hours at 2–8 °C; do not freeze.

Liquichek™ Diabetes Controls:

- After opening vial, stable for 14 days at 2–8 °C.
- Dilute 1:200 prior to analysis (5 µL of control in 1.0 mL of Sample Diluent).

Lyphochek® Diabetes Controls:

- Reconstitute each vial with 0.5 mL of DI water.
- Allow to stand for 5–10 minutes; swirl gently to dissolve.
- Stable for 7 days at 2–8 °C.
- Dilute 1:300 prior to analysis (5 µL of control in 1.5 mL of Sample Diluent).

Whole blood samples:

- Samples should be collected in vacuum collection tubes containing anti-coagulant. For detailed information, see the *Instructions For Use*.
- No sample preparation required.
- If abnormal tube type or height of sample is ≤1 cm, sample may need to be prediluted 1:300 prior to analysis (5 µL of sample in 1.5 mL of Sample Diluent).

Replacing the Reagents

After installing the bottles, the reagents are stable for 90 days at 15–35 °C. A reagent bottle can be removed at any time, except when the bottle is "In Use" in Running state.

1. Remove the empty bottle.
   NOTE: *If the bottle is not empty, touch the reagent indicator in the consumables panel, then touch* ***Remove*** *to depressurize the bottle.*
2. Install a new bottle. The reagent information is automatically updated.

Replacing the Prefilter

Replace the prefilter at 90 days or 2000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the prefilter holder door.
3. Remove the old prefilter.
4. Insert the new prefilter.
5. Close the prefilter holder door. The prefilter information is automatically updated.

Replacing the Analytical Cartridge

Replace the analytical cartridge at 90 days or 10,000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the cartridge holder door.
3. Remove the old cartridge.
4. Insert the new cartridge.
5. Close the cartridge holder door. Test parameters are automatically updated.

Calibration

Calibration must be performed once, following the installation of every new analytical cartridge.

1. Ensure the instrument is in Sleeping or Standby state.
2. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
3. Insert Diabetes Controls (in barcoded adapters) in positions 1–3 and insert the D-100 Calibrator Pack in the dedicated position, with the barcodes facing you.
4. Touch ⏏ **Load**.
5. Touch Calibrate Now.

NOTE: *Racks containing patient samples can be placed in the input area to be automatically processed after calibration has passed.*

6. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
7. Remove the samples from the Stat rack.

Routine Sample Run

1. Check the message panel for red or yellow messages and address as needed.
2. Insert Diabetes Controls (in barcoded adapters) followed by patient samples in racks.
   NOTE: *At least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested.*
3. Place the rack(s) in the input area.
4. Touch Run ►.
5. Load additional racks of samples during the run as needed.
6. Remove the processed racks from the output area.

NOTE: *When the run is complete, the instrument goes to Standby state for approximately 2 hours.*

Running Stat Samples

The D-100 System will analyze Stat samples after completing the last aspirated sample from the routine run.

1. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
2. Insert the sample(s) (i.e., primary sample tubes or prediluted samples in microvial adapters) in positions 1–3 of the Stat rack with the barcode(s) facing you.
3. Touch ⏏ **Load** to process the Stat samples.
4. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
5. Remove the samples from the Stat rack.

NOTE: *If the instrument is not in Running state, Stat samples can be run by loading the Stat area, touching* ⏏ ***Load****, and touching* Run ►.

Viewing Results

1. Touch the **Results** tab.
2. To view a sample result in detail, touch the result row.
3. To filter the results in the table, use the Filter buttons.
   - The filter currently in use is indicated to the right of the ⏬ button.
   - Flagged results can be accessed from the **Home** screen by touching Flagged.

All : Displays results for All processed samples.

: Displays results for Flagged samples only.

★ : Displays results for samples meeting the Favorite filter criteria only.

⏬ : Opens the Filter dialog box for more filtering options.

Releasing or Rejecting Results

To release or reject results in the Results screen:

1. Select the corresponding checkbox for each sample result you want to release.
2. Touch **Release** at the bottom of the screen. The result(s) status indicates .
3. Select the corresponding checkbox for each sample result you want to reject.
4. Touch **Reject** at the bottom of the screen. The result(s) status indicates .

To release or reject results in the Result Details screen:

1. In the **Results** table, touch the sample result row to open the Result Details screen.
2. After reviewing the details, touch **Release** to release the result or **Reject** to reject the result.
3. The system updates the result status (Released or Rejected) accordingly and displays the next sample result.

Label PIN: LB000327revB
Size: 127 x 279 mm
PMS: 347, 2965, Black

BIO-RAD

REF 290-1000

D-100™ Hemoglobin Testing System

LB000327revB

EN: Contains: D-100 Instrument and Accessory Kit
DE: Inhalt: D-100-Gerät und Zubehör-Kit
FR: Contient : un instrument D-100 et un kit d'accessoires
ES: Contiene: Instrumento y kit de accesorios de D-100
IT: Contenuto: strumento D-100 e kit accessori
SE: Innehåller: D-100 instrument och tillbehörssats
DK: Indeholder: D-100 instrument og tilbehørskit
NO: Inneholder: D-100-instrument og tilbehørssett

UDI-DI 00847817023095

SN

CE IVD

−10°C 50°C

20% 80%

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547
FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette

Made in France

Label PIN: LB0003280revA
Size: 119 x 167 mm
PMS: 347, 2965, Black

BIO-RAD

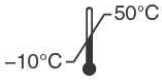
LB0003280revA

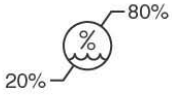
D-100™ Hemoglobin Testing System

Accessory Kit

USE REF 290-1000

CE IVD



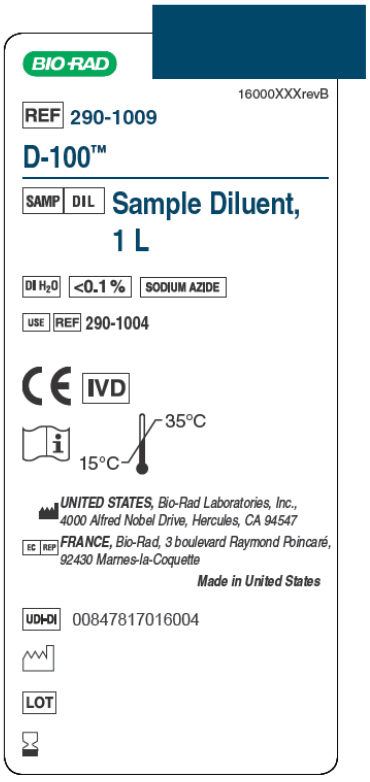


UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in France

LPN: 16000XXXrevB
Size: 2.25" x 5"
PMS: 347, 2965, Black



Label PIN: 16000348revB
Size: 3.625” x 7.5”
PMS: 347, 2965, Black

BIO-RAD

REF 290-1010

D-100™ HbA_{1c} BUF A Elution Buffer A, 2600 mL

16000348revB

CE IVD

15°C 35°C

US: Rx Only

USE REF 290-1004

UDI-DI 00847817025679

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood
Contains Sodium Perchlorate, Succinate Buffer

DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut
Enthält Natriumperchlorat, Succinatpufferr

FR: Pour le dosage de l’hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain
Contient un tampon de succinate et de perchlorate de sodium

ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana
Contiene tampón de perclorato de sodio y succinato sódico.

IT: Per la determinazione quantitativa dell’HbA_{1c} nel sangue intero umano
Contiene un tampone di perclorato e succinato di sodio

SE: För kvantitativ bestämning av HbA_{1c} i humant helblod
Innehåller natriumperklorat- och natriumsuccinatbuffert

DK: Til kvantitativ bestemmelse af HbA_{1c} i humant fuldblod
Indeholder en natriumperchlorat- og succinatbuffer

NO: For kvantifiserende bestemmelse av HbA_{1c} i humant fullblod
Inneholder natriumperklorat, suksinatbuffer

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

Label PIN: 12000939revB
Size: 3.625" x 7.5"
PMS: 347, 2965, Black

BIO-RAD

REF 290-1011

D-100™ HbA_{1c} BUF B Elution Buffer B, 1400 mL

12000939revB

CE IVD

15°C – 35°C

US: Rx Only

USE REF 290-1004

UDI-DI 00847817016028

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood
Contains Sodium Perchlorate, Succinate Buffer

DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut
Enthält Natriumperchlorat, Succinatpufferr

FR: Pour le dosage de l'hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain
Contient un tampon de succinate et de perchlorate de sodium

ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana
Contiene tampón de perclorato de sodio y succinato sódico.

IT: Per la determinazione quantitativa dell'HbA_{1c} nel sangue intero umano
Contiene un tampone di perclorato e succinato di sodio

SE: För kvantitativ bestämning av HbA_{1c} i humant helblod
Innehåller natriumperklorat- och natriumsuccinatbuffert

DK: Til kvantitativ bestemmelse af HbA_{1c} i humant fuldblod
Indeholder en natriumperchlorat- og succinatbuffer

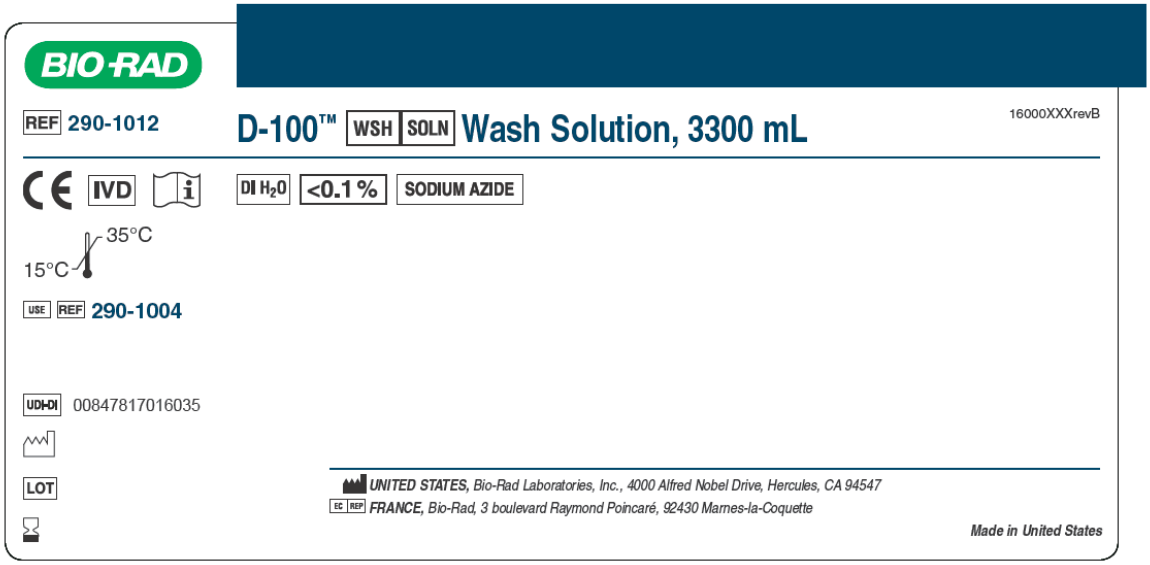
NO: For kvantifiserende bestemmelse av HbA_{1c} i humant fullblod
Inneholder natriumperklorat, suksinatbuffer

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

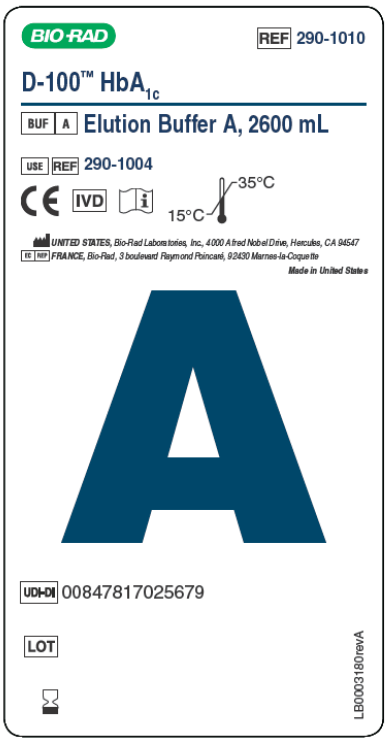
EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

Label PIN: 16000XXXrevB
Size: 3.625” x 7.5”
PMS: 347, 2965, Black



Label PIN: LB0003180revA
Size: 125 x 65 mm
PMS: 2965, 347, Black



Label PIN: LB0003190revA
Size: 125 x 47 mm
PMS: 2965, 347, Black

Label PIN: LB0003200revA
Size: 125 x 88 mm
PMS: 2965, 347, Black

Label PIN: 16000347revB
Size: 5” x 6”
PMS: 347, 2965, Black

BIO-RAD

16000347revB

REF 290-1004

D-100™ HbA_{1c}

ANLT | CRTR | CAL | PACK

Analytical Cartridge/Calibrator Pack

CE IVD

Σ 10000

2°C – 8°C

LATEX

US: Rx Only

UDI-DI 00847817015953

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood / DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut / FR: Pour le dosage de l'hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain / ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana / IT: Per la determinazione quantitativa dell'HbA_{1c} nel sangue intero umano / SE: För kvantitativ bestämning av HbA1c i humant helblod / DK: Til kvantitativ bestemmelse af HbA1c i humant fuldblod / NO: For kvantifiserende bestemmelse av HbA1c i humant fullblod

1 x 1

ANLT | CRTR 1 x 1

CAL | PACK 1 x 1

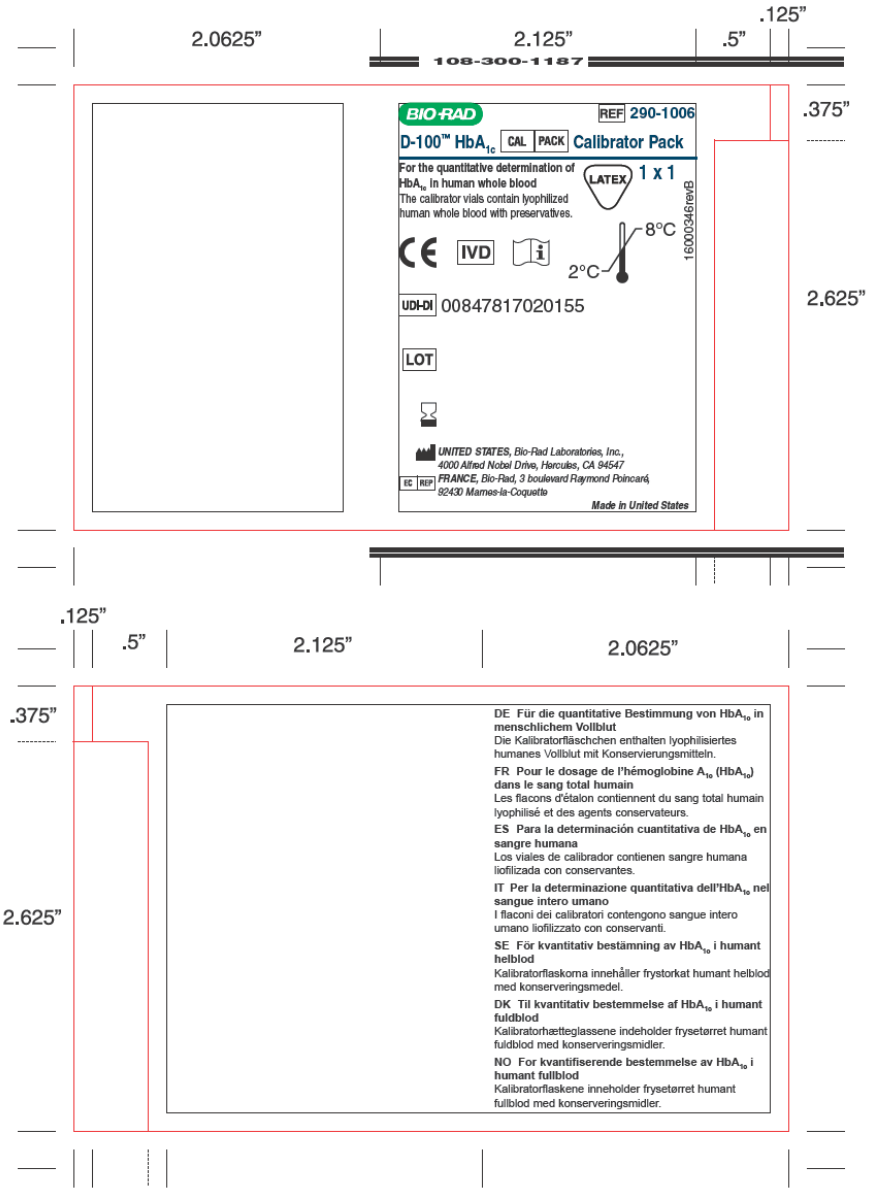
EN: The calibrator vials contain lyophilized human whole blood with preservatives. / DE: Die Kalibratorfläschchen enthalten lyophilisiertes humanes Vollblut mit Konservierungsmitteln. / FR: Les flacons d'étalon contiennent du sang total humain lyophilisé et des agents conservateurs. / ES: Los viales de calibrador contienen sangre humana liofilizada con conservantes. / IT: I flaconi dei calibratori contengono sangue intero umano liofilizzato con conservanti. / SE: Kalibratorrören innehåller frystorkat humant helblod med konserveringsmedel. / DK: Kalibratorhætteglassene indeholder frysetørret humant fuldblod med konserveringsmidler. / NO: Kalibratorflaskene inneholder frysetørret humant fullblod med konserveringsmidler.

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

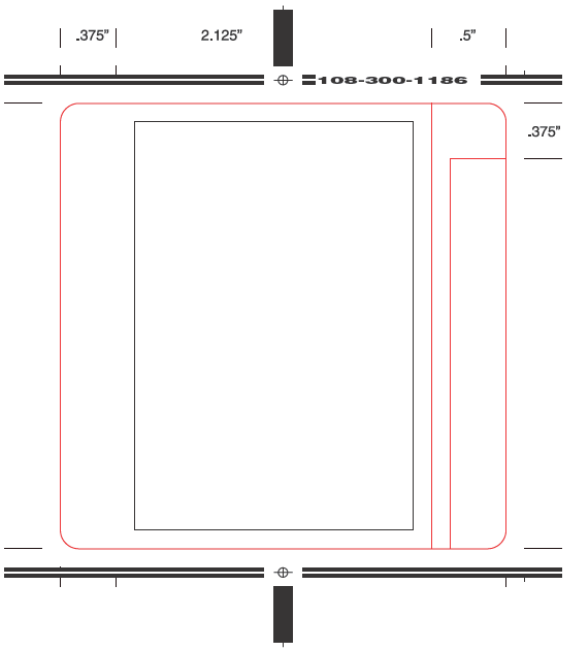
EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

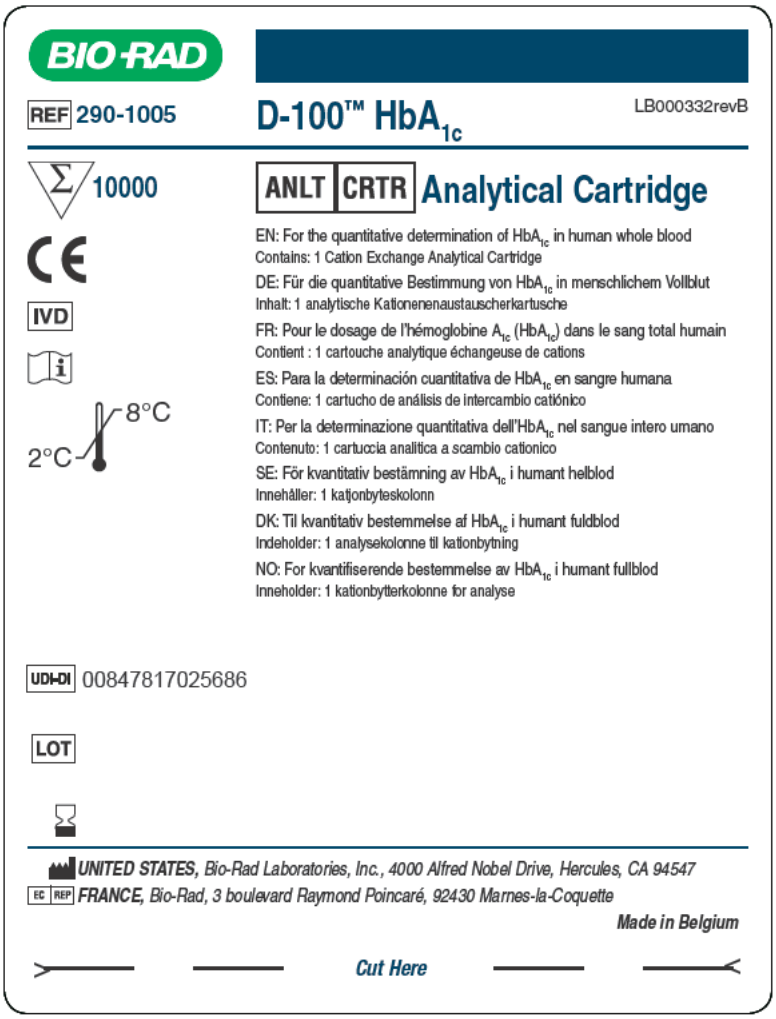
OVERALL SIZE: 4.8125" WIDE X 3.0" HIGH



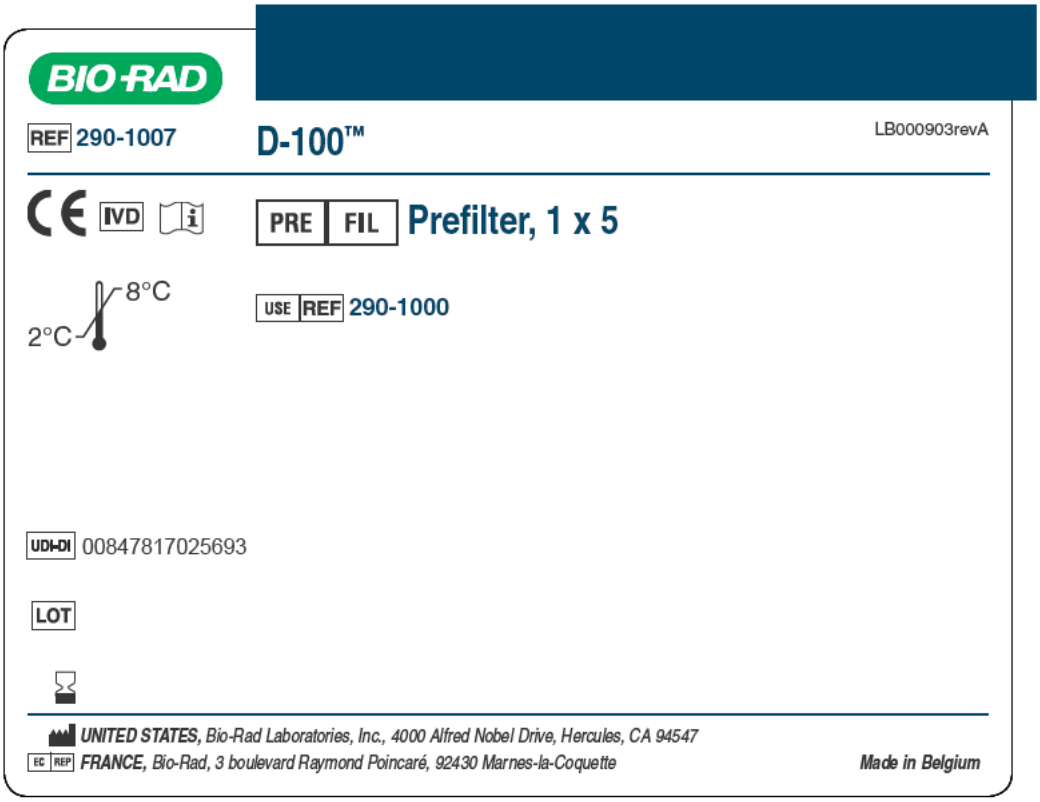
Label PIN: 16000346revB
Size: 3" x 3"
PMS: 347, 2965, Black



LPN: LB000332revB
Size: 4” x 5.25”
PMS: 347, 2965, Black



LPN: LB000903revA
Size: 4” x 5.25”
PMS: 347, 2965, Black



Label PIN: LB0003250revA
Size: 3" x 3"
PMS: 347, 2965, 032, Black

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

BIO-RAD LABORATORIES, INC.
JACKIE BUCKLEY
REGULATORY AFFAIRS REP IV
4000 ALFRED NOBEL DR.
HERCULES CA 94547

December 9, 2015

Re: K151321
Trade/Device Name: D-100™ HbA1c
D-100™ HbA1c Calibrator Pack
Regulation Number: 21 CFR 862.1373
Regulation Name: Glycosylated hemoglobin assay
Regulatory Class: II
Product Code: PDJ, LCP, JIT
Dated: November 06, 2015
Received: November 09, 2015

Dear Jackie Buckley:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

BIO-RAD LABORATORIES, INC.
JACKIE BUCKLEY
REGULATORY AFFAIRS REP IV
4000 ALFRED NOBEL DR.
HERCULES CA 94547

Re: K151321
Trade/Device Name: D-100™ HbA1c
D-100™ HbA1c Calibrator Pack
Regulation Number: 21 CFR 862.1373
Regulation Name: Glycosylated hemoglobin assay
Regulatory Class: II
Product Code: PDJ, LCP, JIT
Dated: November 06, 2015
Received: November 09, 2015

Dear Jackie Buckley:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

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Sincerely yours,

Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Contains Nonbinding Recommendations

Print Form

Acceptance Checklist for Traditional 510(k)s

(Should be completed within 15 days of DCC receipt)

The following information is not intended to serve as a comprehensive review.

510(k) #: K151321 Date Received by DCC: May 21, 2015

Lead Reviewer: Alain Silk

Branch: DDDB Division: DCTD Center/Office: CDRH/OIR

Note: If an element is left blank on the checklist, it does not mean the checklist is incomplete. It means the reviewer did not assess the element during RTA and the element will be assessed during the substantive review.

Preliminary Questions

Answers in the shaded blocks indicate consultations with Center advisor is needed	Yes	No
1) Is the product a device (per section 201(h) of the FD&C Act) or a combination product (per 21 CFR 3.2(e)) with a device constituent part subject to review in a 510(k)? If it appears not to be a device (per section 201(h) of the FD&C Act) or such a combination product, or you are unsure, consult with the CDRH Jurisdictional Officer or the CBER Office Jurisdiction Liaison to determine the appropriate action, and inform division management. *Provide a summary of the Jurisdictional Officer's/Liaison's determination.* If the product does not appear to be a device or such a combination product, mark "No."	X	
Comments?		
2. Is the application with the appropriate Center? If the product is a device or a combination product with a device constituent part, is it subject to review by the Center in which the submission was received? If you believe the application is not with the appropriate Center or you are unsure, consult with the CDRH Jurisdictional Officer or CBER Office Jurisdiction Liaison to determine the appropriate action and inform your division management. *Provide a summary of the Jurisdictional Officer's/Liaison's determination.* If application should not be reviewed by your Center mark "No."	X	
Comments?		
3) If a Request for Designation was submitted for the device or combination product with a device constituent part and assigned to your center, identify the RFD # and confirm the following: a) Is the device or combination product the same (e.g., design, formulation) as that presented in the RFD submission? b) Are the indications for use for the device or combination product identified in the 510(k) the same as those identified in the RFD submission ? If you believe the product or the indications presented in the 510(k) have changed from the RFD, or you are unsure, consult with the CDRH Jurisdictional Officer or appropriate CBER Jurisdiction Liaison to determine the appropriate action and inform your division management. Provide summary of Jurisdictional Officer's/Liaison's determination. If the answer to either question is no, mark "No." If there was no RFD, skip this question.		
Comments?		
4) Is this device type eligible for a 510(k) submission? If a 510(k) does not appear to be appropriate (e.g., Class III type and PMA required, or Class I or II type and 510(k)-exempt), you should consult with the CDRH 510(k) Program Director or appropriate CBER staff during the acceptance review. If 510(k) is not the appropriate regulatory submission, mark "No."	X	
Comments?		

5) Is there a pending PMA for the same device with the same indications for use? If yes, consult division management and the CDRH 510(k) Program Director or appropriate CBER staff to determine the appropriate action.		X
Comments?		
6) If clinical studies have been submitted, is the submitter the subject of an Application Integrity Policy (AIP)? If yes, consult with the CDRH Office of Compliance/Division of Bioresearch Monitoring (OC/DBM - BIMO) or CBER Office of Compliance and Biologics Quality/Division of Inspections and Surveillance/Bioresearch Monitoring Branch (OCBQ/DIS/BMB) to determine the appropriate action. Check on web at http://www.fda.gov/ICECI/EnforcementActions/ApplicationIntegrityPolicy/ucm134453.htm		X
Comments?		

If the answer to 1 or 2 appears to be "No," then stop review of the 510(k) and issue the "Original Jurisdictional Product" letter.
If the answer to 3a or 3b appears to be "No," then stop the review and contact the CDRH Jurisdictional Officer or CBER Office of Jurisdiction Liaison.
If the answer to 4 is "No," the lead reviewer should consult division management and other Center resources to determine the appropriate action.
If the answer to 5 is "Yes," then stop review of the 510(k), contact the CDRH 510(k) Staff and PMA Staff, or appropriate CBER staff.
If the answer to 6 is "Yes," then contact CDRH/OC/DBM-BIMO or CBER/OCBQ/DIS/BMB, provide a summary of the discussion with the BIMO Staff, and indicate BIMO's recommendation/action.

Organizational Elements

Failure to include these items alone generally should not result in an RTA designation.

	Yes	No
1) Submission contains a Table of Contents	X	
2) Each section is labeled (e.g., headings or tabs designating Device Description section, Labeling section, etc.)	X	
3) All pages of the submission are numbered.	X	
4) Type of 510(k) is identified (i.e., traditional, abbreviated, or special)	X	
Comments?		

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
A. Administrative				
1) All content used to support the submission is written in English (including translations of test reports, literature articles, etc.)	X			
2) Submission identifies the following (such as in CDRH Premarket Review Submission Cover Sheet (Form 3514) or 510(k) cover letter):	X			
a) Device trade name or proprietary name	X			
b) Device common name	X			
c) Device class and panel or Classification regulation or Statement that device has not been classified with rationale for that conclusion	X			
3) Submission contains Indications for Use Statement with Rx and/or OTC designation (see also 21 CFR 801.109).	X			
4) Submission contains 510(k) Summary or 510(k) Statement	X			
a) Summary contains all elements per 21 CFR 807.92 *(See also* 510(k) Summary Checklist*)*	X			
b) Statement contains all elements per 21 CFR 807.93			X	
5) Submission contains Truthful and Accuracy Statement per 21 CFR 807.87(k) *See recommended* format.	X			
6) Submission contains Class III Summary and Certification. *See recommended* content			X	
7) Submission contains clinical data			X	
8) If submission references use of a national or international standard as part of demonstration of substantial equivalence, submission contains Standards Data Report for 510(k)s (Form 3654) or includes detailed information about how and the extent to which the standard has been followed.	X			
9) The submission identifies prior submissions for the same device for which FDA provided feedback related to the data or information needed to support substantial equivalence (e.g., submission numbers for Pre-Submission, IDE, prior not substantially equivalent (NSE) determination, prior 510(k) that was deleted or withdrawn) or states that there were no prior submissions for the subject device.		X		X
a) If there were prior submissions, the submitter has identified where in the current submission any issues related to a determination of substantial equivalence outlined in prior communications are addressed. For additional information regarding the Pre-Submission process, please refer to the Draft Guidance "Medical Devices: The Pre-Submission Program and Meetings with FDA Staff." Once finalized, this guidance will represent the Agency's current thinking on this topic.		X		

(b) (5)

[redacted]

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
B. Device Description				
10)				
a) If there are requirements regarding the device description, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes device description information to establish that the submitter has followed the device-specific requirement.	X			
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes device description information to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			X	
11) Descriptive information is present and consistent within the submission (e.g., the device description section is consistent with the device description in the labeling), including:				
a) A description of the principle of operation and mechanism of action for achieving the intended effect.	X			
b) A description of proposed conditions of use, such as surgical technique for implants; anatomical location of use; user interface; how the device interacts with other devices; and/or how the device interacts with the patient.	X			
c) A list and description of each device for which clearance is requested.	X			
12) Submission contains representative engineering drawing(s), schematics, illustrations and/or figures of the device that are clear, legible, labeled, and include dimensions.	X			
13) If device is intended to be marketed with multiple components, accessories, and/or as part of a system				
a) Submission includes a list of all components and accessories to be marketed with the subject device.	X			
b) Submission includes a description (as detailed in item 11(a) and (b) and 12 above) of each component or accessory.	X			
c) A 510(k) number is provided for each component or accessory that received a prior 510(k) clearance.			X	
C. Substantial Equivalence Discussion				
14) Submitter has identified a predicate device.	X			X
a) Predicate's 510(k) number, trade name, and model number (if applicable) provided. For predicates that are preamendments devices, information is provided to document preamendments status. *Information regarding* documenting preamendment status *is available online.*	X			
b) The identified predicate(s) is consistent throughout the submission (i.e., the predicate(s) identified in the Substantial Equivalence section is the same as that listed in the 510(k) Summary (if applicable) and that used in comparative performance testing.		X		

(b) (5)

[redacted]

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
(b) (5) [redacted]				
15) Submission includes a comparison of the following for the predicate(s) and subject device				
a) Indications for Use	X			
b) Technology, including features, materials, and principles of operation	X			
16) Submission includes an analysis of why any differences between the subject device and predicate(s) do not render the device NSE (e.g., does not constitute a new intended use; and any differences in technological characteristics are accompanied by information that demonstrates the device is as safe and effective as the predicate and do not raise different questions of safety and effectiveness than the predicate), affect safety or effectiveness, or raise different questions of safety and effectiveness (see section 513(i)(1)(A) of the FD&C Act and 21 CFR 807.87(f))	X			
D. Proposed Labeling (see also 21 CFR part 801)				
X If *in vitro* diagnostic (IVD) device, criteria 17 & 19 may be omitted.				
18) If indicated for prescription use, labeling includes the prescription use statement (see 21 CFR 801.109(b)(1)) or "Rx only" symbol [See also Alternative to Certain Prescription Device Labeling Requirements]		X		X
(b) (5) [redacted]				
20)				
a) If there are requirements regarding labeling, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes labeling to establish that the submitter has followed the device-specific requirement.	X			
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes labeling to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.	X			
c) If there is a special controls document applicable to the device, the submission includes labeling to establish that the submitter has complied with the particular mitigation measures set forth in the special controls document or uses alternative mitigation measures but provides a rationale to demonstrate that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.	X			
21) If the device is an in vitro diagnostic device, provided labeling includes all applicable information required per 21 CFR 809.10.	X			X
(b) (5) [redacted]				
E. Sterilization				
If IVD device and sterilization is not applicable, select "N/A" and criteria below will be omitted from checklist.			X	

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
F. Shelf Life				
26) Proposed shelf life/expiration date stated	X			X
(b) (5) [redacted]				
27) For sterile device, submission includes summary of methods used to establish that device will remain sterile through the proposed shelf life or a rationale for why testing to establish shelf life is not applicable.			X	
28) Submission includes summary of methods used to establish that device performance is not adversely affected by aging or includes a rationale for why the storage conditions are not expected to affect device safety or effectiveness.	X			
G. Biocompatibility				
If IVD device, select "N/A" and the below criteria will be omitted from checklist.			X	
H. Software				
Submission states that the device: (one of the below must be checked)				
X does contain software/firmware.				
does not contain software/firmware.				
Information regarding whether the device contains software is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				
32) Submission includes a statement of software level of concern and rationale for the software level of concern.	X			
33) All applicable software documentation provided based on level of concern identified by the submitter, as described in Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, or the submitter has provided an alternative approach with a rationale.	X			
I. EMC and Electrical Safety				
Submission states that the device: (one of the below must be checked)				
X does require EMC and Electrical Safety evaluation.				
does not require EMC and Electrical Safety evaluation.				
Information regarding whether the device requires EMC and Electrical Safety evaluation is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
34) Submission includes evaluation of electrical safety (e.g., per IEC 60601-1, or equivalent FDA-recognized standard, and if applicable, the device-specific standard), OR submission includes electrical safety evaluation using methods or standards that are not FDA-recognized and submission includes information to establish that the submitter has otherwise met the applicable statutory or regulatory criteria through this alternative approach (i.e., the submitter has identified alternate methods or standards with a rationale).	X			X
(b) (5) [redacted]				
35) Submission includes evaluation of electromagnetic compatibility (e.g., per IEC 60601-1-2 or equivalent FDA-recognized standard and if applicable, the device-specific standard) OR submission includes electromagnetic compatibility evaluation using methods or standards that are not FDA-recognized and submission includes information to establish that the submitter has otherwise met the applicable statutory or regulatory criteria through this alternative approach (i.e., the submitter has identified alternate methods or standards with a rationale).	X			X
(b) (5) [redacted]				
J. Performance Data - General If IVD device, select "N/A" and the below criteria will be omitted from checklist. Performance data criteria relating to IVD devices will be addressed in Section K.			X	
K. Performance Characteristics - In Vitro Diagnostic Devices Only **(Also see 21 CFR 809.10(b)(12))**				
Submission states that the device: (one of the below must be checked)				
X is an in vitro diagnostic device.				
is not an in vitro diagnostic device.				
40) Submission includes the following studies, as appropriate for the device type, including associated protocol descriptions, study results and line data:				
a) Precision/reproducibility	X			
b) Accuracy(includes as appropriate linearity; calibrator or assay traceability; calibrator and/or assay stability protocol and acceptance criteria; assay cut-off; method comparison or comparison to clinical outcome; matrix comparison; and clinical reference range or cutoff.	X			
c) Sensitivity (detection limits, LoB, LoD, LoQ where relevant for the device type).			X	
d) Analytical specificity	X			
41)				

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
a) If there are requirements regarding performance data, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes performance data to establish that the submitter has followed the device-specific requirement.	X			
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes performance data to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			X	
c) If there is a special controls document applicable to the device, the submission includes performance data to establish that the submitter has complied with the particular mitigation measures set forth in the special controls document or uses alternative mitigation measures but provides a rationale to demonstrate that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.	X			

Decision: (•) Accept ( ) Refuse to Accept

If Accept, notify applicant.

If Refuse to Accept, notify applicant in writing and include a copy of this checklist.

Digital Signature Concurrence Table

Reviewer Sign-Off	Alain D. Silk -S 2015.06.01 12:41:38 -04'00'
Branch Chief Sign-Off (digital signature optional)*	
Division Sign-Off (digital signature optional)*	

* Branch and Division review of checklist and concurrence with decision required. Branch and Division digital signature optional.

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center WO66 G609
Silver Spring, MD 20993 0002

BIO-RAD LABORATORIES, INC.
JACKIE BUCKLEY
REGULATORY AFFAIRS REP IV
4000 ALFRED NOBEL DR.
HERCULES CA 94547

December 9, 2015

Re: K151321
Trade/Device Name: D-100™ HbA1c
D-100™ HbA1c Calibrator Pack
Regulation Number: 21 CFR 862.1373
Regulation Name: Glycosylated hemoglobin assay
Regulatory Class: II
Product Code: PDJ, LCP, JIT
Dated: November 06, 2015
Received: November 09, 2015

Dear Jackie Buckley:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151321

Device Name
D-100™ HbA1c
D-100™ HbA1c Calibrator Pack

Indications for Use *(Describe)*
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100™ HbA1c test is intended for Professional Use Only.

Calibrators:
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c(HbA1c) in human whole blood.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151321_____.

Date Summary prepared: Nov. 4, 2015

1. **Applicant Name:**
   Bio-Rad Laboratories, Inc.
   Clinical Diagnostics Group
   4000 Alfred Nobel Drive
   Hercules, California 94547

2. **Contact Person(s):**
   Jackie Buckley, Regulatory Affairs Representative IV
   Telephone Number: (510) 741-5309
   FAX: (510) 741-6471
   E-Mail: jackie_buckley@bio-rad.com

   Alfred Evans, RA/QA Director
   Telephone Number: (510) 741-4579
   FAX: (510) 741-6471
   E-Mail:al_evans@bio-rad.com

3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

4. **Predicate Device:**

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

5. Description of the Device:

The Bio-Rad D-100™ HbA1c utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative are percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution. Single use.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.05% sodium azide as a preservative.

D-100 ™ Wash Solution. Each bottle contains 3300 mL of deionized water with <0.05% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:
The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	**Predicate Device 510(k) Number**
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 and 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c** (K)151321	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack** (K)151321	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) 070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots, three D-100™ I Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 day.
NGSP results are shown in Tables 3-6. IFCC results are shown in Tables 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.7%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.7%	0.7%	0.7%	0.7%	0.7%	0.6%	0.8%	0.7%	0.7%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.2%	0.3%	0.2%	0.1%	0.3%	0.3%	0.1%	0.2%
Between-Lot	1.2%	0.8%	1.3%	1.1%	1.0%	0.6%	1.4%	0.8%	0.6%
Total Precision	1.5%	1.1%	1.5%	1.3%	1.2%	0.9%	1.6%	1.0%	0.9%

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	10.0%	14.8%
Repeatability	1.1%	0.9%	0.8%	0.9%	1.0%	0.9%	1.0%	1.0%	1.0%
Between-Run	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.1%
Between-Day	0.6%	0.2%	0.5%	0.5%	0.3%	0.3%	0.2%	0.4%	0.3%
Between-Lot	1.2%	0.2%	1.5%	0.6%	0.0%	0.3%	1.5%	0.1%	0.5%
Total Precision	1.7%	1.0%	1.8%	1.2%	1.0%	1.0%	1.8%	1.1%	1.1%

Table 5: Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4%	9.4%	5.1%	6.6%	8.1%	12.0%	5.3%	9.9%	14.7%
Repeatability	1.0%	1.0%	1.0%	1.1%	0.9%	0.9%	1.0%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.5%	0.4%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%
Between-Lot	1.6%	1.0%	1.5%	1.5%	1.2%	1.1%	1.7%	1.0%	1.1%
Total Precision	1.9%	1.4%	1.9%	2.0%	1.6%	1.5%	2.0%	1.4%	1.4%

Table 6: Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5%	9.4%	5.2%	6.6%	8.1%	12.0%	5.3%	9.9%	14.8%
Repeatability	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.9%	0.9%	0.8%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.6%	0.3%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%	0.3%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	1.4%	0.7%	1.5%	1.1%	0.9%	0.7%	1.5%	0.7%	0.8%
Total Precision	1.7%	1.2%	1.7%	1.5%	1.3%	1.2%	1.8%	1.2%	1.2%

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2%	0.9%	1.2%	1.0%	0.9%	0.8%	1.3%	0.8%	0.8%
Between-Run	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	0.9%	0.3%	0.5%	0.4%	0.2%	0.4%	0.6%	0.2%	0.3%
Between-Lot	2.0%	1.1%	2.2%	1.6%	1.3%	0.7%	2.3%	1.0%	0.7%
Total Precision	2.5%	1.4%	2.5%	1.9%	1.6%	1.1%	2.7%	1.3%	1.1%

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6%	1.2%	1.8%	1.7%	1.3%	1.1%	1.6%	1.2%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.6%	0.8%	0.8%	0.7%	0.5%	0.7%	0.5%	0.5%
Between-Lot	2.6%	1.2%	2.6%	2.3%	1.6%	1.3%	2.9%	1.2%	1.2%
Total Precision	3.2%	1.8%	3.3%	2.9%	2.1%	1.8%	3.4%	1.7%	1.7%

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5%	1.1%	1.5%	1.4%	1.2%	1.0%	1.5%	1.1%	1.0%
Between-Run	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Between-Day	1.0%	0.4%	0.7%	0.6%	0.5%	0.4%	0.6%	0.4%	0.4%
Between-Instrument	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Between-Lot	2.2%	1.0%	2.5%	1.7%	1.2%	0.9%	2.6%	0.9%	0.9%
Total Precision	2.9%	1.5%	3.0%	2.3%	1.7%	1.4%	3.1%	1.5%	1.4%

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 0.94mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	3.50	3.43	0.07
Level 2	5.16	5.14	0.02
Level 3	6.82	6.85	-0.03
Level 4	8.48	8.54	-0.06
Level 5	10.13	10.21	-0.08
Level 6	11.79	11.88	-0.09
Level 7	13.44	13.53	-0.09
Level 8	15.09	15.17	-0.08
Level 9	16.74	16.79	-0.05
Level 10	18.39	18.41	-0.02
High, Level 11	20.04	20.01	0.03

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Control, Level 1	14.78	13.85	0.92
Level 2	32.93	32.56	0.37
Level 3	51.07	51.12	-0.28
Level 4	69.19	69.55	-0.36
Level 5	87.30	87.83	-0.54
Level 6	105.38	105.98	-0.60
Level 7	123.46	123.99	-0.53
Level 8	141.51	141.86	-0.34
Level 9	159.55	159.59	-0.04
Level 10	177.57	177.18	0.39
High, Level 11	195.58	194.63	0.94

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.5% to 20.0% HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-

Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method are summarized in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

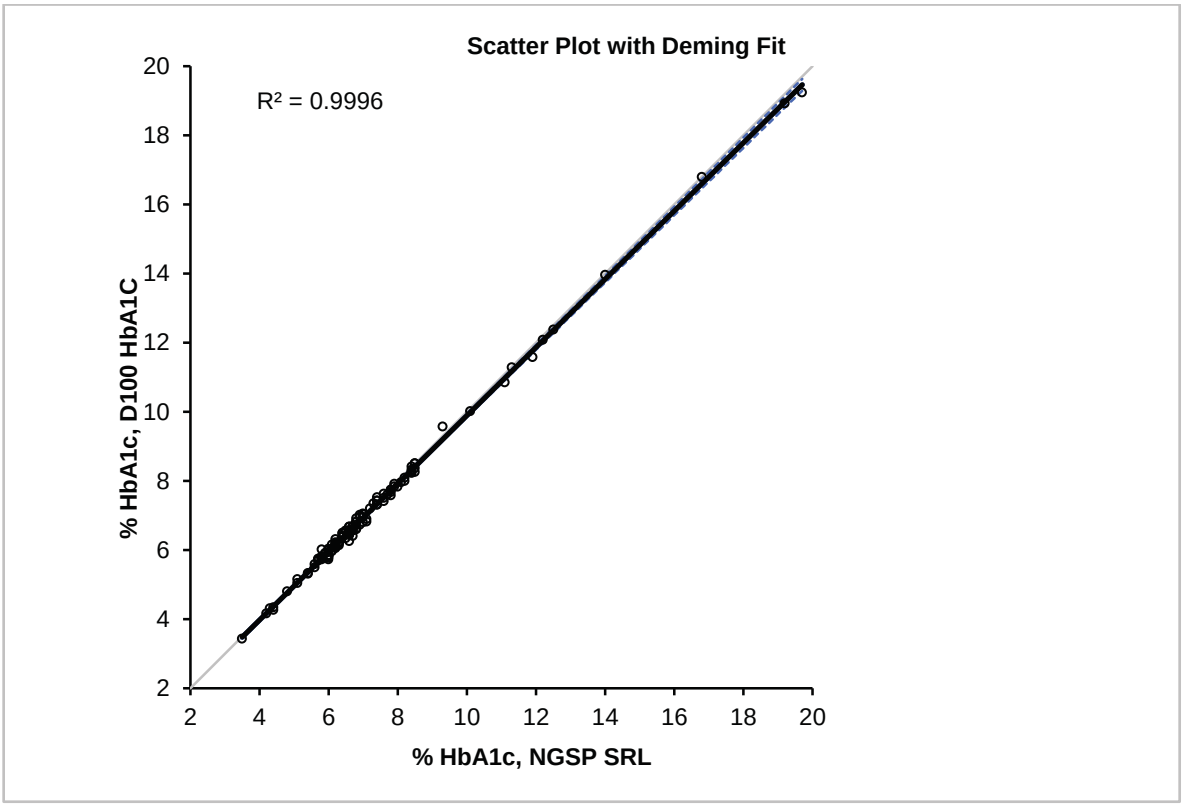

Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.047	-0.85

6.5	-0.066	-0.98
8.0	-0.090	-1.11
12.0	-0.190	-1.57

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-0.85	1.7	4.2
6.5	-0.98	1.5	3.9
8.0	-1.11	1.3	3.6
12.0	-1.57	1.2	3.9

d. Traceability, Stability, Expected Values (calibrators)

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator Materials:
Value assignment for D-100™ HbA1c Calibrators are traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day. On-board stability for the D-100 HbA1c calibrator pack and reagents demonstrated 90 days stability on the D-100 Hemoglobin Testing System.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure

form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18 on the following page.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	SI units
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of

~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 1200 mg/dL of glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 8% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative %Bias (Range of %Bias) for HbA1c	Relative %Bias (Range of %Bias) for HbA1c
	~6.5%	~9.0%
HbS	-0.6 (-5.8 to 5.5)	-1.5 (-3.3 to -0.1)
HbC	-1.3 (-4.0 to 1.3)	-3.9 (-5.5 to -2.4)
HbD	-4.7 (-6.7 to -1.1)	-4.4 (-6.3 to -.2.4)
HbE	-2.7 (-6.7 to 1.6)	-1.3 (-2.0 to -0.6)
HbA2	-1.3 (-5.1 to 0.5)	3.4 (2.8 to 4.1)
HbF	-2.3 (-4.1 to -0.7)	-3.5 (-4.2 to -2.8)

2. Matrix comparison
The data supports the use of the following blood collection tubes with the D-100™ HbA1c test in Table 21.

Table 21: Anticoagulant

K_2-EDTA
K_3.EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes are presented in Table 22.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

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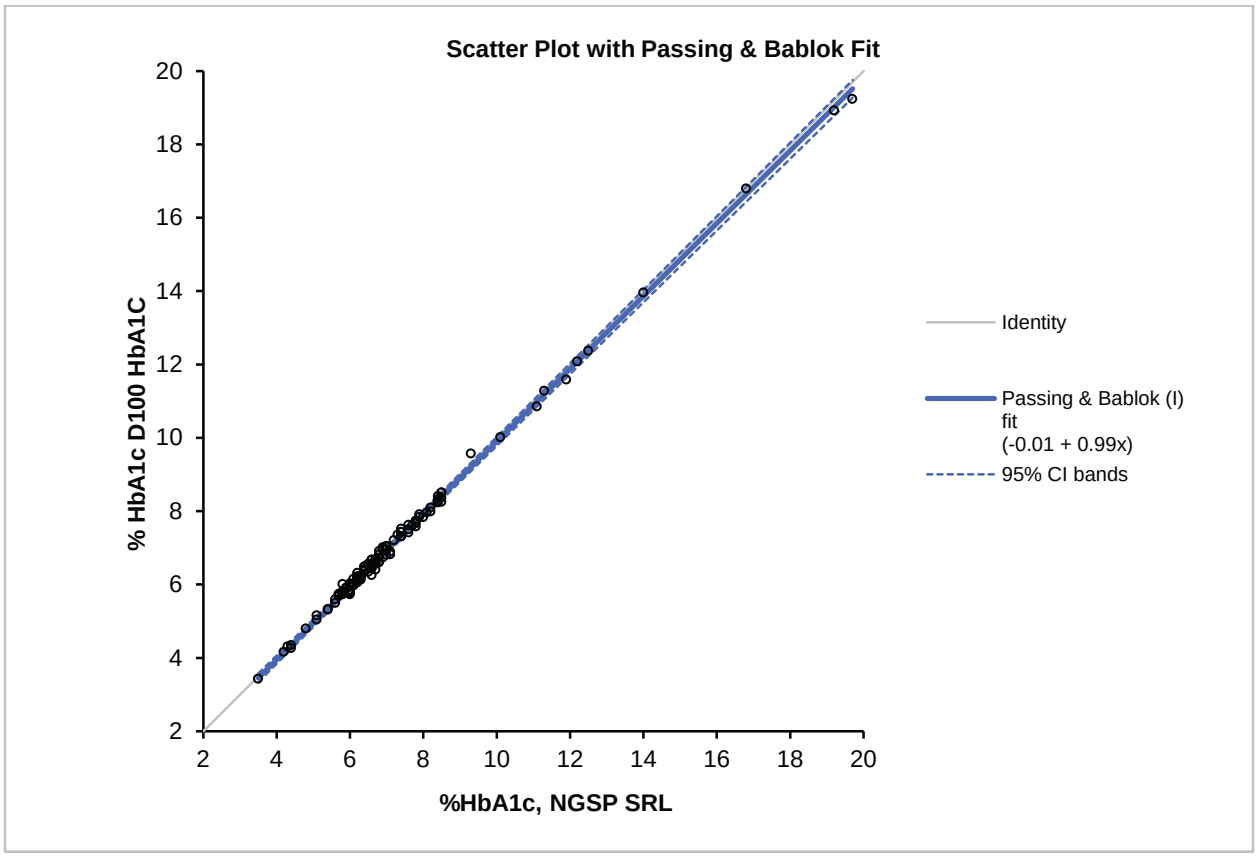

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Sincerely,
Jackie Buckley
RA Rep IV

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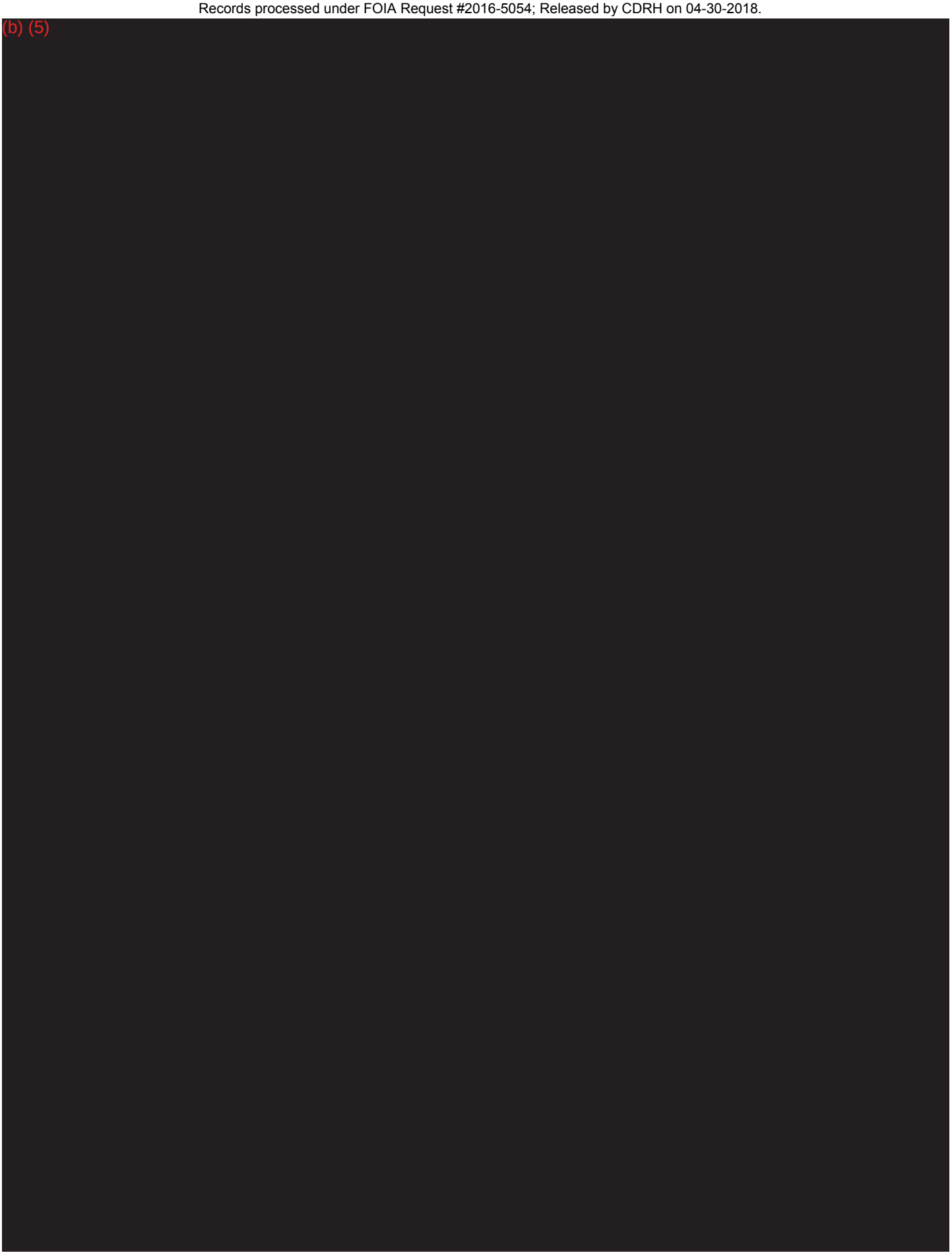

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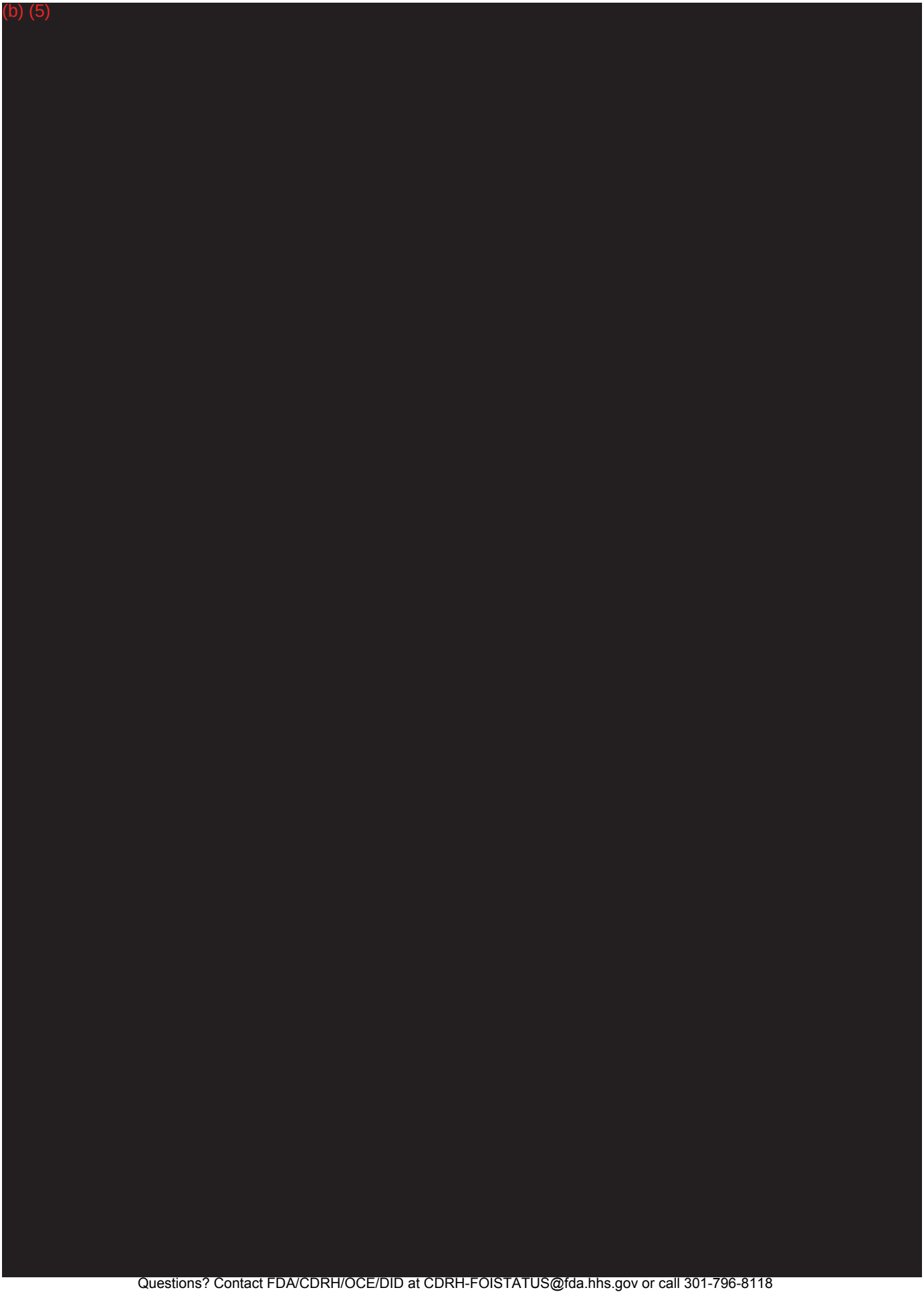

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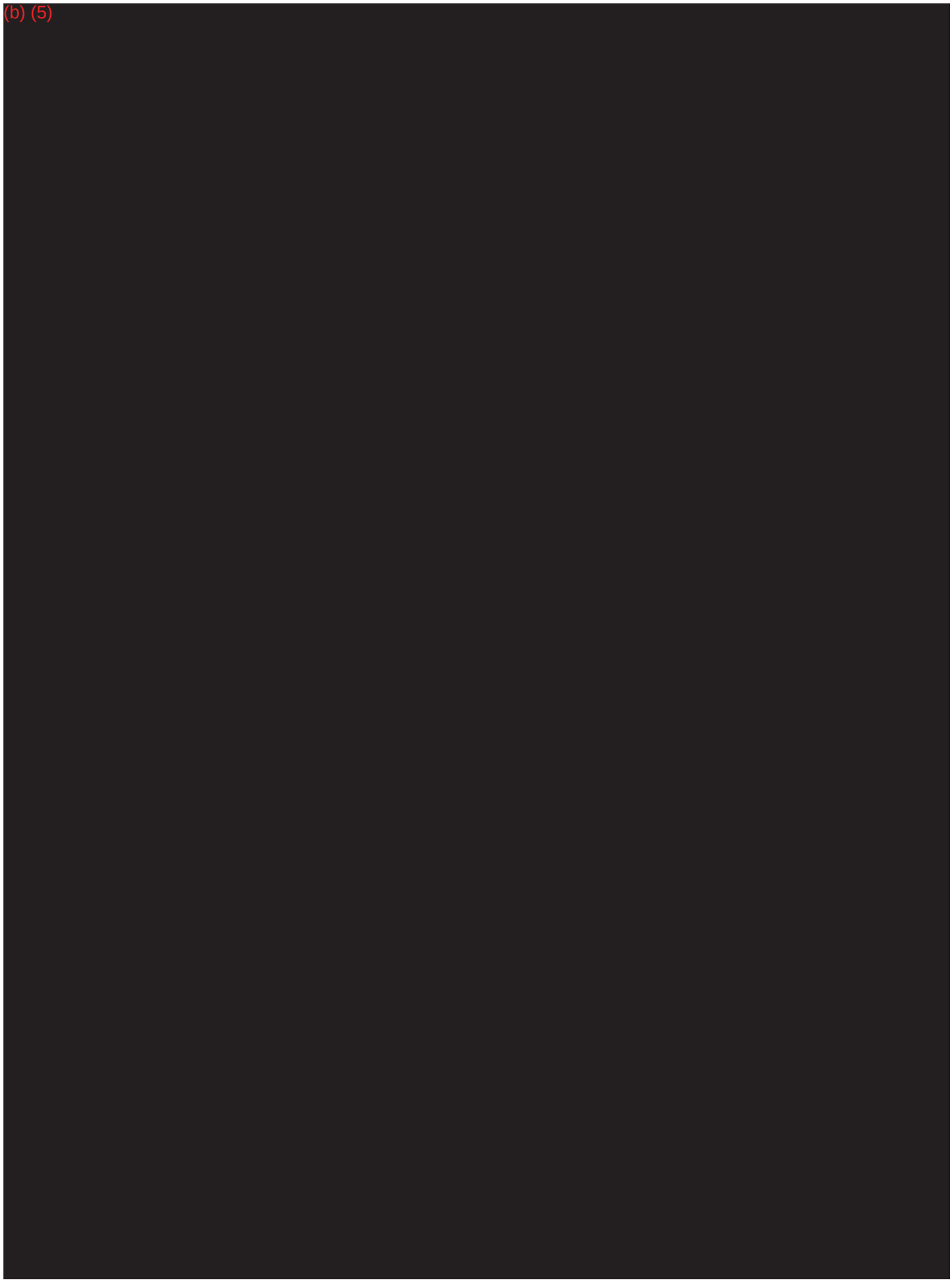

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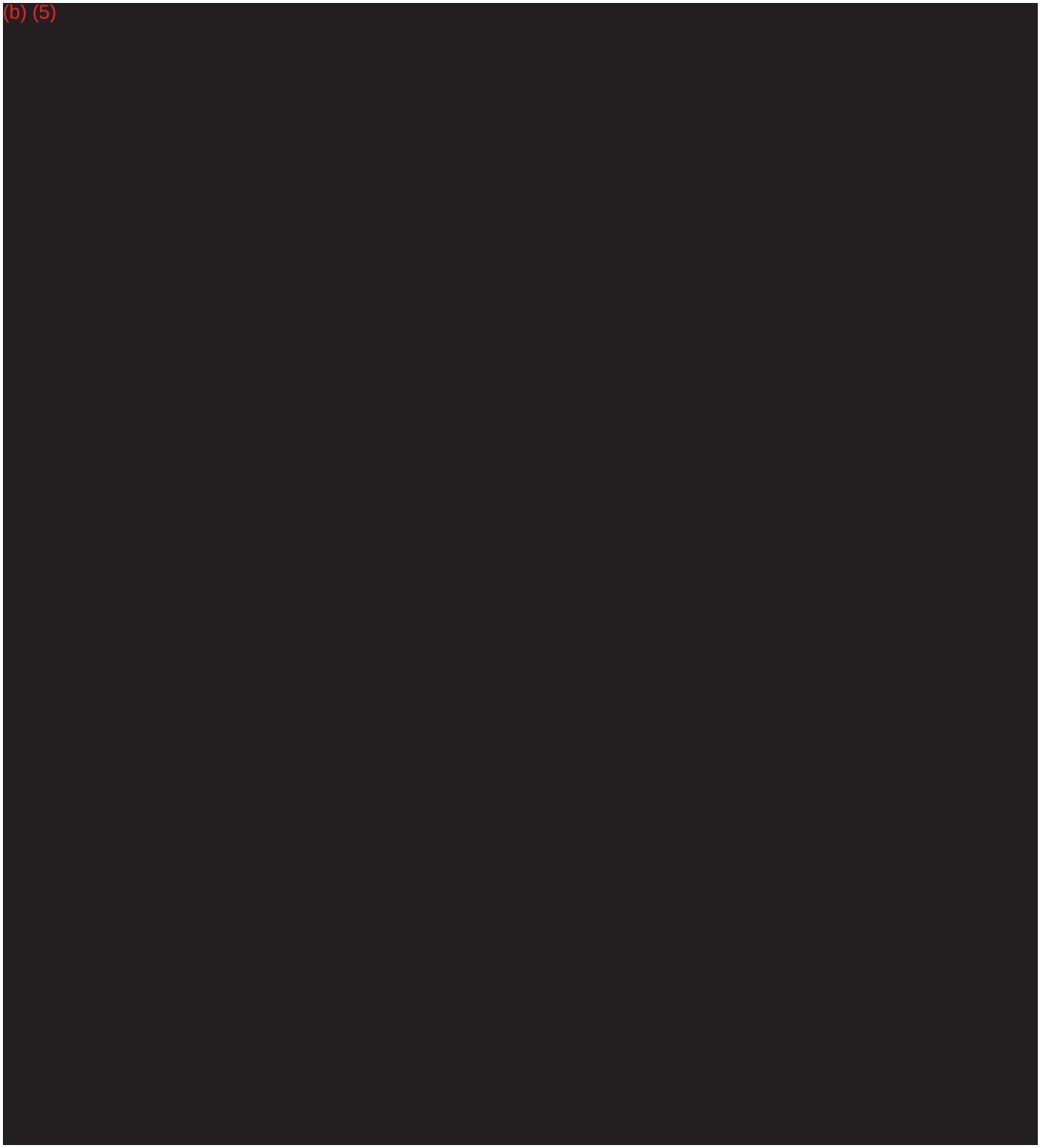

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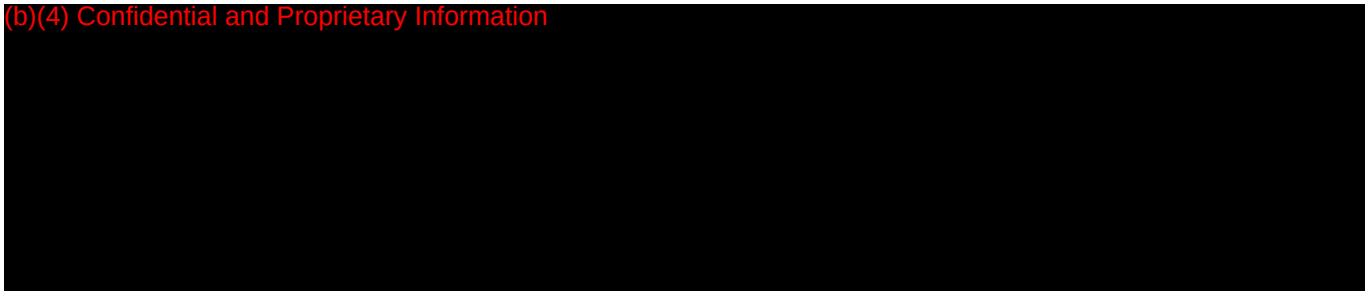

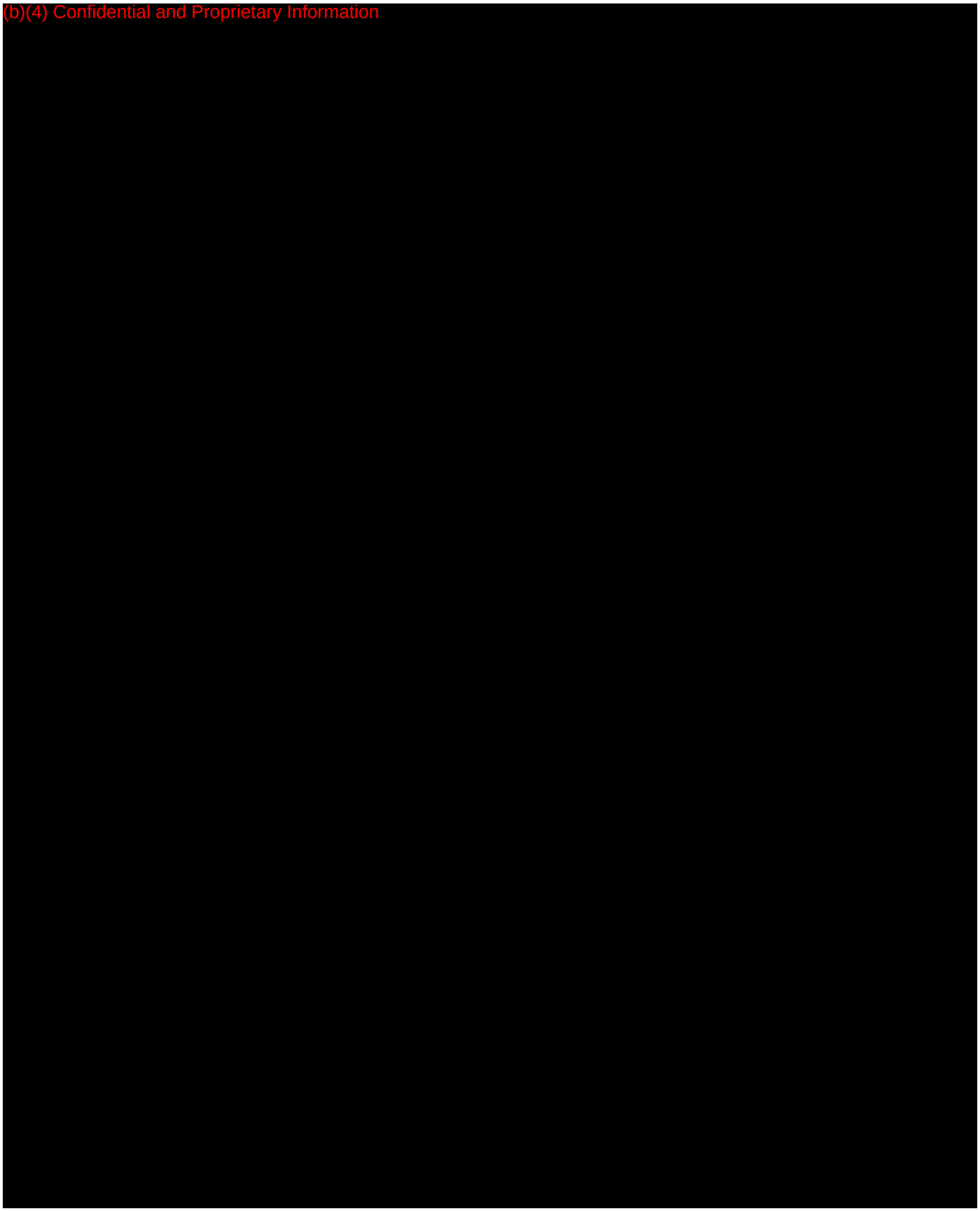

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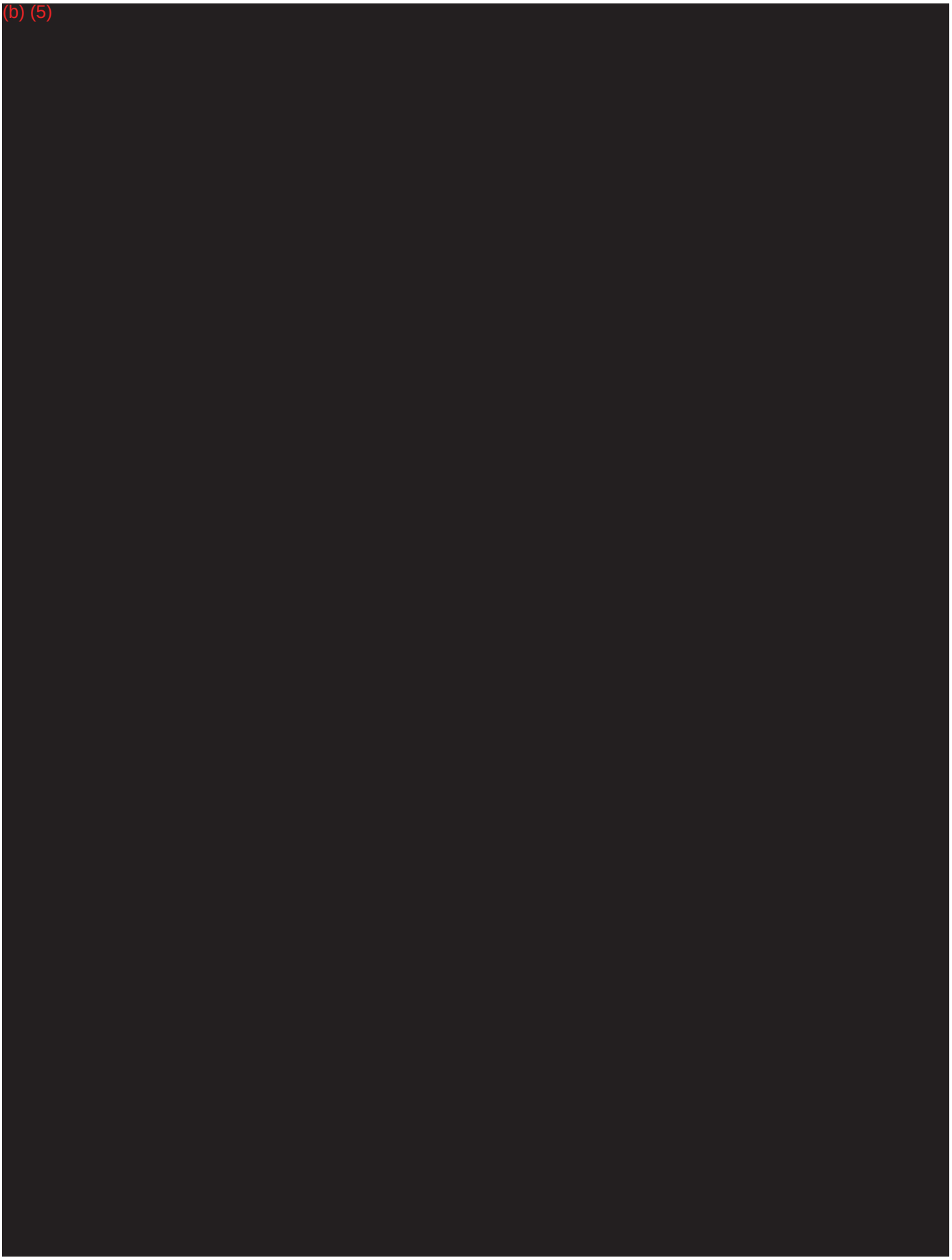

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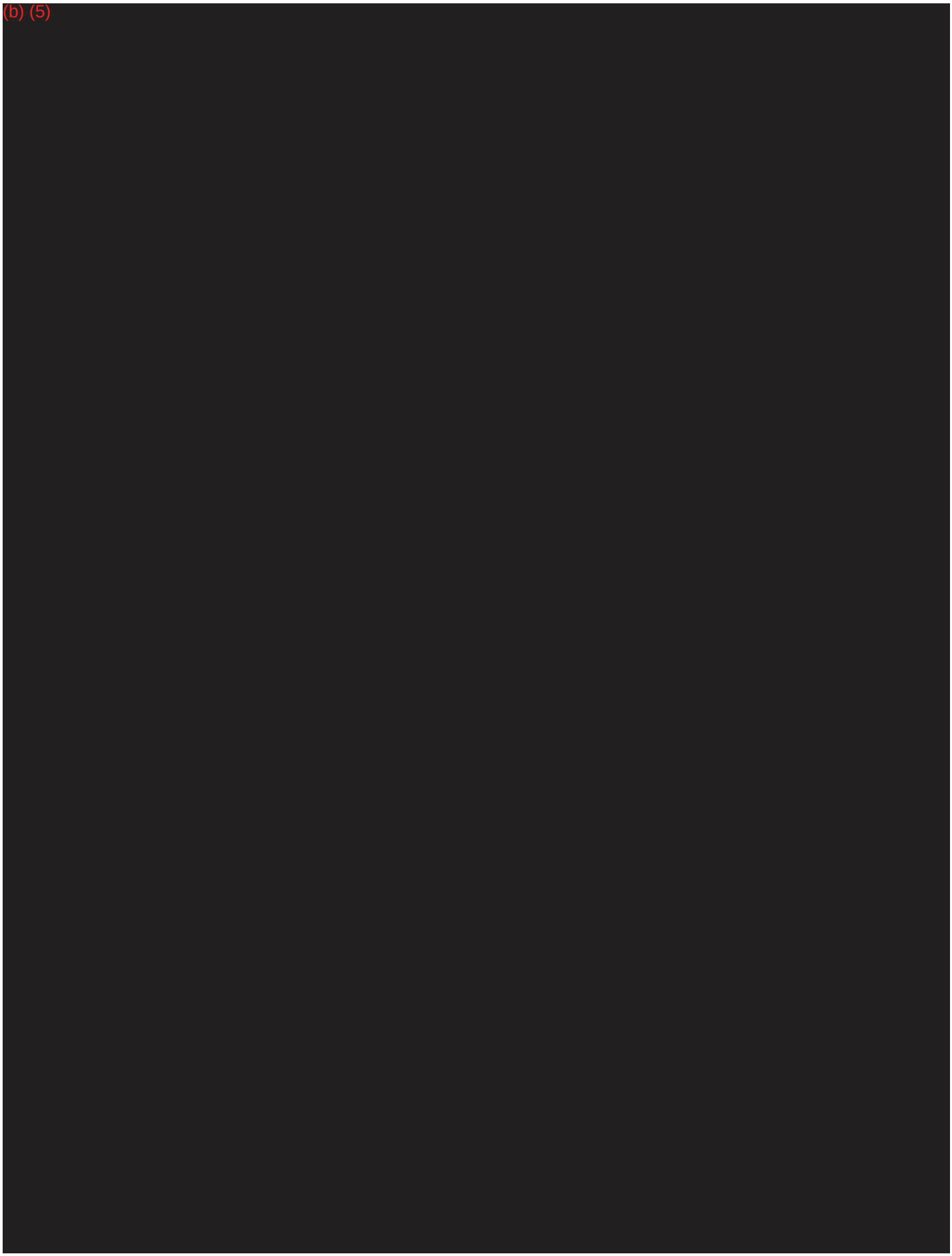

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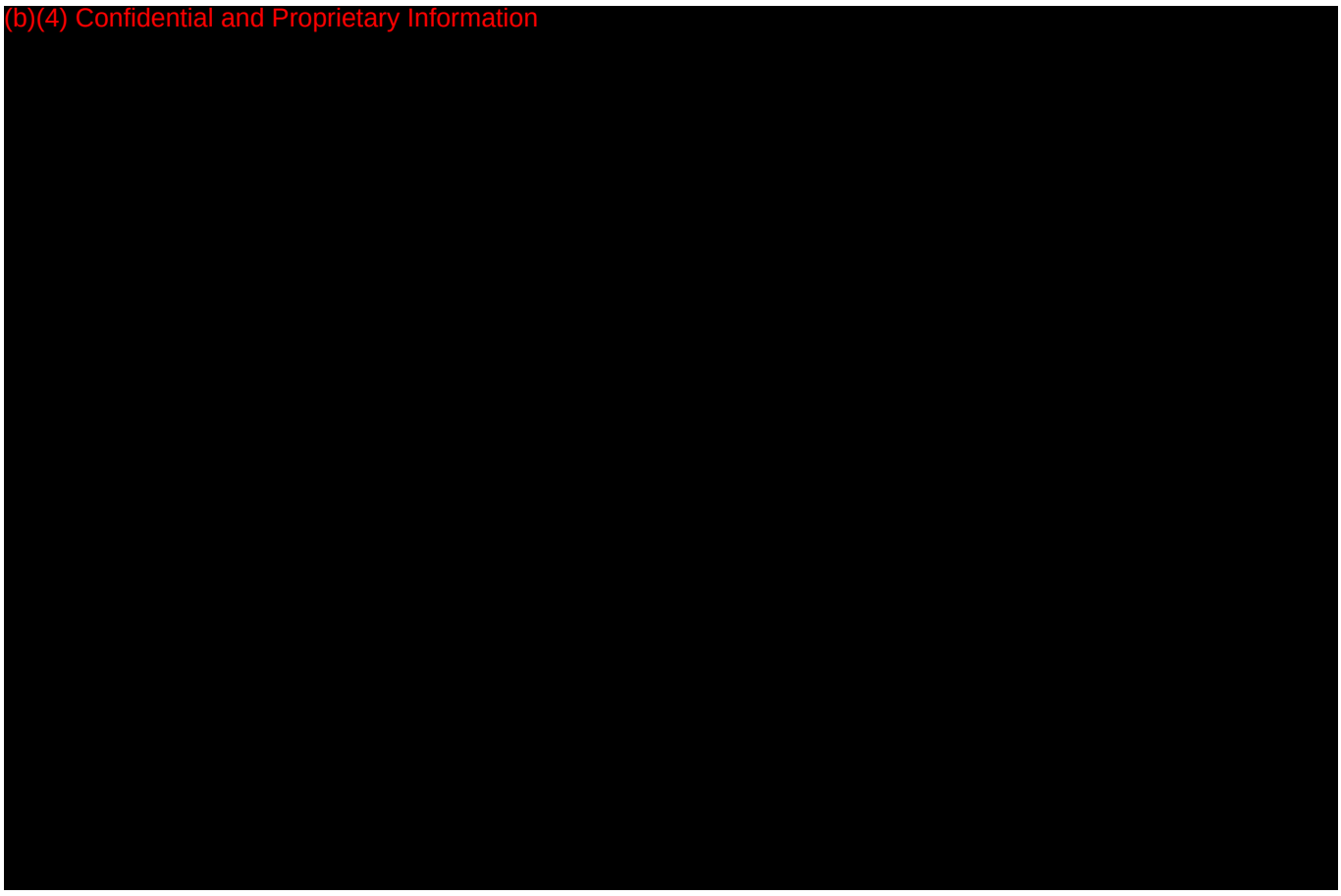

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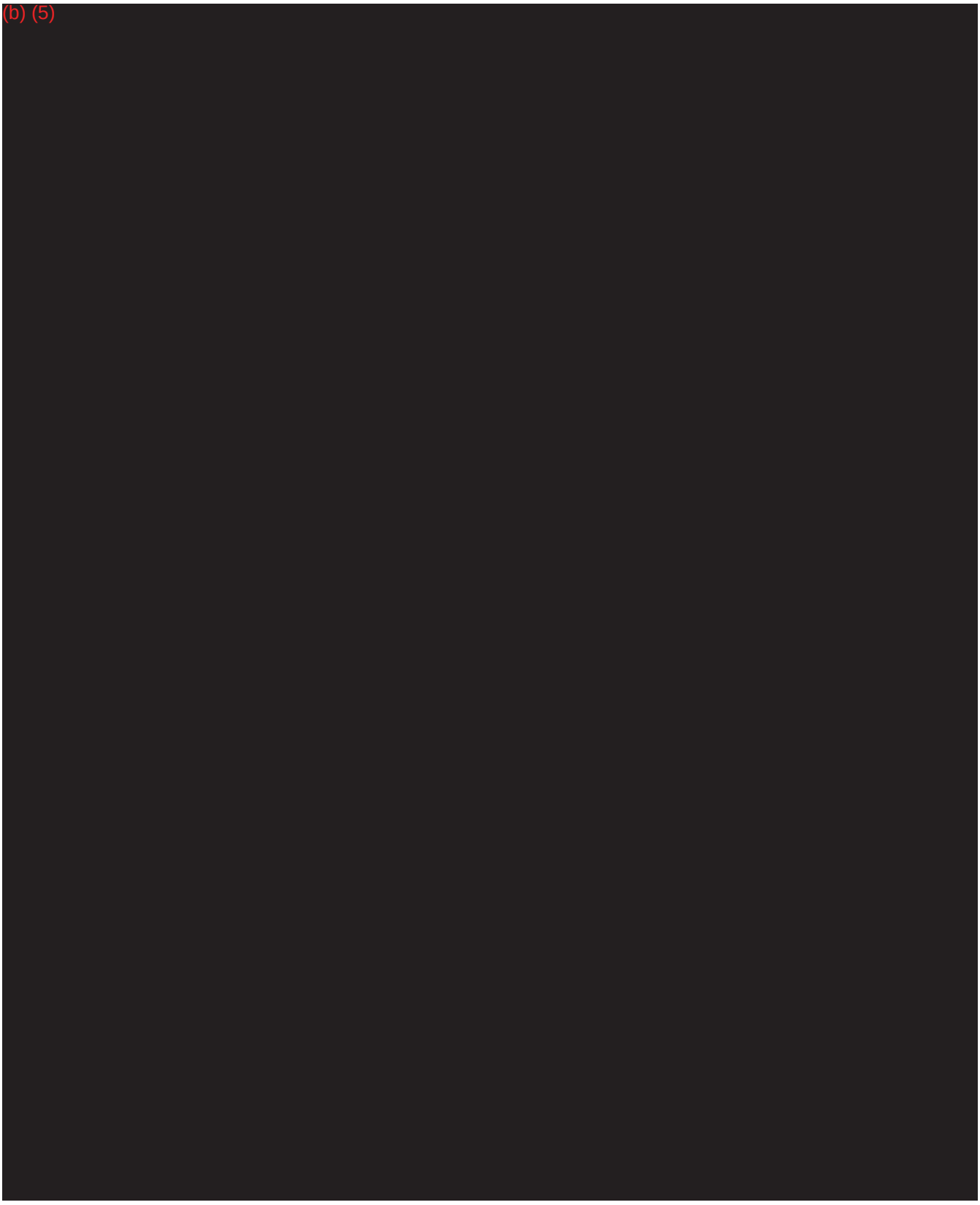

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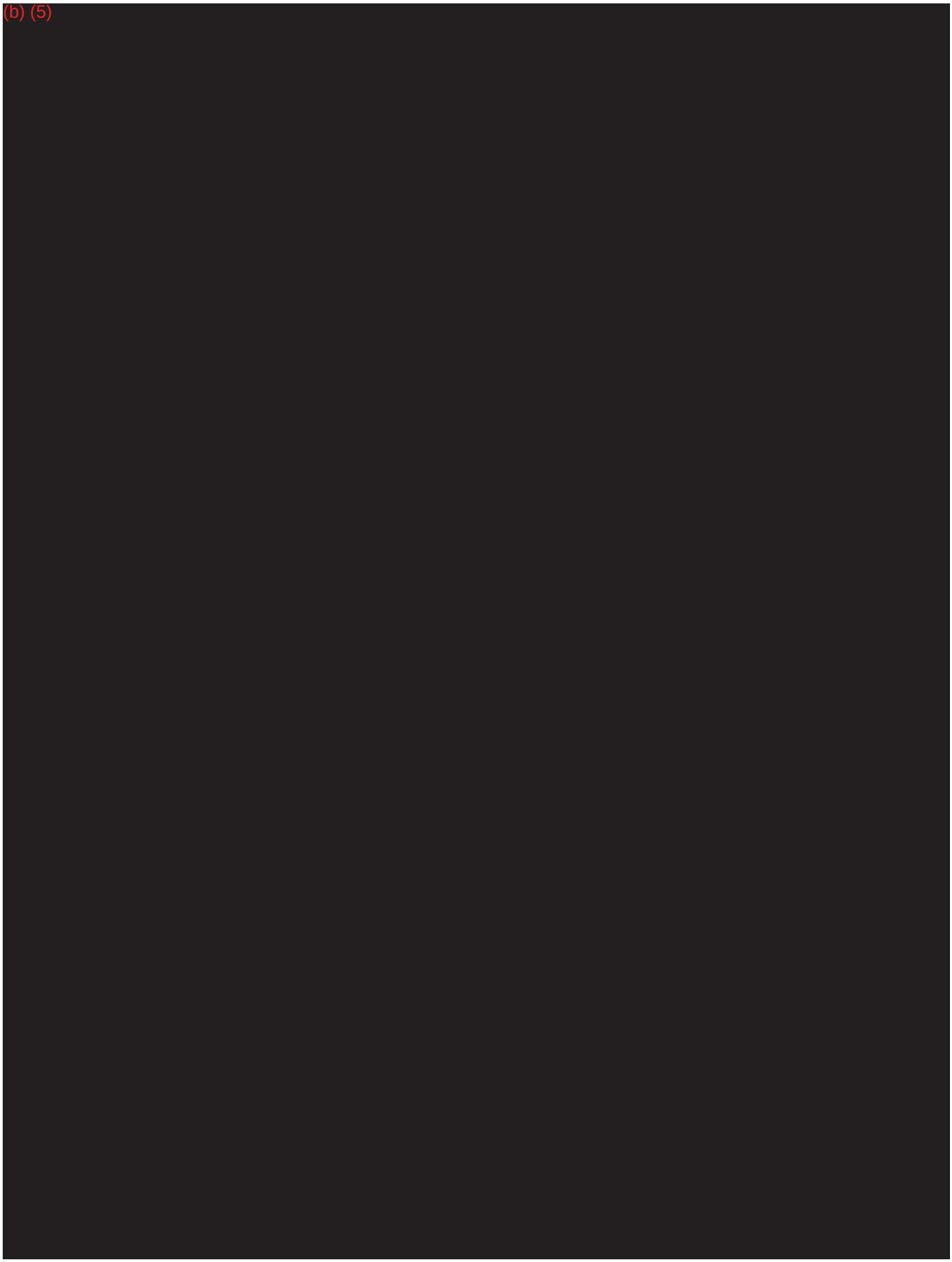

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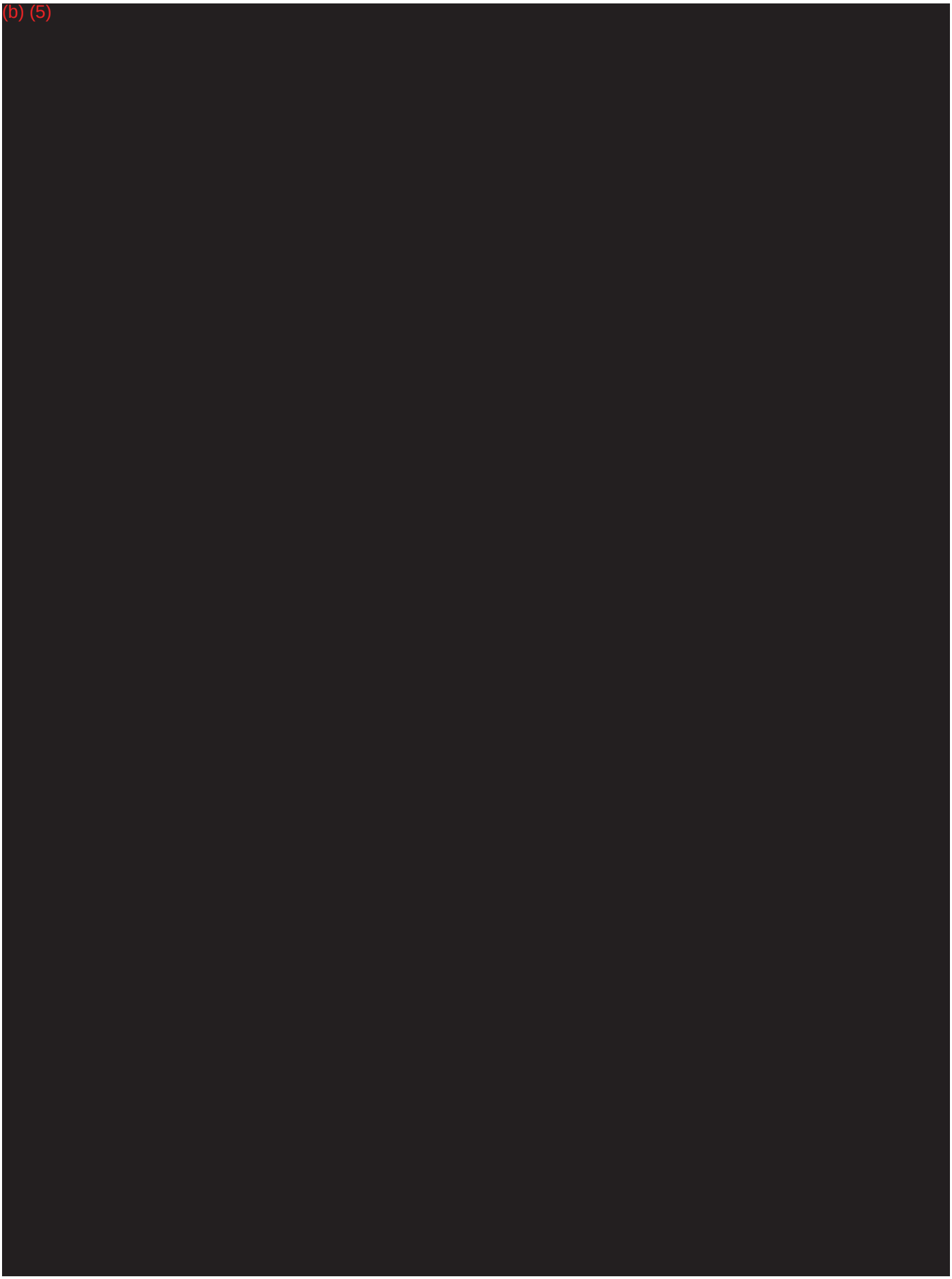

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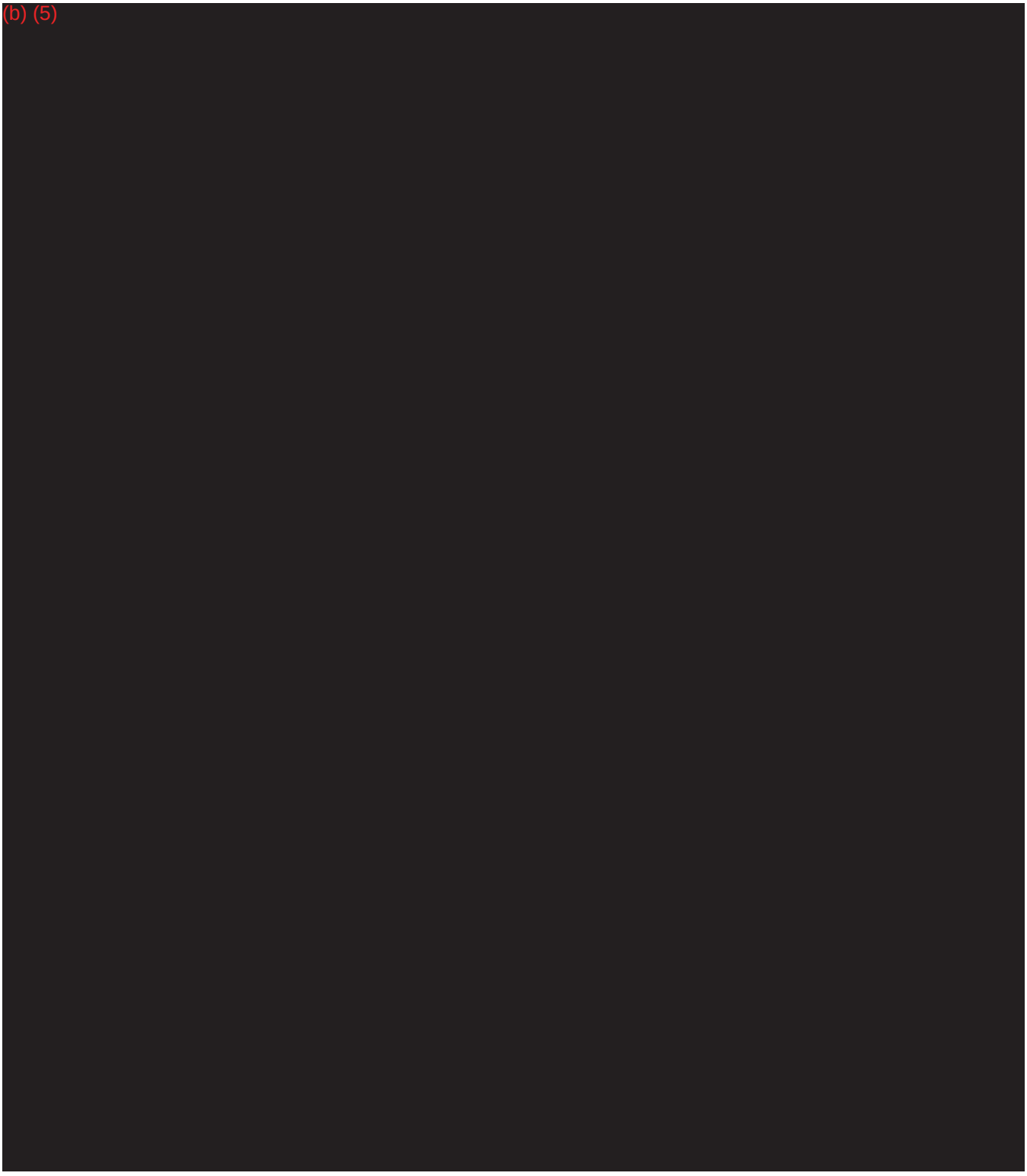

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additional recommendations.

Thank you,

Alain

Alain Silk | FDA/CDRH/OIR/DCTD | tel: 301-796-2129

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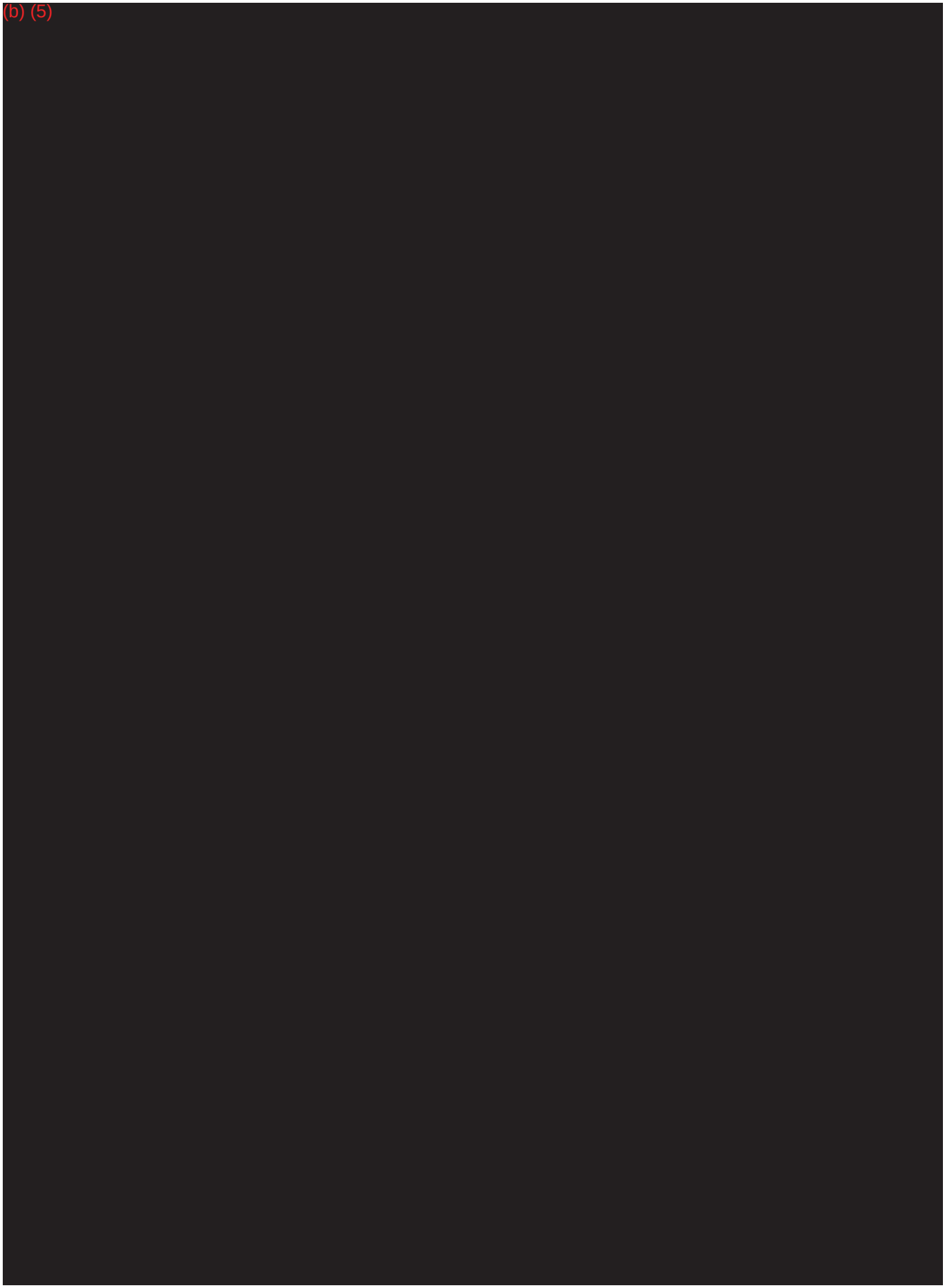

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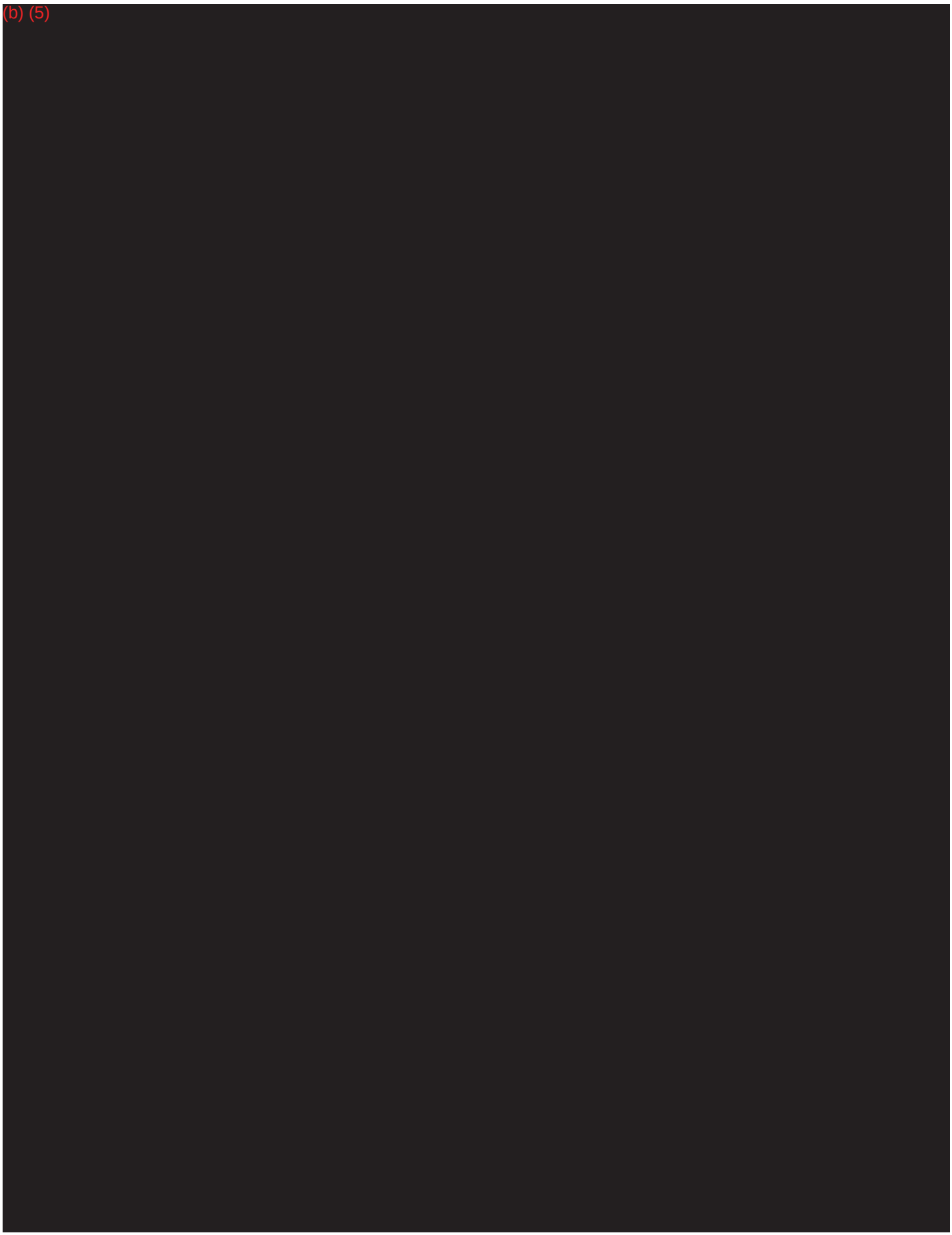

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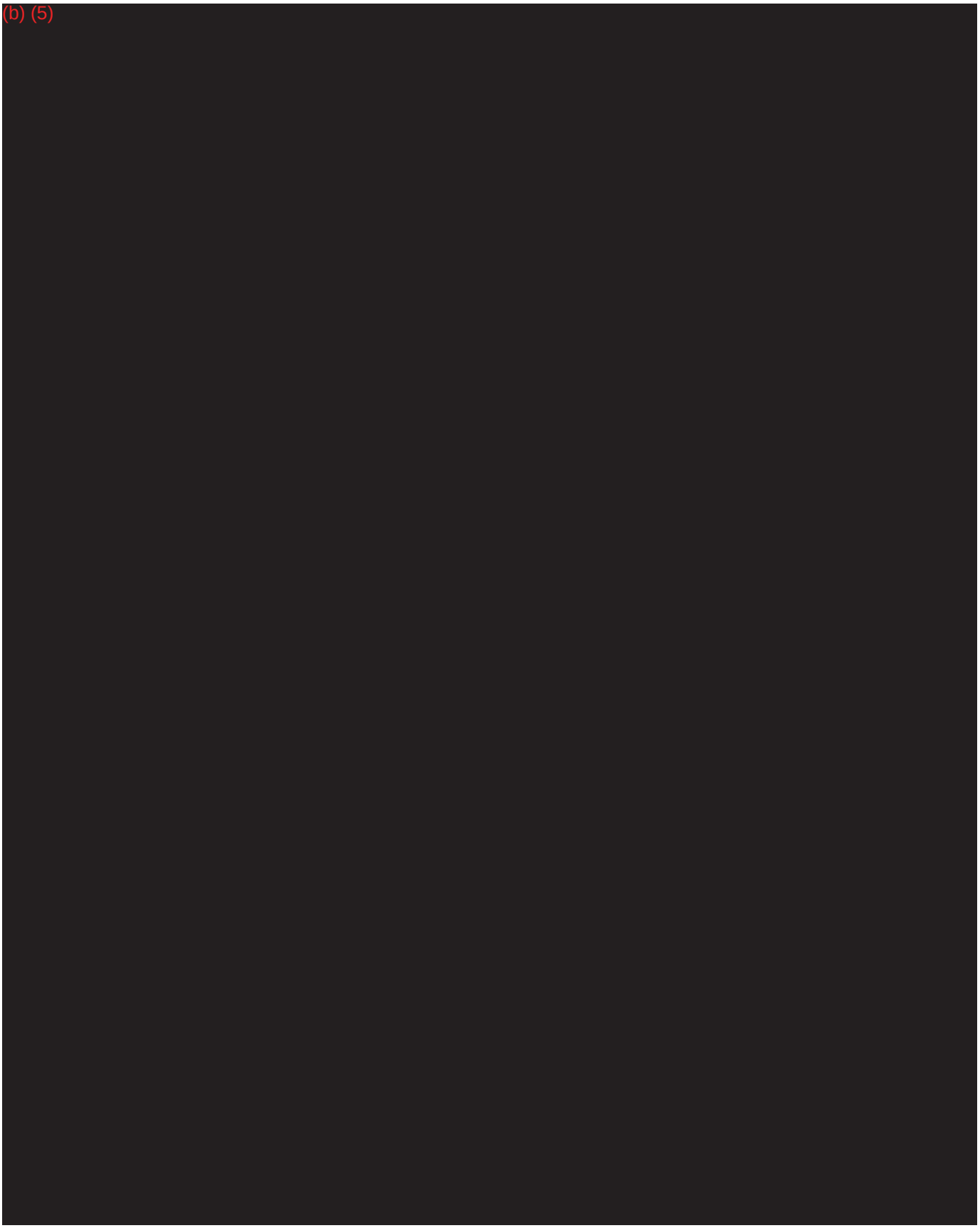

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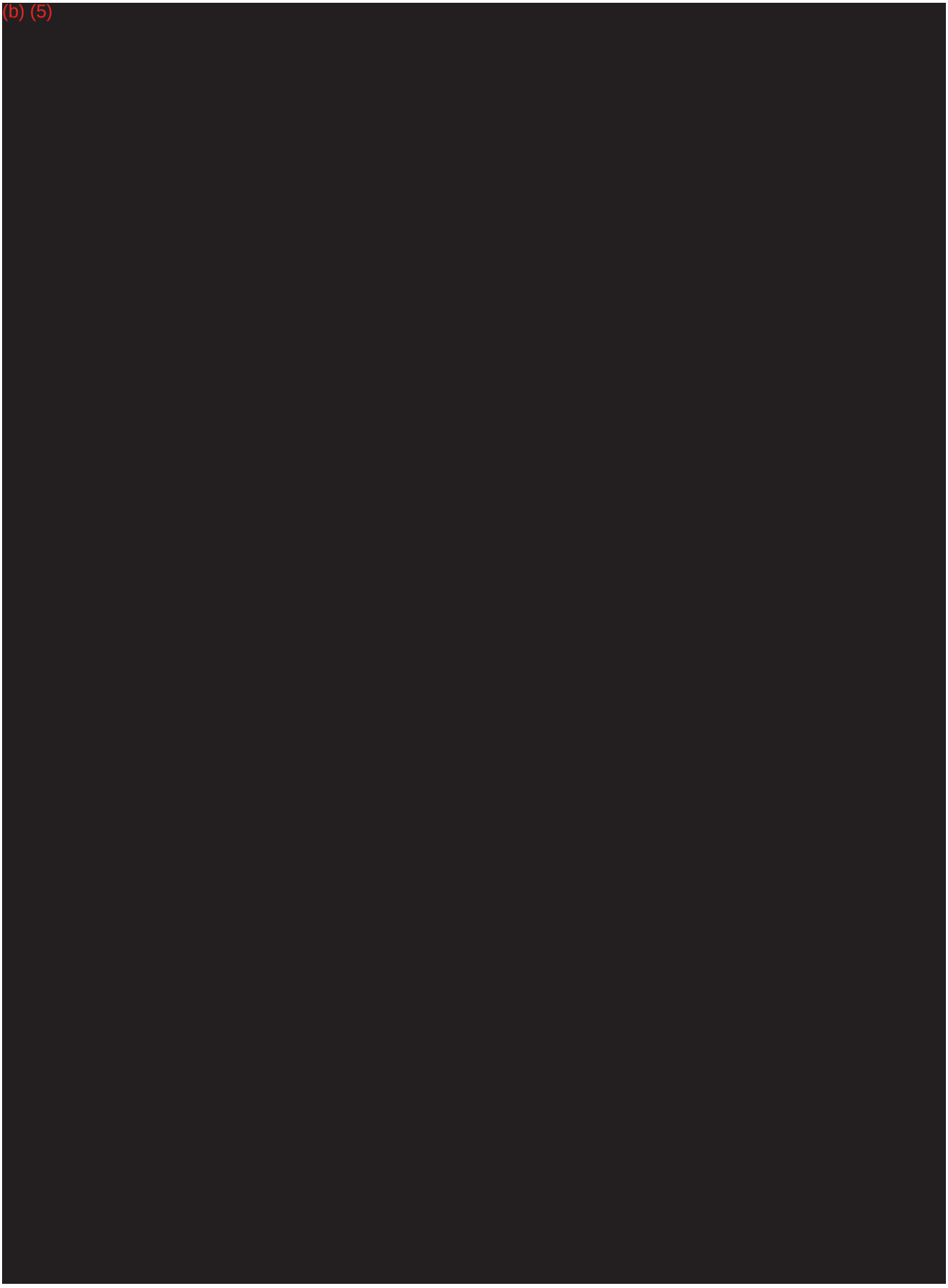

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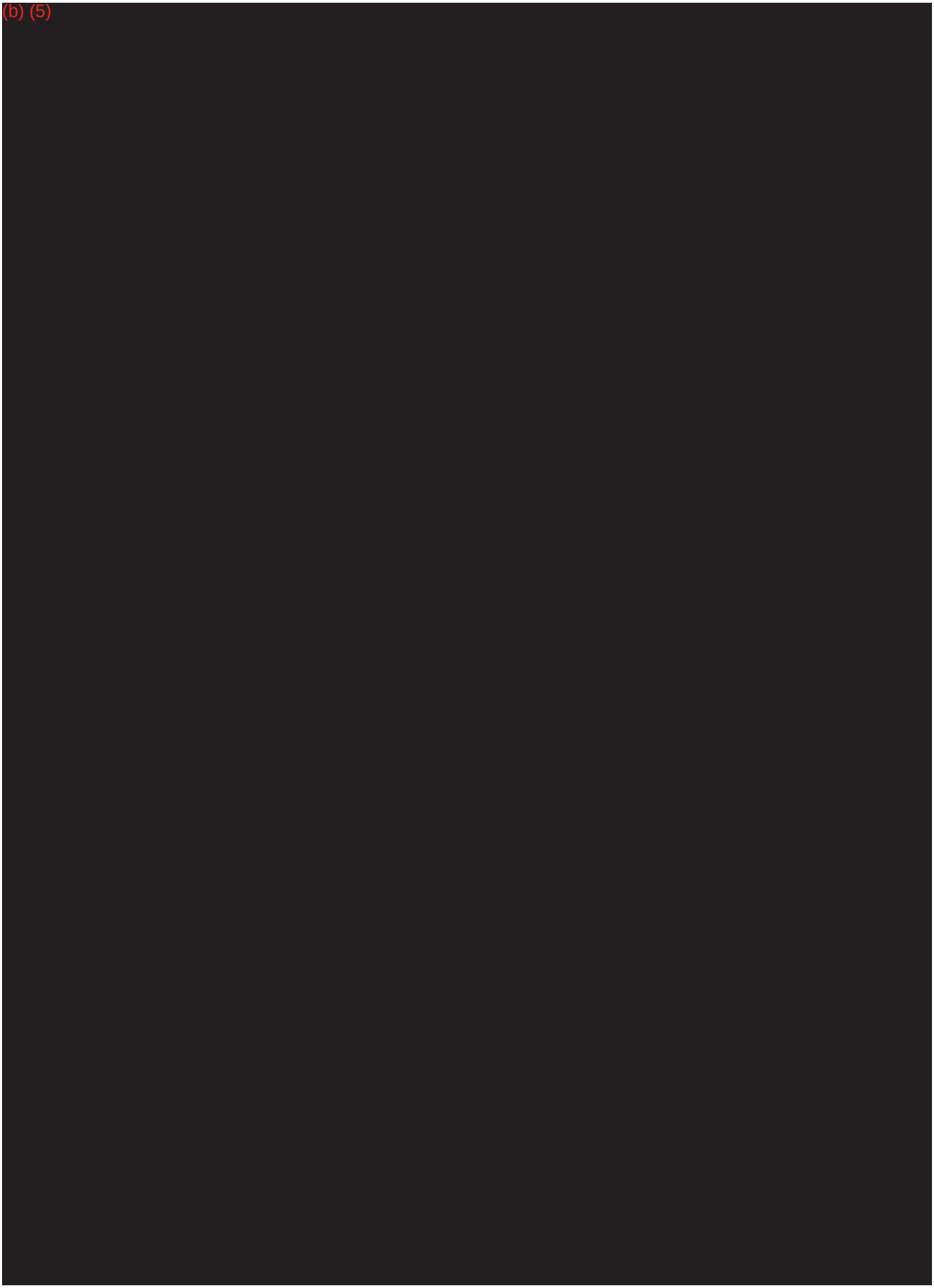

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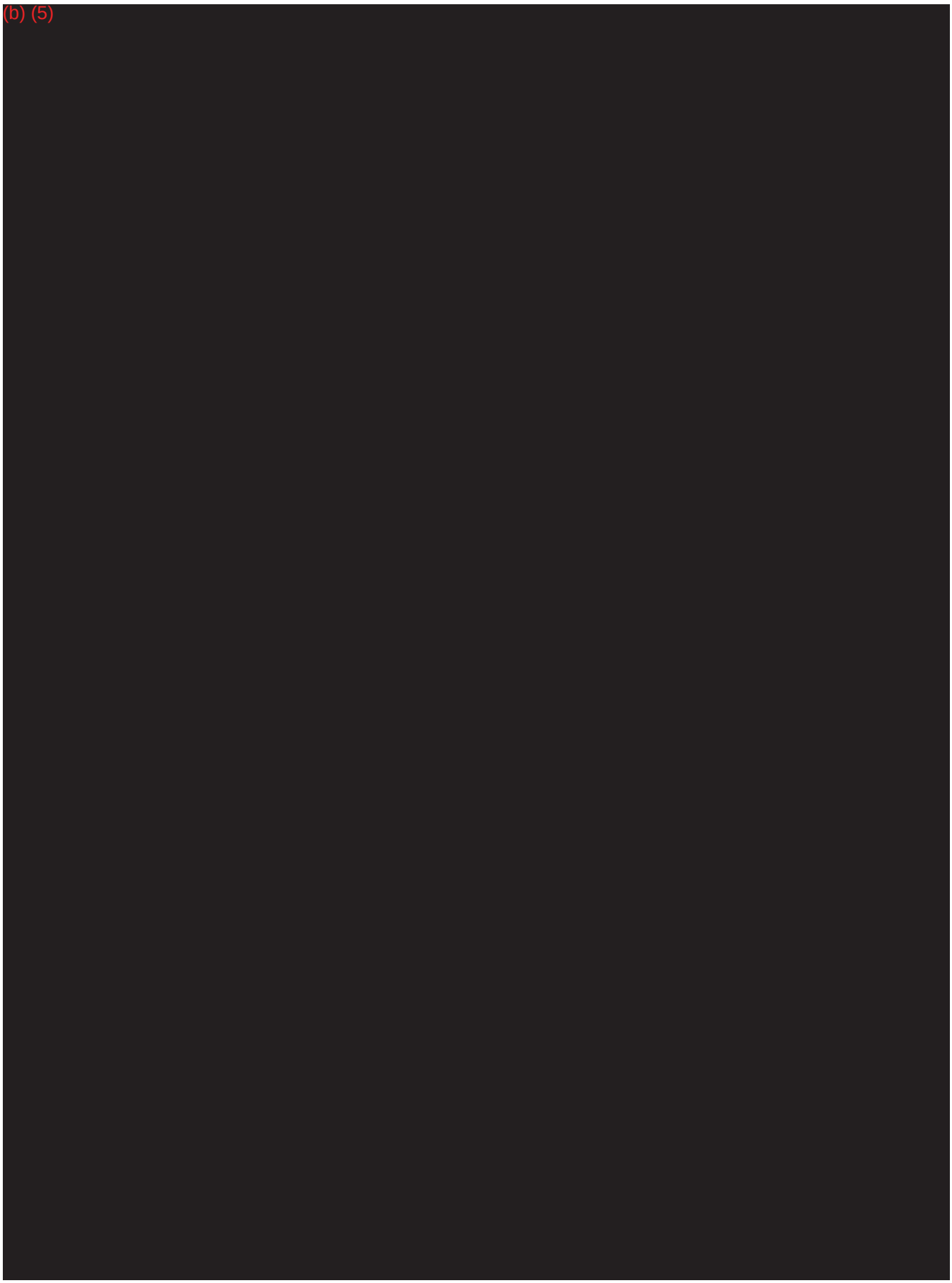

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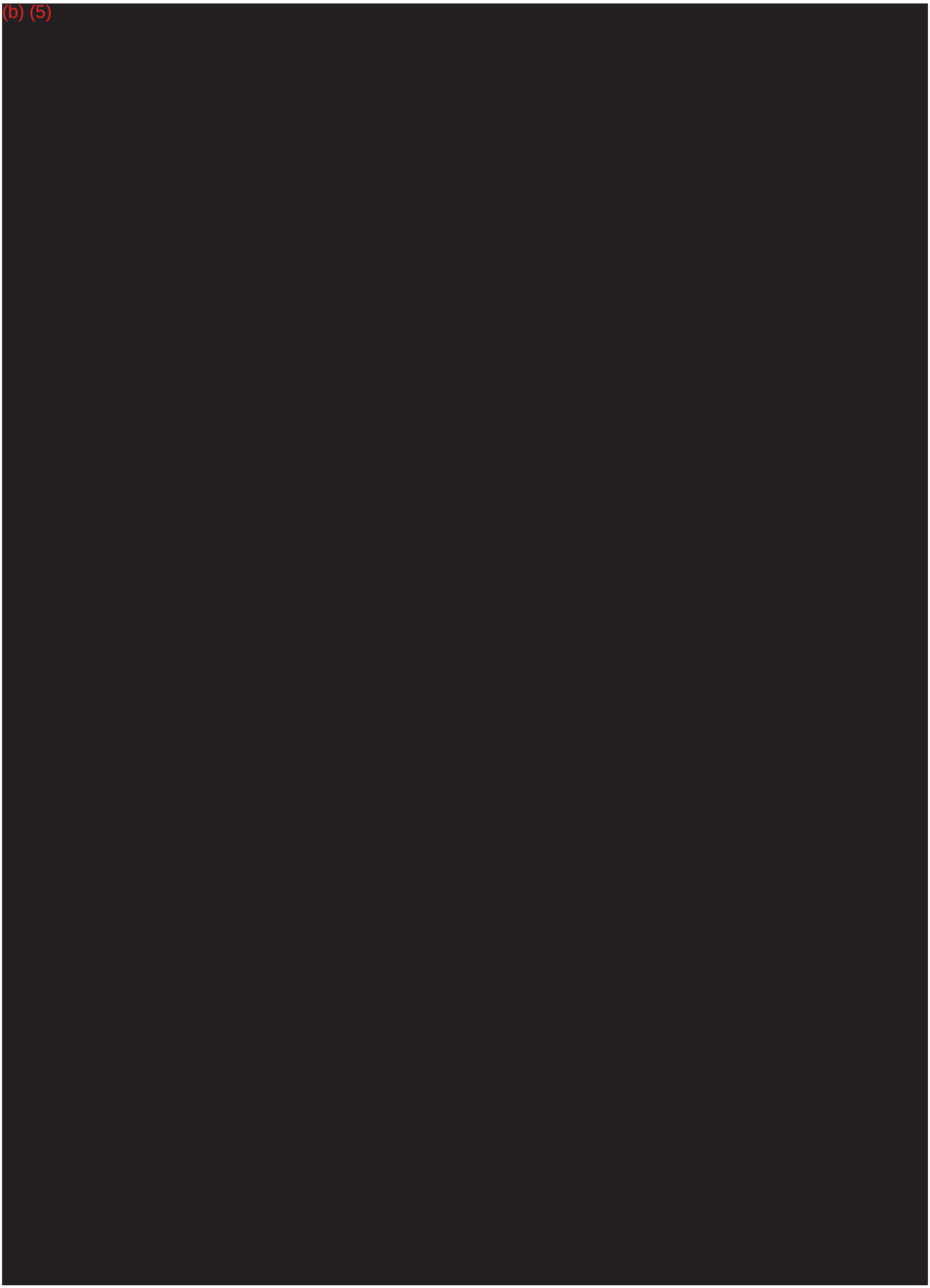

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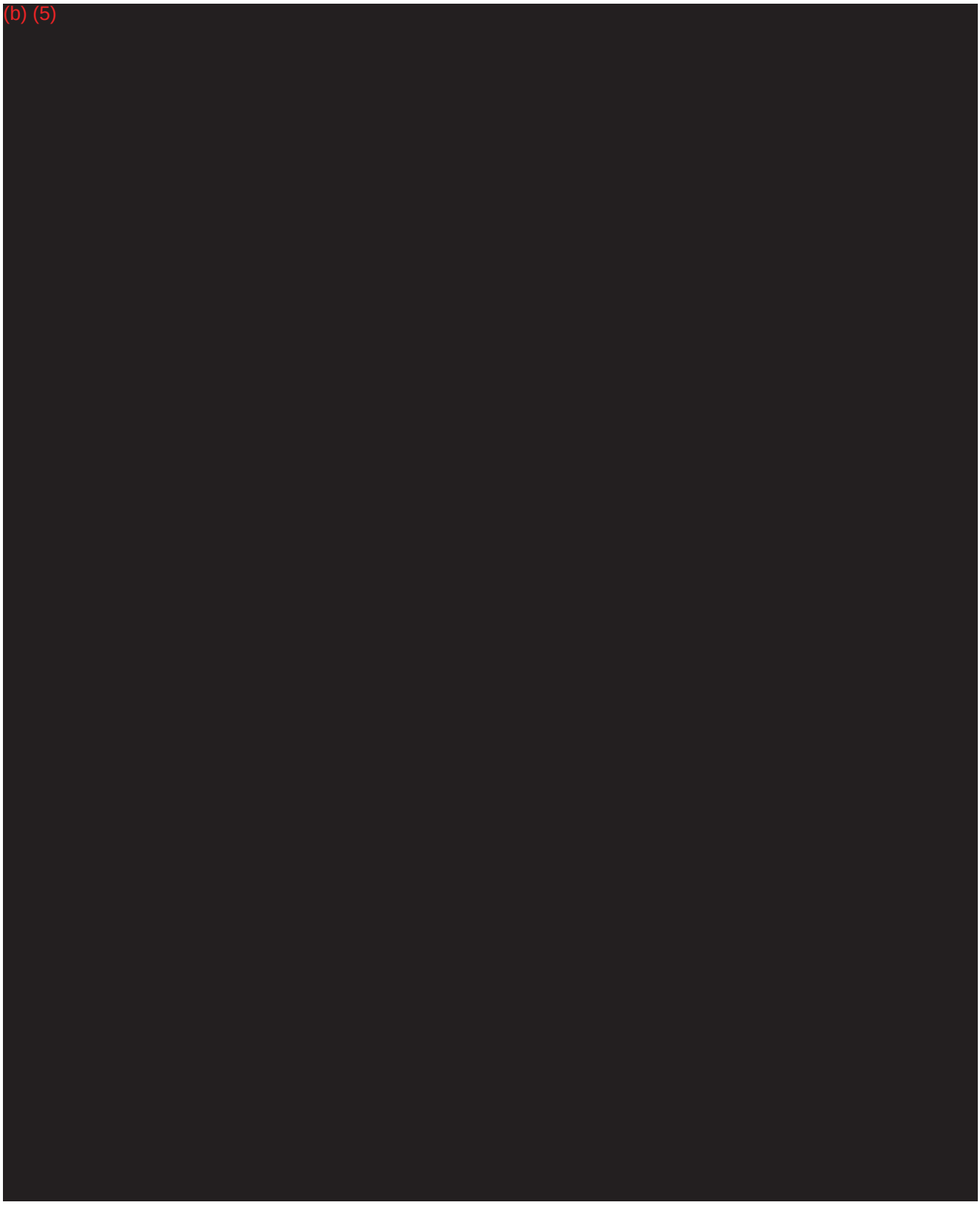

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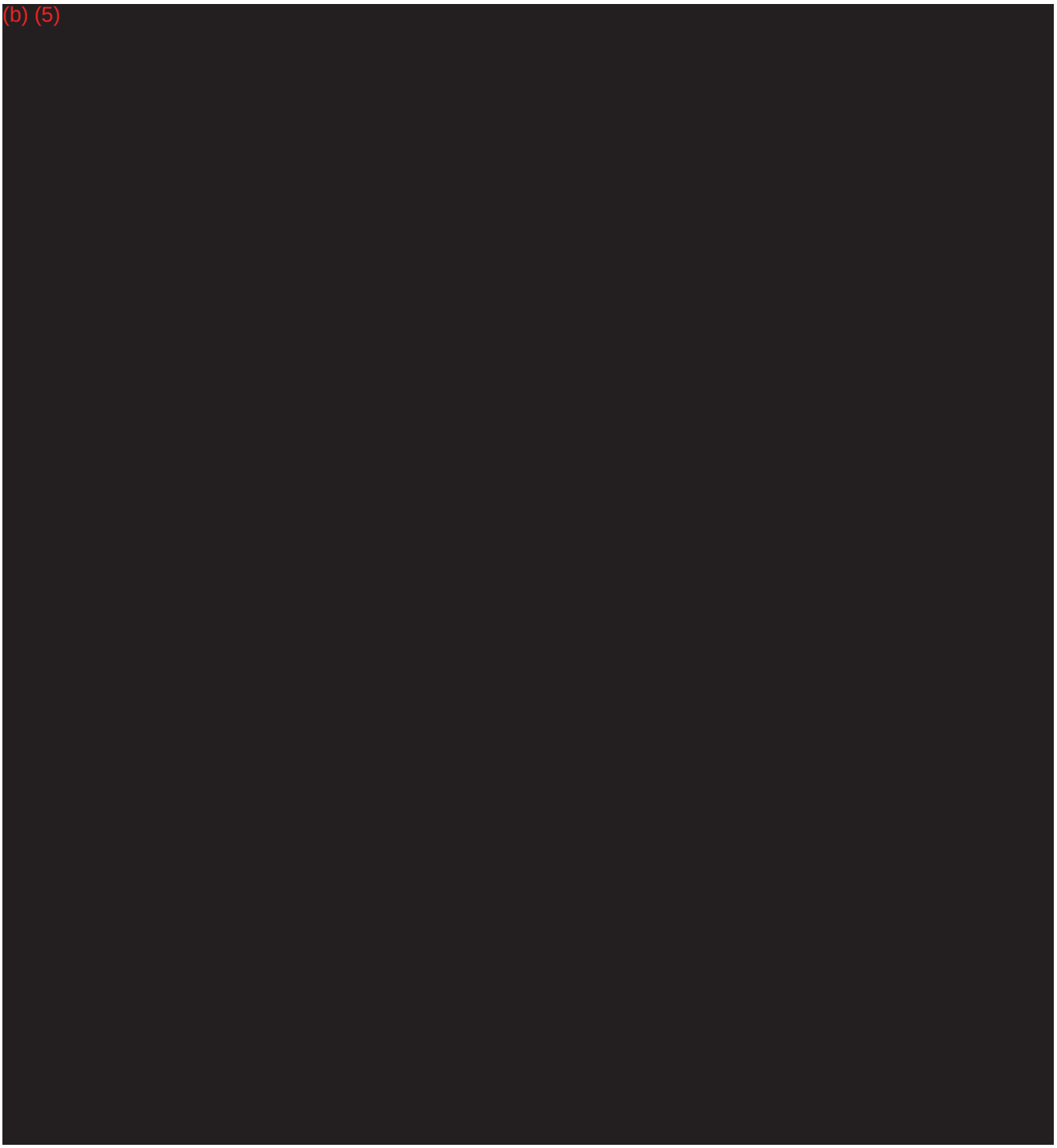

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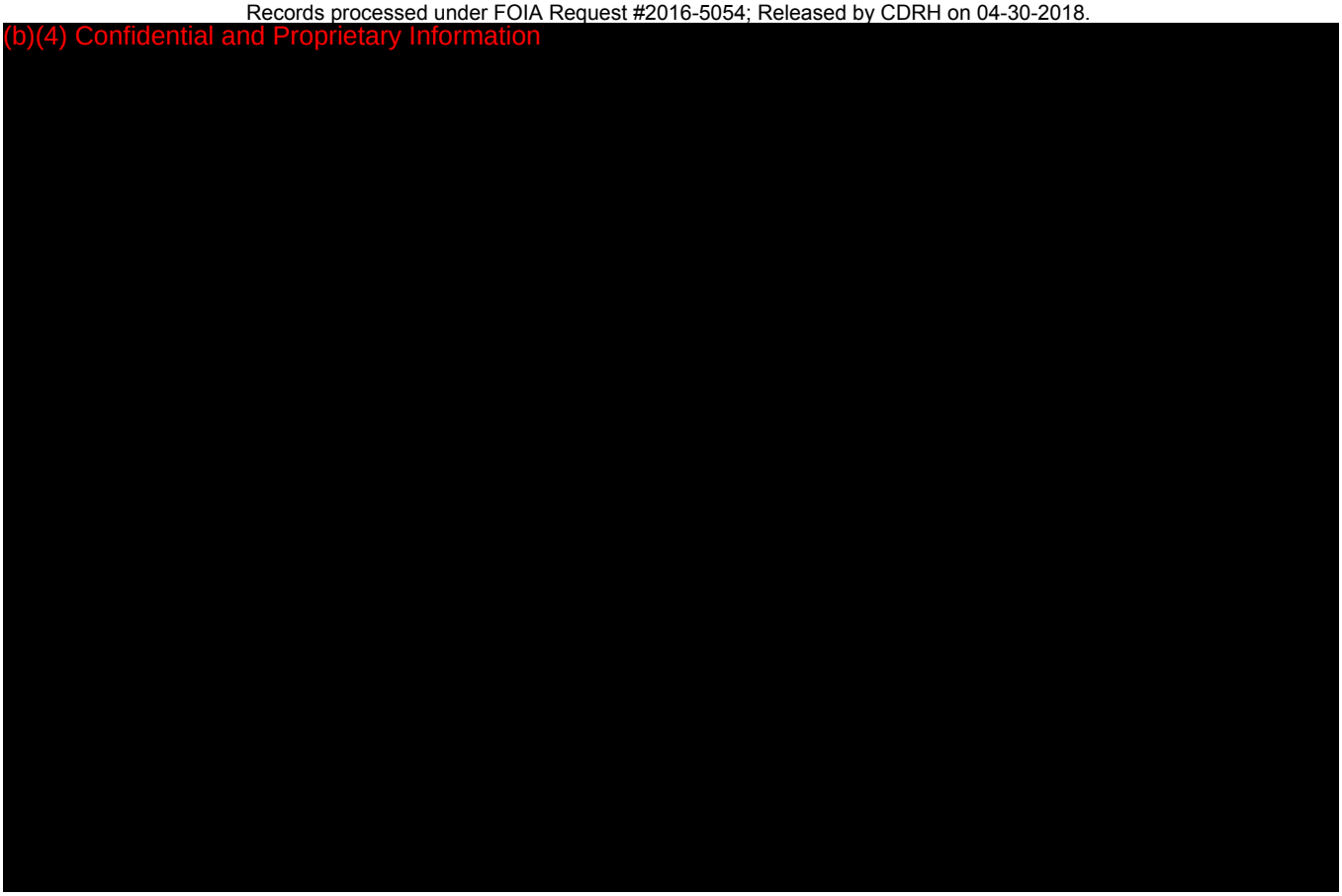

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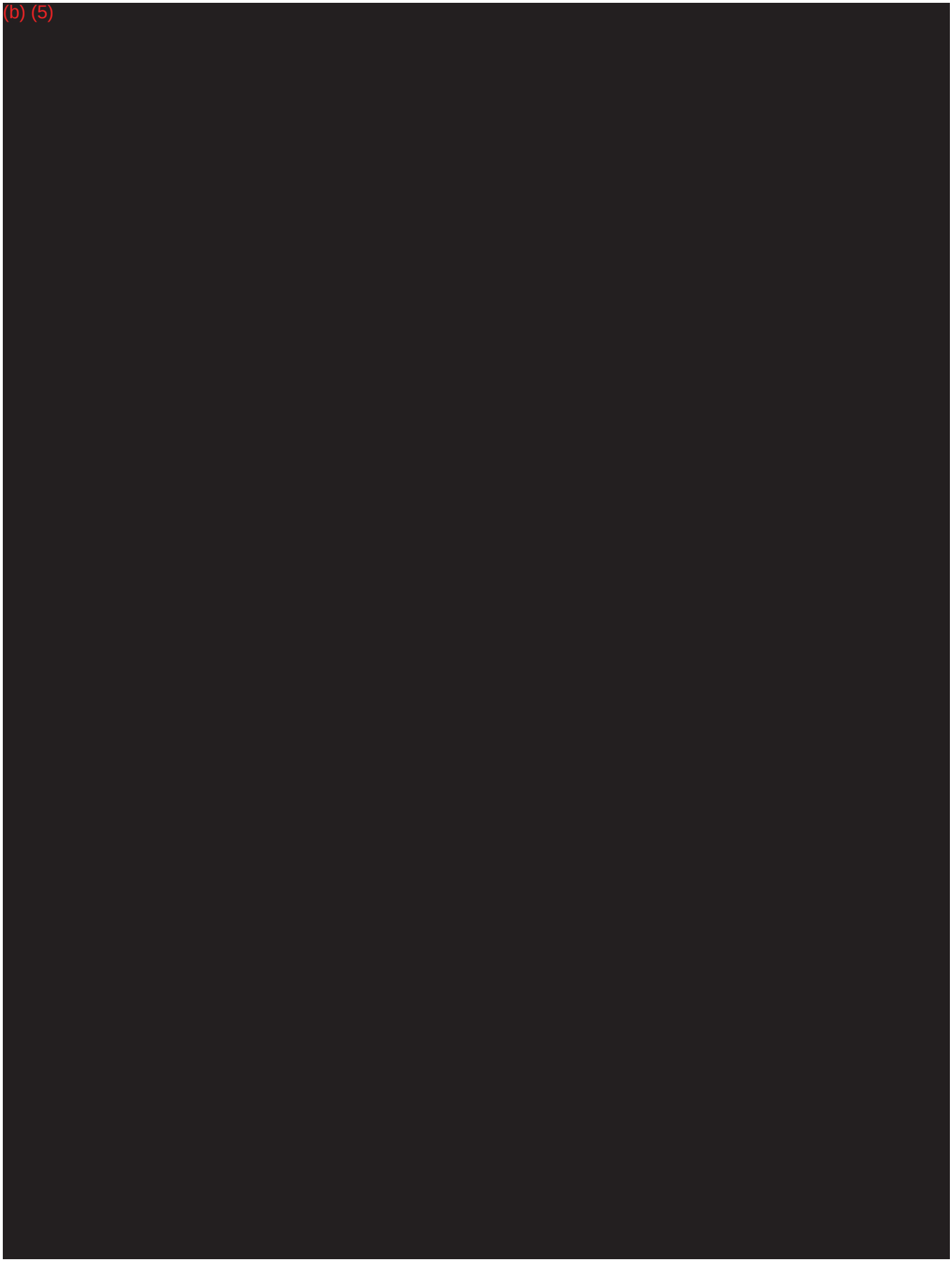

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K151321

BIO-RAD

Bio-Rad
Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

May 18, 2015 VIA FEDERAL EXPRESS

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Attention: OIR – Chemistry and Toxicology Devices (DCTD) - Chemistry Panel 75

RE:K151321 Replacement eCopy and Form 3514
DEVICE: D-100™ Hemoglobin Testing System, D-100™ HbA1c, D-100™ Calibrator Pack, Hemoglobin Capillary Collection System

There was an error in FDA form 3514, Section F. This 510(k) submission is for the D-100™ Hemoglobin Testing System, D-100™ HbA1c reagent components, D-100™ Calibrator Pack and the Hemoglobin Capillary Collection System not the device listed on the eCopy Hold letter.

This regulatory packet holds a CD containing the updated eCopy which is an exact duplication of the printed paper copies previous sent. The updated eCopy paper copy of FDA form 3514 is enclosed to be substituted in the submission.

Please contact Jackie Buckley at Bio-Rad Laboratories, Inc. by phone at 510-741-5309 with any questions.

Sincerely,

Jackie Buckley
Regulatory Affairs IV
Bio-Rad Laboratories, Inc.
Clinical Systems Division
4000 Alfred Noble Drive
Hercules, CA 94547
Enc.

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K151321

BIO-RAD

Bio-Rad
Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

May 18, 2015

VIA FEDERAL EXPRESS

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Regulatory Affairs IV
Bio-Rad Laboratories, Inc.
Clinical Systems Division
4000 Alfred Noble Drive
Hercules, CA 94547
Enc.

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K151321

BIO-RAD

Bio-Rad Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

May 15, 2015 VIA FEDERAL EXPRESS

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Attention: OIR – Chemistry and Toxicology Devices (DCTD) - Chemistry Panel 75

RE: Traditional 510(k) Submission Document: D-100™ Hemoglobin Testing System, D-100™ HbA1c, D-100™ Calibrator Pack, Hemoglobin Capillary Collection System Class II Classification: PDJ, LCP, JIT and JKA.

In accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act ("FDC Act"), I am submitting to you on behalf of the Sponsor, Bio-Rad Laboratories, Inc., Hercules, CA 94547, a traditional 510(k) submission for the D-100™ Hemoglobin Testing System, D-100™ HbA1c, D-100™ Calibrator Pack and Hemoglobin Capillary Collection System. This submission has been written to demonstrate substantial equivalence to the predicate and the additional requirements from the FDA Special Controls for HbA1c for Diabetes Diagnosis.

To conform with the Food and Drug Administration's August 12, 2005, "*Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s*", D-100™Hemoglobin Testing System, D-100™HbA1c Test, and D-100™Calibrators the principal factors concerning the design and use of the are set forth in the following table of FDA questions:

Question	YES	NO
Is the device intended for prescription use (21 CFR 801 Subpart D)?	X	
Is the device intended for over-the-counter use (21 CFR 807 Subpart C)?		X

Does the device contain components derived from a tissue or other biologic source?		X
Is the device provided sterile?		X
Is the device intended for single use?		X
Is the device a reprocessed single use device?		X
If yes, does this device type require reprocessed validation data?		N/A
Does the device contain a drug?		X
Does the device contain a biologic?		X
Does the device use software?*	X	
Does the submission include clinical information? (method comparison)*	X	
Is the device implanted?		X

*Clinical samples only

The D-100™ HbA1c test on the D-100™ Hemoglobin Testing System is a HPLC reagent/instrument system for the quantitative determination (IFCC mmol/mol and NGSP %) of HbA1c in human whole blood samples. The purpose of this 510(k) is to demonstrate the safety and effectiveness of the D-100™ HbA1c Test on the D-100™ Hemoglobin Testing System to support the Intended Use/Indications for Use allowing the D-100™HbA1c test to be used as an aid in the diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes and monitoring long-term glycemic control in individuals with diabetes mellitus.

In accordance with instructions in the Food and Drug Administration Special Controls for HbA1c for Diabetes Diagnosis, there are six requirements that must be met by an *in vitro* diagnostic device measuring HbA1c in order for the FDA to clear that device for the diagnosis of diabetes mellitus. As supported by information filed in this submission document, the D-100™ HbA1c test meets all the stated requirements for clearance of the product for this diagnostic purpose.

D™-100 HbA1c on the D-100™ Hemoglobin Testing Systems

Status of Special Controls Requirements for HbA1c for Diabetes Diagnosis

Satisfies Requirements	Requirement	Submission Location
YES	Device must have initial and annual standardization verification by a certifying glycohemoglobin standardization organization deemed acceptable by the FDA.	Section F
YES	Performance testing of device precision must, at a minimum, use blood samples with concentrations near 5.0%, 6.5%, 8.0% and 12% hemoglobin A1c. Testing must evaluate precision over a minimum of 20 days using at least 3 lots of the device and 3 instruments, as applicable.	Section M and Appendix I

YES	Performance testing of accuracy must include a minimum of 120 blood samples that span the measuring interval of the new device and compare results of the new device to results of the standardized method. Results must demonstrate little or no bias versus the standardized method.	Section N and Appendix J
YES	Total error of the new device must be evaluated using single measurements by the new device compared to results of the standardized test method, and this evaluation must demonstrate a total error less than or equal to 6%.	Section N and Appendix J
YES	Performance testing must demonstrate that there is little to no interference from common hemoglobin variants, including Hemoglobin C, Hemoglobin D, Hemoglobin E, Hemoglobin A2 and Hemoglobin S.	Section K and L and Appendices G and H
N/A	When assay interference from Hemoglobin F or interference with other hemoglobin variants with low frequency in the population is observed, a warning statement must be placed in a black box and must appear in all labeling material for these devices describing the interference and any affected population.	See Section L and Appendix H

Bio-Rad Laboratories, Inc. considers its intent to market the D-100™ HbA1c on the D-100™ Hemoglobin Testing System as confidential commercial information. The Company has not disclosed its intent to market this device for this additional intended use to anyone, except its employees, others with a financial interest in the Company, its advertising or law firms, and its consultants. The Company, therefore, requests that the FDA not disclose the existence of this document until such time as the final action on the 510(k) submission is taken. Bio-Rad Laboratories, Inc. requests the confidentiality afforded submissions of this type as defined in 21 CFR 812.38.

This regulatory packet holds one hard copy original, one copy of that original, and a CD containing the required eCopy which is an exact duplication of the same files printed on the paper copies. The required eCopy statement is attached directly to the eCopy disc folder located at the front cover of the ORIGINAL hard copy document. The document is arranged in alphabetical order by section with Appendices containing more information when a more detailed or lengthy explanatory document is required.

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We trust that the information provided in this 510(k) notice is sufficient for the agency to find the D-100™HbA1c test on the D-100™Hemoglobin Testing System substantially equivalent to its predicate device. If there are any questions or additional information is needed, please contact Jackie Buckley at Bio-Rad Laboratories, Inc. by phone at 510-741-5309 or by email at jackie_buckley@bio-rad.com. If Jackie Buckley is unavailable, please contact Alfred Evans via phone at 510-741-4579 or by email at al_evans@bio-rad.com.

Sincerely,

Jackie Buckley
Regulatory Affairs IV
Bio-Rad Laboratories, Inc.
Clinical Systems Division
4000 Alfred Noble Drive
Hercules, CA 94547

Enc.

Traditional 510(k)

D-100™ Hemoglobin Testing System

D-100™ HbA1c

D-100™ Calibrator Pack

Hemoglobin Capillary Collection Kit

Bio-Rad Laboratories, Inc.

Clinical Systems Division

Hercules, CA

May 15, 2015

Version 1.0

SECTION A:

Bio-Rad Laboratories, Inc. Cover Letter

Traditional 510(k)

D-100™ Hemoglobin Testing System

D-100™ HbA1c Test

D-100™ Calibrator Pack

Hemoglobin Capillary Collection Kit

BIO-RAD

Bio-Rad Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

May 15, 2015 VIA FEDERAL EXPRESS

U.S. Food and Drug Administration
Center for Devices and Radiological Health
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In accordance with instructions in the Food and Drug Administration Special Controls for HbA1c for Diabetes Diagnosis, there are six requirements that must be met by an *in vitro* diagnostic device measuring HbA1c in order for the FDA to clear that device for the diagnosis of diabetes mellitus. As supported by information filed in this submission document, the D-100™ HbA1c test meets all the stated requirements for clearance of the product for this diagnostic purpose.

D™-100 HbA1c on the D-100™ Hemoglobin Testing Systems

Status of Special Controls Requirements for HbA1c for Diabetes Diagnosis

Satisfies Requirements	Requirement	Submission Location
YES	Device must have initial and annual standardization verification by a certifying glycohemoglobin standardization organization deemed acceptable by the FDA.	Section F
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YES	Performance testing of accuracy must include a minimum of 120 blood samples that span the measuring interval of the new device and compare results of the new device to results of the standardized method. Results must demonstrate little or no bias versus the standardized method.	Section N and Appendix J
YES	Total error of the new device must be evaluated using single measurements by the new device compared to results of the standardized test method, and this evaluation must demonstrate a total error less than or equal to 6%.	Section N and Appendix J
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Sincerely,

Jackie Buckley
Regulatory Affairs IV
Bio-Rad Laboratories, Inc.
Clinical Systems Division
4000 Alfred Noble Drive
Hercules, CA 94547

Enc.

SECTION B:

Form 3514 (CDRH Premarket Review Submission Cover Sheet)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

CDRH PREMARKET REVIEW SUBMISSION COVER SHEET

Form Approval
OMB No. 0910-0120
Expiration Date: December 31, 2013
See PRA Statement on page 5.

Date of Submission	User Fee Payment ID Number	FDA Submission Document Number *(if known)*
May 15, 2015	(b) (4)	

SECTION A TYPE OF SUBMISSION

PMA	PMA & HDE Supplement	PDP	510(k)	Request for Feedback
☐ Original Submission	☐ Regular (180 day)	☐ Original PDP	☒ Original Submission:	☐ Pre-Submission
☐ Premarket Report	☐ Special	☐ Notice of Completion	☒ Traditional	☐ Informational Meeting
☐ Modular Submission	☐ Panel Track (PMA Only)	☐ Amendment to PDP	☐ Special	☐ Submision Issue Meeting
☐ Amendment	☐ 30-day Supplement		☐ Abbreviated (Complete section I, Page 5)	☐ Day 100 Meeting
☐ Report	☐ 30-day Notice		☐ Additional Information	☐ Agreement Meeting
☐ Report Amendment	☐ 135-day Supplement		☐ Third Party	☐ Determination Meeting
☐ Licensing Agreement	☐ Real-time Review			☐ Study Risk Determination
	☐ Amendment to PMA & HDE Supplement			☐ Other *(specify):*
	☐ Other			

IDE	Humanitarian Device Exemption (HDE)	Class II Exemption Petition	Evaluation of Automatic Class III Designation (De Novo)	Other Submission
☐ Original Submission	☐ Original Submission	☐ Original Submission	☐ Original Submission	☐ 513(g)
☐ Amendment	☐ Amendment	☐ Additional Information	☐ Additional Information	☒ Other *(describe submission):* Q140167
☐ Supplement	☐ Supplement			
	☐ Report			
	☐ Report Amendment			

Have you used or cited Standards in your submission? ☒ Yes ☐ No *(If Yes, please complete Section I, Page 5)*

SECTION B SUBMITTER, APPLICANT OR SPONSOR

Company / Institution Name	Establishment Registration Number *(if known)*		
Bio-Rad Laboratories, Inc.	2915274		
Division Name *(if applicable)*	**Phone Number *(including area code)***		
Clinical System Division	510-741-5309		
Street Address	**FAX Number *(including area code)***		
4000 Alfred Nobel Dr.	510-741-3954		
City	**State / Province**	**ZIP/Postal Code**	**Country**
Hercules	CA	94547	USA
Contact Name			
Jackie Buckley			
Contact Title	**Contact E-mail Address**		
Regulatory Affairs Rep IV	jackie_buckley@bio-rad.com		

SECTION C APPLICATION CORRESPONDENT (e.g., consultant, if different from above)

Company / Institution Name			
Division Name *(if applicable)*	**Phone Number *(including area code)***		
Street Address	**FAX Number *(including area code)***		
City	**State / Province**	**ZIP Code**	**Country**
Contact Name			
Contact Title	**Contact E-mail Address**		

PSC Publishing Services (301) 443-6740 EF

SECTION D1 REASON FOR APPLICATION - PMA, PDP, OR HDE

- ☐ New Device
- ☐ Withdrawal
- ☐ Additional or Expanded Indications
- ☐ Request for Extension
- ☐ Post-approval Study Protocol
- ☐ Request for Applicant Hold
- ☐ Request for Removal of Applicant Hold
- ☐ Request to Remove or Add Manufacturing Site

- ☐ Change in design, component, or specification:
  - ☐ Software / Hardware
  - ☐ Color Additive
  - ☐ Material
  - ☐ Specifications
  - ☐ Other *(specify below)*

- ☐ Location change:
  - ☐ Manufacturer
  - ☐ Sterilizer
  - ☐ Packager

- ☐ Process change:
  - ☐ Manufacturing ☐ Packaging
  - ☐ Sterilization
  - ☐ Other *(specify below)*

- ☐ Labeling change:
  - ☐ Indications
  - ☐ Instructions
  - ☐ Performance Characteristics
  - ☐ Shelf Life
  - ☐ Trade Name
  - ☐ Other *(specify below)*

- ☐ Report Submission:
  - ☐ Annual or Periodic
  - ☐ Post-approval Study
  - ☐ Adverse Reaction
  - ☐ Device Defect
  - ☐ Amendment

- ☐ Response to FDA correspondence:

- ☐ Change in Ownership
- ☐ Change in Correspondent
- ☐ Change of Applicant Address

- ☐ Other Reason *(specify)*:

SECTION D2 REASON FOR APPLICATION - IDE

- ☐ New Device
- ☐ New Indication
- ☐ Addition of Institution
- ☐ Expansion / Extension of Study
- ☐ IRB Certification
- ☐ Termination of Study
- ☐ Withdrawal of Application
- ☐ Unanticipated Adverse Effect
- ☐ Notification of Emergency Use
- ☐ Compassionate Use Request
- ☐ Treatment IDE
- ☐ Continued Access

- ☐ Change in:
  - ☐ Correspondent / Applicant
  - ☐ Design / Device
  - ☐ Informed Consent
  - ☐ Manufacturer
  - ☐ Manufacturing Process
  - ☐ Protocol - Feasibility
  - ☐ Protocol - Other
  - ☐ Sponsor

- ☐ Report submission:
  - ☐ Current Investigator
  - ☐ Annual Progress Report
  - ☐ Site Waiver Report
  - ☐ Final

- ☐ Response to FDA Letter Concerning:
  - ☐ Conditional Approval
  - ☐ Deemed Approved
  - ☐ Deficient Final Report
  - ☐ Deficient Progress Report
  - ☐ Deficient Investigator Report
  - ☐ Disapproval
  - ☐ Request Extension of Time to Respond to FDA
  - ☐ Request Meeting
  - ☐ Request Hearing

- ☐ Other Reason *(specify)*:

SECTION D3 REASON FOR SUBMISSION - 510(k)

- ☒ New Device
- ☐ Additional or Expanded Indications
- ☐ Change in Technology

- ☐ Other Reason *(specify)*:

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS

Product codes of devices to which substantial equivalence is claimed

1	LCP	2	PDJ	3	JIT	4	JKA
5		6		7		8	

Summary of, or statement concerning, safety and effectiveness information

☒ 510 (k) summary attached
☐ 510 (k) statement

Information on devices to which substantial equivalence is claimed *(if known)*

	510(k) Number		Trade or Proprietary or Model Name		Manufacturer
1	K090699	1	VARIANT II TURBO HbA1c Kit - 2.0	1	Bio-Rad Laboratories, Inc.
2	K142448	2	VARIANT II TURBO HbA1c Kit - 2.0	2	Bio-Rad Laboratories, Inc.
3	K070452	3	VARIANT II Hemoglobin A1c Kit	3	Bio-Rad Laboratories, Inc.
4	K090737	4	Hemoglobin Capillary Collection System	4	Bio-Rad Laboratories, Inc.
5		5		5	
6		6		6	

SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS

Common or usual name or classification name

Hemoglobin A1c or HbA1c or A1c

	Trade or Proprietary or Model Name for This Device		Model Number
1	D-100 HbA1c Analytical Cartridge/Calibrator Pack and D-100 HbA1c Calibrator Pack	1	290-1004 and 290-1006
2	D-100 HbA1c Elution Buffer A and D-100 HbA1c Elution Buffer B	2	290-1010 and 290-1011
3	D-100 Sample Diluent and D-100 Wash Solution	3	290-1009 and 290-1012
4	D-100 Hemoglobin Testing System	4	290-1000
5	Hemoglobin Capillary Collection System	5	196-2051, 196-2052, 196-2053

FDA document numbers of all prior related submissions *(regardless of outcome)*

1	2	3	4	5	6
7	8	9	10	11	12

Data Included in Submission

☒ Laboratory Testing ☐ Animal Trials ☐ Human Trials

SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS

Product Code	C.F.R. Section *(if applicable)*	Device Class
PDJ & LCP & JIT	21 CFR Section 862.1373 and Section 21 CFR 862.1150	☐ Class I ☒ Class II
Classification Panel		☐ Class III ☐ Unclassified
Chemistry (75)		

Indications *(from labeling)*

The D-100 HbA1c is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP%) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System. The test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. Measurement of hemoglobin A1c is effective in monitoring long-term glycemic control in individuals with diabetes mellitus. The D-100 HbA1c is intended for Professional Use Only.

The D-100 HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

Note: Submission of the information entered in Section H does not affect the need to submit device establishment registration.	FDA Document Number *(if known)*

SECTION H — MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION

☒ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number	☒ Manufacturer ☐ Contract Sterilizer ☐ Contract Manufacturer ☐ Repackager / Relabeler
Company / Institution Name: Bio-Rad Laboratories, Inc.		Establishment Registration Number: 2915274
Division Name *(if applicable)*: Clinical System Division		Phone Number *(including area code)*: 510-741-5309
Street Address: 4000 Alfred Nobel Dr.		FAX Number *(including area code)*: 510-741-3954
City: Hercules	State / Province: CA	ZIP Code: 94547 — Country: USA
Contact Name: Jackie Buckley	Contact Title: Regulatory Affairs Representative IV	Contact E-mail Address: jackie_buckley@bio-rad.com

☐ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number	☐ Manufacturer ☐ Contract Sterilizer ☐ Contract Manufacturer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number
Division Name *(if applicable)*		Phone Number *(including area code)*
Street Address		FAX Number *(including area code)*
City	State / Province — ZIP Code — Country	
Contact Name	Contact Title	Contact E-mail Address

☐ Original ☐ Add ☐ Delete	Facility Establishment Identifier (FEI) Number	☐ Manufacturer ☐ Contract Sterilizer ☐ Contract Manufacturer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number
Division Name *(if applicable)*		Phone Number *(including area code)*
Street Address		FAX Number *(including area code)*
City	State / Province — ZIP Code — Country	
Contact Name	Contact Title	Contact E-mail Address

SECTION I UTILIZATION OF STANDARDS

Note: Complete this section if your application or submission cites standards or includes a *"Declaration of Conformity to a Recognized Standard"* statement.

	Standards No.	Standards Organization	Standards Title	Version	Date
1	EP07-A2	CSLI	Interference Testing in Clinical Chemistry, Approved Guidelines, Second Edition	Vol 25, No. 27	Nov. 23, 2005
2	Standards No. EP09-A2IR	Standards Organization CSLI	Standards Title Method Comparison Study and Bias Estimation using Patient Samples: Approved Guideline - Second Edition	Version Vol 22, No. 19	Date UNK
3	Standards No. EP05-A2	Standards Organization CSLI	Standards Title Evalution of Precision Performance of Clinical Chemistty Devices; Approved Guidelines	Version Vol 24, No. 25	Date Aug. 2004
4	Standards No. EP06-A	Standards Organization CSLI	Standards Title Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach: Approved Guidelines	Version Vol 23, No. 16	Date April 2003
5	Standards No. EP14-A2	Standards Organization CSLI	Standards Title Evaluation of Matrix Effects; Approved Guidelines	Version Vol 25, No. 4	Date Jan. 2005
6	Standards No.	Standards Organization	Standards Title	Version	Date
7	Standards No.	Standards Organization	Standards Title	Version	Date

Please include any additional standards to be cited on a separate page.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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SECTION C:

Table of Contents

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SECTION D:

Device Description

DEVICE DESCRIPTION

GENERAL DEVICE DESCRIPTION

The Bio-Rad D-100™ HbA1c on the D-100™ Hemoglobin Testing System utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative area percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c components which constitute the test is designed to be used on the D-100™ Hemoglobin Testing System.

SOFTWARE DESCRIPTION

The D-100 Hemoglobin Testing System software has been developed with “Moderate Concern” guidelines discussed in the United States Food and Drug Administration (May 11, 2005) *Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices* (online) http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089593.pdf as well as guidelines discussed in the following US FDA guidance documents-

- United States Food and Drug Administration (January 11, 2002) *General Principles of Software Validation; Final Guidance for Industry and FDA Staff* (online) http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085371.pdf

- United States Food and Drug Administration (September 9, 1999) Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices(online) http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073779.pdf

- United States Food and Drug Administration (October 2, 2014) Content of Premarket Submissions for Management of Cybersecurity in Medical Devices(online) http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM356190.pdf

All software documents required for the 510(k) submission are located in the Software Appendices in S to CC in Vol004.

KIT COMPONENTS DESCRIPTION

The following components are sold individually and used in combination to perform the D-100 HbA1c test:

Component part number	Quantity	Description
290-1004	1 each	**D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack**. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 ™ HbA_{1c} Calibrator Pack.** One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 ™ Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 ™ Cleaning Tube.** One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 ™ Sample Diluent.** Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 ™ HbA_{1c} Elution Buffer A.** Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 ™ HbA_{1c} Elution Buffer B.** Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 ™ HbA_{1c} Wash Solution.** Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

DEVICE TESTING

Safety and EMC testing reports can be found in Appendices DD to FF in Vol005.

SECTION E:

Proposed Intended

Use/Indications for Use

FORM FDA 3881

(b)(4) Confidential and Proprietary Information - Draft

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Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

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SECTION F:

NGSP Certification

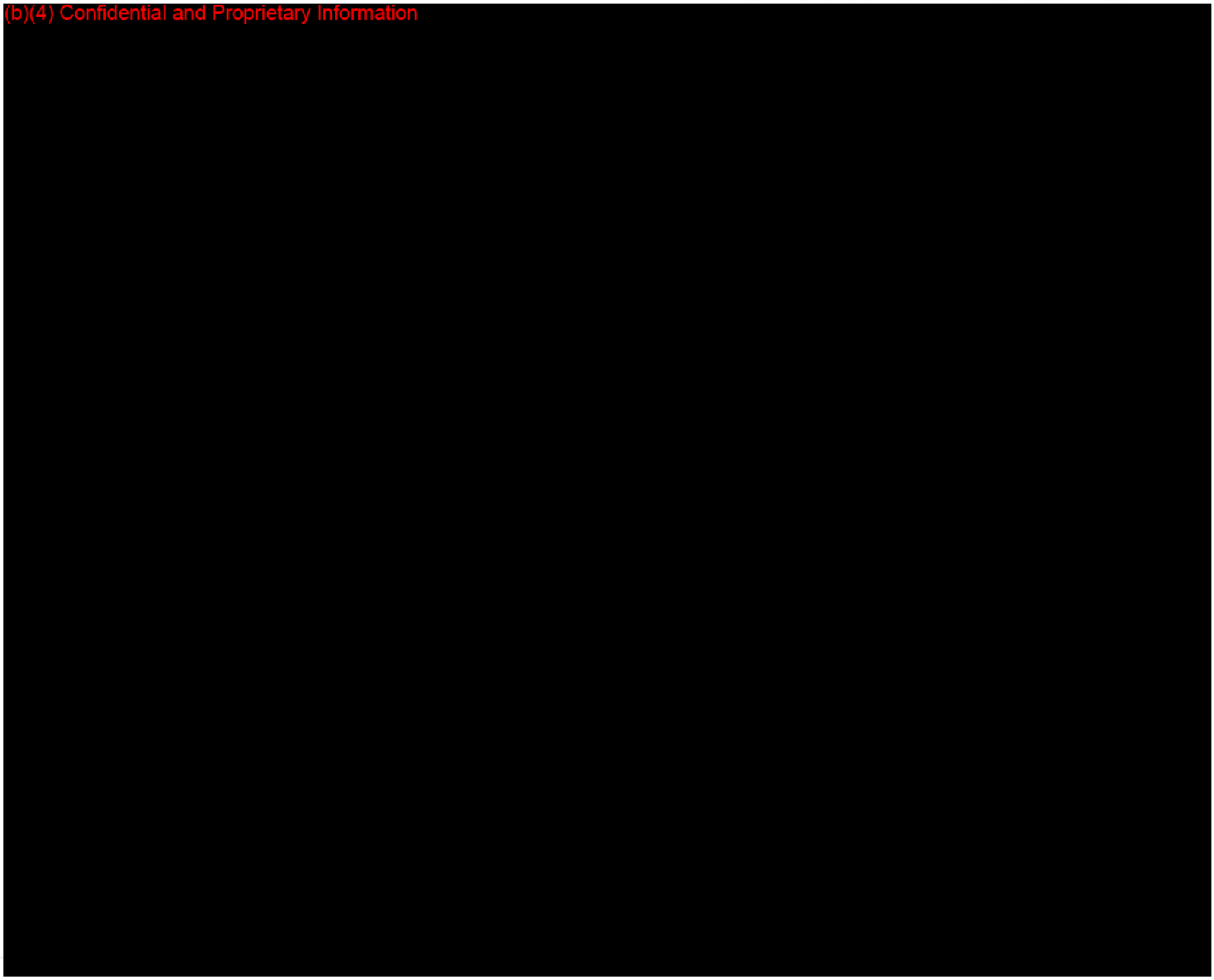
D-100™ HbA1c

NGSP CERTIFICATION: D-100™ HbA1c

The D-100 HbA1c test has completed the NGSP Certification of Traceability in 2014. This certification indicates that the Bio-Rad D-100™ HbA1c has met the requirements of the NGSP to produce Hemoglobin A1c test results standardized to those of the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) which established the direct relationships between HbA1c levels and outcome risks in patients with diabetes.

It is the intent of Bio-Rad Laboratories, Inc. to continue to use the NGSP Certification Program and to reapply when the current certification expires.

The most recent certification document for D-100™ HbA1c, valid through December 1 2015, is filed immediately following this sheet.



SECTION G:

Predicate Device Discussion

PREDICATE DEVICE

Predicate Device Trade Name: VARIANT™ II TURBO HbA1c Kit – 2.0

510(k) Number: K142448

Classification: Class II

Product Code: PDJ, LCP

Instrument: VARIANT™ II TURBO Hemoglobin Testing System

Justification for Predicate Device Choice:

1. The Intended Use and Indications for Use for the VARIANT II TURBO HbA1c Kit – 2.0 is the same as that proposed for the D-100™ HbA1c test, therefore, this previously cleared device is identified as a viable predicate device.

2. The technological characteristics of the VARIANT II TURBO HbA1c Kit – 2.0 and the D-100 HbA1c on the D-100™ Hemoglobin Testing System are the same. The D-100 HbA1c on the D-100 Hemoglobin Testing System and the VARIANT II TURBO HbA1c Kit – 2.0 on the VARIANT II TURBO Hemoglobin Testing System both utilize the principles of ion-exchange high-performance liquid chromatography (HPLC).

3. Both assays are standardized to NGSP test methods. This standardization assures that results from the VARIANT II TURBO HbA1c Kit – 2.0 and the D-100 HbA1c will correlate well. Test and predicate devices were not directly compared. The Test device was compared to standardized results from the NGSP network.

4. Elution Buffer A, Elution Buffer B, Wash Solution and Sample Diluent have the same reagent formulation in both the VARIANT II TURBO HbA1c Kit – 2.0 and D-100™ HbA1c. The Analytical cartridge chemistry is the same for both the VARIANT II TURBO HbA1c Kit – 2.0 and D-100™ HbA1c.

5. Performance characteristics for the VARIANT II TURBO HbA1c Kit – 2.0 and the D-100 HbA1c on the D-100 Hemoglobin Testing System both meet the criteria set forth in the Special Control Regulation for “Hemoglobin A1c Devices with a Diagnostic Claim” from the US Food and Drug Administration. Satisfying the same criteria for performance characteristics makes the VARIANT II TURBO HbA1c Kit – 2.0 an appropriate predicate device.

See **Appendix A** for a copy of the Instructions for Use for the predicate device VARIANT II TURBO HbA1c Kit – 2.0 IFU and the FDA 510(k) Summary for that device.

SECTION H:

510(K) Summary of Safety and Effectiveness Document

510(k) Summary (Summary of Safety and Effectiveness)

This Summary of 510(k) Safety and Effectiveness is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: _____.

Date Summary prepared: May 15, 2015

1. **Applicant Name:**
   Bio-Rad Laboratories, Inc.
   Clinical Diagnostics Group
   4000 Alfred Nobel Drive
   Hercules, California 94547

2. **Contact Person(s):**
   Jackie Buckley, Regulatory Affairs Representative IV
   Telephone Number: (510) 741-5309
   FAX: (510) 741-6471
   E-Mail: jackie_buckley@bio-rad.com

   Alfred Evans, RA/QA Director
   Telephone Number: (510) 741-4579
   FAX: (510) 741-6471
   E-Mail:al_evans@bio-rad.com

3. **Device Name/Trade Name:**
   Reagents:
   Trade Name: D-100™ HbA1c
   Classification Name: Assay, Glycosylated Hemoglobin
   Common Name: HbA1c
   Product Code: PDJ, LCP
   C.F.R Section: 21 CFR 862.1373
   Device classification: Class II
   Panel Classification: Chemistry

   Calibrators:
   Trade Name: D-100™ HbA1c Calibrator Pack
   Classification Name: Calibrator, Secondary
   Common Name: Calibrator
   Product Code: JIT
   C.F.R Section: 21 CFR 862.1150
   Device classification: Class II
   Panel Classification: Clinical Chemistry

   Tubes
   Trade Name: Hemoglobin Capillary Collection Kit
   Classification Name: Tubes, Vials, Systems, Serum Separators, Blood Collection
   Product Class: JKA
   C.F.R. Section: 21 CFR 862.1675
   Device classification: Class II

4. Predicate Device(s):

Predicate Device Name	Predicate Device 510(k) Number
VARIANT II TURBO HbA1c Kit -2.0	K142448
VARIANT II Hemoglobin A1c Calibrators	K070452
Hemoglobin Capillary Collection Kit	K142448

5. Description of the Device:

The Bio-Rad D-100™ HbA1c on the D-100™ Hemoglobin Testing System utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative area percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

The D-100™ HbA1c test is designed to be used on the D-100™ Hemoglobin Testing System.

Reagents:
The D-100™ HbA1c reagents contain the following components:

Description
D-100 ™ HbA_{1c} Analytical Cartridge/Calibrator Pack. One pack consisting of: ▪ Cation exchange cartridge. 10,000 tests each ▪ Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
D-100 ™ Prefilters. 2000 tests each. Package of 5.
D-100 ™ Cleaning Tube. One microvial containing 1.5 mL of a liquid cleaning solution.
D-100 ™ Sample Diluent. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.

D-100 ™ HbA_{1c} Elution Buffer A. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
D-100 ™ HbA_{1c} Elution Buffer B. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.1% sodium azide as a preservative.
D-100 ™ HbA_{1c} Wash Solution. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

Calibrator:
Each Calibrator Pack contains Calibrator values which have been value assigned using secondary calibrators that are traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

Description
D-100 ™ HbA_{1c} Calibrator Pack. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.

6. Indications for Use:

The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100™ Hemoglobin Testing System.

This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.

Measurement of hemoglobin A1c is effective in monitoring long-term glycemic control in individuals with diabetes mellitus.

The Bio-Rad D-100™ HbA1c test is intended for Professional Use Only.

Calibrators
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100™ Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c (HbA1c) in human whole blood.

Hemoglobin Capillary Collection System (HCCS)
The Hemoglobin Capillary Collection System (HCCS) is intended for the collection of human whole blood for the percent determination of hemoglobin A1c using Bio-Rad HPLC methods.

7. Substantial Equivalence Information:

Predicate Device Information:

Predicate Device Name	Predicate Device 510(k) Number
VARIANT™ II TURBO HbA1c Kit -2.0	K142448
VARIANT™ II Hemoglobin A1c Calibrators	K070452

The comparison of the technological characterizes of the D-100 HbA1c assay (candidate assay) utilizes principles of ion-exchange high-performance liquid chromatography (HPLC) similar to the same technology of the VARIANT II TURBO HbA1c Kit – 2.0 (predicate device).
Tables 1 & 2 provide the similarities and differences between the candidate assay and the predicate assay.

Table 1: Reagent Similarities and Differences

Reagent Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c**	**Predicate Device: VARIANT™ II TURBO HbA1c Kit – 2.0** (K)142448
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %)
Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Interval	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Specimen Type	Same	Human Whole blood
Assay Principle	Same	Ion exchange HPLC
Matrices	K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin Hemoglobin Capillary Collection Kit	K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Standardization	Same	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)

Table 2: Calibrator Similarities and Differences

Calibrator Similarities and Differences		
Features	**Candidate Device: D-100™ HbA1c Calibrator Pack**	**Predicate Device: VARIANT™ II Hemoglobin A1c Calibrators** (K) K070452
Intended Use	Same	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood
Levels	Same	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.
Standardization/Traceability	Same	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.

8. Summary of Nonclinical Performance Data:

a. Precision/Reproducibility:

The precision of the D-100™ HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were utilized in the study. In addition, five quality control materials were also tested. Precision was evaluated using three reagent lots and three D-100™ Hemoglobin Testing Systems. The samples were run in duplicate in 2 runs per day for 20 days.
NGSP results are shown in Tables 3-6. IFCC results are shown on Table 7-10.

Table 3: Instrument 1 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5	9.4	5.2	6.7	8.1	12.0	5.3	9.9	14.8
Repeatability	0.7	0.7	0.7	0.7	0.7	0.6	0.8	0.7	0.7
Between-Run	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.6	0.2	0.3	0.2	0.1	0.3	0.3	0.1	0.2
Between-Lot	1.2	0.8	1.3	1.1	1.0	0.6	1.4	0.8	0.6
Total Precision	1.5	1.1	1.5	1.3	1.2	0.9	1.6	1.0	0.9

Table 4: Instrument 2 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5	9.4	5.2	6.6	8.1	12.0	5.3	10.0	14.8
Repeatability	1.1	0.9	0.8	0.9	1.0	0.9	1.0	1.0	1.0
Between-Run	0.0	0.3	0.0	0.0	0.2	0.0	0.0	0.1	0.1
Between-Day	0.6	0.2	0.5	0.5	0.3	0.3	0.2	0.4	0.3
Between-Lot	1.2	0.2	1.5	0.6	0.0	0.3	1.5	0.1	0.5
Total Precision	1.7	1.0	1.8	1.2	1.0	1.0	1.8	1.1	1.1

Table 5: Instrument 3 (% CV by Sample (NGSP)

Variation Source	Instrument ID: SM98								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4	9.4	5.1	6.6	8.1	12.0	5.3	9.9	14.7
Repeatability	1.0	1.0	1.0	1.1	0.9	0.9	1.0	0.9	0.8
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.6	0.5	0.4	0.5	0.5	0.4	0.4	0.4	0.4
Between-Lot	1.6	1.0	1.5	1.5	1.2	1.1	1.7	1.0	1.1
Total Precision	1.9	1.4	1.9	2.0	1.6	1.5	2.0	1.4	1.4

Table 6: Instruments Combined (% CV by Sample (NGSP)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP %)	5.5	9.4	5.2	6.6	8.1	12.0	5.3	9.9	14.8
Repeatability	0.9	0.9	0.9	0.9	0.9	0.8	0.9	0.9	0.8
Between-Run	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Between-Day	0.6	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.4	0.7	1.5	1.1	0.9	0.7	1.5	0.7	0.8
Total Precision	1.7	1.2	1.7	1.5	1.3	1.2	1.8	1.2	1.2

Table 7: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID:SM93								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.5	79.3	33.2	49.4	65.4	108.1	34.5	85.0	137.7
Repeatability	1.2	0.9	1.2	1.0	0.9	0.8	1.3	0.8	0.8
Between-Run	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.9	0.3	0.5	0.4	0.2	0.4	0.6	0.2	0.3
Between-Lot	2.0	1.1	2.2	1.6	1.3	0.7	2.3	1.0	0.7
Total Precision	2.5	1.4	2.5	1.9	1.6	1.1	2.7	1.3	1.1

Table 8: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Variation Source	Instrument ID: SM94								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.4	79.2	32.9	49.0	65.1	108.0	34.5	85.4	137.9
Repeatability	1.7%	1.2%	1.4%	1.4%	1.3%	1.1%	1.7%	1.3%	1.1%
Between-Run	0.0%	0.4%	0.0%	0.0%	0.3%	0.0%	0.0%	0.1%	0.1%
Between-Day	1.0%	0.3%	0.8%	0.7%	0.4%	0.4%	0.3%	0.5%	0.4%
Between-Lot	2.1%	0.3%	2.6%	1.0%	0.0%	0.4%	2.5%	0.1%	0.6%
Total Precision	2.9%	1.4%	3.1%	1.8%	1.4%	1.2%	3.1%	1.4%	1.3%

Table 9: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	Instrument ID: SM95								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.0	79.1	32.7	48.9	64.9	107.7	34.1	84.8	137.6
Repeatability	1.6	1.2	1.8	1.7	1.3	1.1	1.6	1.2	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	1.0	0.6	0.8	0.8	0.7	0.5	0.7	0.5	0.5
Between-Lot	2.6	1.2	2.6	2.3	1.6	1.3	2.9	1.2	1.2
Total Precision	3.2	1.8	3.3	2.9	2.1	1.8	3.4	1.7	1.7

Table 10: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol)

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC)	36.3	79.2	33.0	49.1	65.1	107.9	34.3	85.1	137.8
Repeatability	1.5	1.1	1.5	1.4	1.2	1.0	1.5	1.1	1.0
Between-Run	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Between-Day	1.0	0.4	0.7	0.6	0.5	0.4	0.6	0.4	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.2	1.0	2.5	1.7	1.2	0.9	2.6	0.9	0.9
Total Precision	2.9	1.5	3.0	2.3	1.7	1.4	3.1	1.5	1.4

b. Linearity

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; A Statistical Approach. Linearity across the reportable range was performed using low (3.5%HbA1c) and high (20%HbA1c) EDTA whole blood patient samples. These samples were mixed together in varying ratios. The measured values were compared to the expected values based upon the dilution factor. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated.

% HbA1c (NGSP) using the D-100™ HbA1c test has been demonstrated linear from 3.5 – 20.0% HbA1c with the maximum measured difference of

± 0.09% between the predicted 1st and 2rd order results as shown in Table 11 below. Mmol/mol HbA1c (IFCC) has been demonstrated as linear from 15 – 195 mmol/mol with the maximum measured difference of ± 0.9% (or +/- 1.0 mmol/mol) as shown in Table 12 below.

Table 11: Results of Linearity Study (NGSP %)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Level 1	3.50	3.42	0.08
Level 2	5.16	5.13	0.03
Level 3	6.82	6.83	0.00
Level 4	8.48	8.51	-0.03
Level 5	10.14	10.19	-0.05
Level 6	11.79	11.85	-0.05
Level 7	13.45	13.49	-0.05
Level 8	15.10	15.13	-0.03
Level 9	16.75	16.75	-0.00
Level 10	18.40	18.36	0.04
Level 11	20.04	19.96	0.09

Table 12: Results of Linearity Study (IFCC mmol/mol)

Sample Pool	Predicted 1st Order	Predicted 2nd Order	Difference
Level 1	14.8	13.9	0.9
Level 2	32.9	32.6	0.3
Level 3	51.1	51.1	-0.3
Level 4	69.2	69.5	-0.3
Level 5	87.3	87.8	-0.5
Level 6	105.4	106.0	-0.6
Level 7	123.5	124.0	-0.5
Level 8	141.5	141.9	-0.4
Level 9	159.5	159.6	-0.1
Level 10	177.6	177.2	0.4
Level 11	195.6	194.6	1.0

c. Method Comparison

A Method comparison study was performed per CLSI EP09-A2, Method Comparison and Bias Estimation Using Patient Samples. 129 variant-free whole blood EDTA samples ranging from 3.4% to 19.2% (14-187 mmol/mol) HbA1c were evaluated using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System. Samples were tested in a single determination over a 4 day period. The results were compared to testing performed at a secondary NGSP SRL reference laboratory using a cleared HPLC-based HbA1c assay. The distribution of samples spanned the measuring interval listed in Table 13.

Table 13: Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100™ HbA1c versus the NGSP SRL reference method. Deming (weighted), Passing-Bablok and Linear regression analyses were performed for the D-100™ HbA1c on the D-100 Hemoglobin Testing System versus the reference G8 HPLC method and the results are presented in Table 14.

Table 14: Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	0.0223	-0.0684 - 0.1131	0.9867	0.9736 – 0.9999
Passing-Bablok	-0.0091	-0.0803 – 0.0763	0.9909	0.9789 – 1.0026

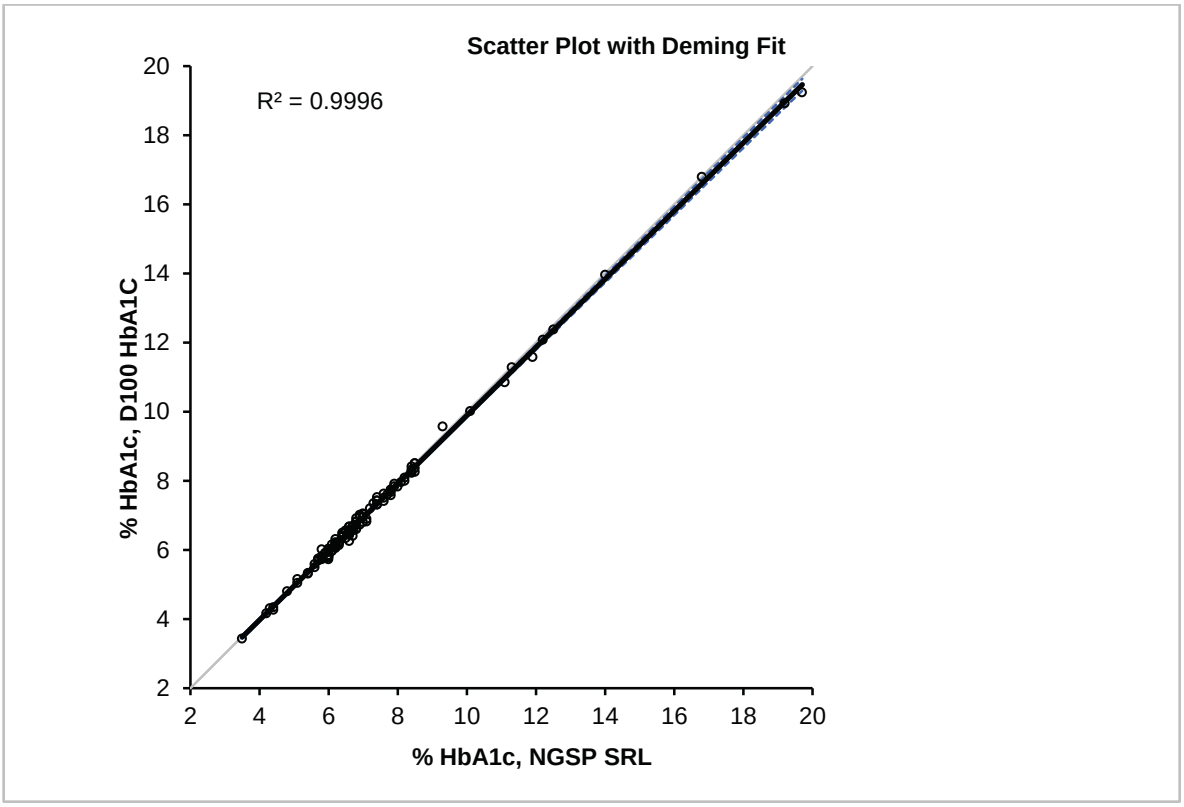


Figure 1: Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c.

(1) The following biases between D-100 HbA1c versus NGSP SRL Method (Reference method) were observed in Table 15.

Table 15: Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.042	-0.84
6.5	-0.063	-0.97
8.0	-0.085	-1.06
12.0	-0.142	-1.18

Total Error Decision Levels

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in the Table 16.

Table 16: Total Error Estimation

% A1c – Decision Level	% Absolute Bias	% CV	% TE
5.0	0.84	1.7	4.1
6.5	0.97	1.5	3.9
8.0	1.06	1.3	3.6
12.0	1.18	1.2	3.5

d. Traceability, Stability, Expected Values R e s u l t s

The D-100 HbA1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The D-100 HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org. The derived results of (%) from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

D-100 HbA1c Calibrator Pack:
Value assignment for D-100™ HbA1c Calibrators is traceable to IFCC reference method and can be transferred to DCCT/NGSP by calculation.

Stability:
Shelf life claims: Un-opened calibrators can be stored at 2-8°C until the expiration date or for 24 months.
Open-vial claims: The recommended storage condition for in-use calibrators is one day.
On-board stability claims: The recommended storage conditions for the D-100 HbA1c Calibrator Pack demonstrated 90 days stability.

e. Analytical specificity:

i.) Endogenous Interference

An Endogenous Interference study was performed per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of HbA1c of ~8.0%.

Conjugated bilirubin, unconjugated bilirubin and glucose, available in pure form, were obtained and stock solutions prepared at 10x the intended test concentration. The 10x stock solution of the test substance was pipetted into a low whole blood sample pool (at ~6.5% HbA1c) and a high whole blood sample pool (~8.0% HbA1c), making the test pool. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™Hemoglobin Testing System.

Rheumatoid factor, lipemia and total protein were not available as pure standards therefore serum samples with known concentration of these compounds were used. The test pool was prepared by mixing the serum sample known to have a high test substance concentration with a whole blood non-variant sample such that the concentration of test substance in the final mixture would be at the desired level. Ten replicates of each pool prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a ± 7% change in %HbA1c value from the control. Results in Table 17 showed no significant interference up to the stated concentrations.

Table 17: Endogenous Interference Study Results

	Concentration	
Endogenous substance	**Conventional (US) units**	**SI Units**
Lipemia (Intralipid)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/mL
Total protein	21 g/dL	210 g/L

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a low level whole blood sample with a concentration ~6.5%HbA1c and a high level whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in Table 18. Ten replicates of each drug prepared with the test and control samples were analyzed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in Table 18.

Table 18: Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference	
	Conventional (US) units	**SI units**
Acetylcysteine	166 mg/dL	10.2 mmol/L
Ampicillin-Na	1000 mg/dL	28.65 mmol/L
Ascorbic acid	300 mg/dL	17.05 mmol/L
Cefoxitin	2500 mg/dL	58.55 mmol/L
Heparin	5000 U/L	5000 U/L
Levodopa	20 mg/dL	1015 µmol/L
Methyldopa	20 mg/dL	948 µmol/L
Metronidazole	200 mg/dL	11.7 mmol/L
Doxycyclin	50 mg/dL	1124 µmol/L
Acetylsalicylic acid	1000 mg/dL	55.51 mmol/L
Rifampicin	64 mg/L	78 µmol/L
Cyclosporine	5 mg/L	4 µmol/L
Acetaminophen	200 mg/L	1323 µmol/L
Ibuprofen	500 mg/L	2427 µmol/L
Theophylline	100 mg/L	556 µmol/L
Phenylbutazone	400 mg/L	1299 µmol/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c

were evaluated using a low level whole blood EDTA sample with a concentration ~6.5%HbA1c and a high level whole blood EDTA sample with a concentration of ~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the low and high level blood samples and each sample was analyzed using ten replicates each in the same analytical run on the D-100™ Hemoglobin Testing System with the D-100™HbA1c.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- up to 50 mg/dL does not interfere with this assay.
- Carbamylated Hb – up to 5% does not interfere with this assay.
- Labile A1c- up to 7% glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using specific variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Two whole blood EDTA patient samples containing an HbA1c ~6.5% and ~ 9% and the appropriate hemoglobin variant were tested. Testing of the samples containing hemoglobin variants S, C, E, D , A2 and F were performed in duplicate. Testing of the samples was performed using the D-100™ HbA1c on the D-100™ Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent. Table 19 contains the number of samples, range of samples and concentration of samples used in the Hemoglobin Variant Study. Table 20 contains the results for the Hemoglobin Variant study bias.

Table 19: Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	20	28.7 – 40.2	5.6 – 9.6
HbC	20	34.4 – 44.1	5.0 – 10.7
HbD	20	36.6 – 43.4	5.8 – 8.6
HbE	20	25.5 – 32.5	5.9 – 8.3
HbA2	25	5.0 - 13.3	5.0 - 14.5
HbF	30	4.1 – 30.2	4.4 - 14.4

Table 20: Hemoglobin Variant Study Bias Results

Hemoglobin Variant	Percent Relative Bias to Comparative Method	
	HbA1c Concentration	
	~6.5%	~9.0%
HbS	-0.6	-1.5
HbC	-1.3	-3.9
HbD	-4.7	-4.4
HbE	-2.7	-1.3
HbA2	-1.3	3.4
HbF	-2.3	-3.5

2. Matrix comparison

The data supports the use of the following whole blood collection tubes in Table 2 and the Hemoglobin Capillary Collection System (HCCS).

Table 21: Anticoagulant

K_2-EDTA
K3-EDTA
Potassium Oxalate/Sodium Fluoride
Sodium Citrate
Sodium Heparin
Lithium Heparin

3. Expected Values/Reference Range
Hemoglobin A1c expected values range was cited from American Diabetes Association Standards of Medical Care in Diabetes 2010, 33 (Supplement 1), S62-S69 for Diagnosis of Diabetes.

Table 22: Hemoglobin A1c Expected Values

Hemoglobin A1c		**Suggested Diagnosis**
NGSP %	**IFCC mmol/mol**	
≥6.5	≥47	Diabetic
5.7 – 6.4	39-46	Pre-Diabetic
<5.7	<39	Non-Diabetic

Conclusion:

The information and data in this 510(k) document demonstrate that the D-100™ HbA1c test as performed on the D-100™Hemoglobin Testing System is an accurate, reliable, precise test that correlates well with current cleared methods and NGSP standardized testing for the quantitation of HbA1c. The contents of this submission demonstrates that the D-100™ HbA1c test as performed on the D-100™ Hemoglobin Testing System is substantially equivalent to its predicate device, VARIANT II TURBO HbA1c Kit – 2.0 and, therefore, safe and effective for its intended use. The performance criteria as stipulated by the Special Controls requirements for HbA1c systems that diagnose diabetes have clearly been met. The D-100™ HbA1c must be found to be substantially equivalent to the predicate and, therefore, cleared by the agency for the intended use requested.

SECTION I:

Draft Labeling for D-100

DRAFT Labeling

D-100™ HbA_{1c} and D-100™ Hemoglobin Testing System

Instructions for Use (IFU):

The DRAFT Instructions for Use (IFU) document for the D-100 HbA1c with the monitoring and diagnostic claim is filed in **Appendix B** in this document. Data presented in this DRAFT IFU represents information pertaining to the D-100 HbA1c and D-100 Hemoglobin Testing System.

Operation Manual:

Appendix C contains the DRAFT version of the Operation Manual for the D-100 Hemoglobin Testing System.

Quick Guide:

Appendix D contains the DRAFT version of the Quick Guide for the D-100 HbA1c.

Component Labels:

Appendix E contains the DRAFT labels for each component.

SECTION J:

Interference Studies

(b)(4) Confidential and Proprietary Information - Interference Studies

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SECTION U:

Standards Data Reports

Forms FDA 3654

Form Approved: OMB No. 0910-0120; Expiration Date: 1/31/2017

Department of Health and Human Services
Food and Drug Administration

STANDARDS DATA REPORT FOR 510(k)s

(To be filled in by applicant)

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

CLSI guidance document, EP05-A2, Vol. 24 No. 25, "Evaluation of Precision Performance of Quantitative Measurements Methods;Approved Guidelines - Second Edition"

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3]	#7-110	
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☐	☒
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☒	☐
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☐	☒
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☐	☒
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
Were there any exclusions from the standard? If yes, report these exclusions in the summary report table.	☐	☒
Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: Pre Submission Q140167

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

[3] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

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[6] The online search for CDRH Guidance Documents can be found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm

EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
CLSI guidance document, EP05-A2, Vol. 24 No. 25, "Evaluation of Precision Performance of Quantitative Measurements Methods;Approved Guidelines - Second Edition"

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
All		☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION
Followed the recommendation of the guideline to design the study necessary to evaluate precision.

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.

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Department of Health and Human Services
Food and Drug Administration

STANDARDS DATA REPORT FOR 510(k)s

(To be filled in by applicant)

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

CLSI guidance document, EP06-A, Vol 23, No. 16 (Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach: Approved Guideline)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3]	#7-193	
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☐	☒
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☒	☐
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☐	☒
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☐	☒
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
Were there any exclusions from the standard? If yes, report these exclusions in the summary report table.	☐	☒
Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: Pre Submission Q140167

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

[3] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
CLSI guidance document, EP06-A, Vol 23, No. 16 (Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach: Approved Guideline)

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
All		☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION
Followed the recommendation of the guideline to design the study necessary to evaluate Linearity.

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.

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Department of Health and Human Services
Food and Drug Administration

STANDARDS DATA REPORT FOR 510(k)s

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

CLSI guidance document, EP07-A2 (Interference Testing in Clinical Chemistry: Approved Guideline- Second Edition)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3]	#7-127	
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☐	☒
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☒	☐
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☐	☒
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☐	☒
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
Were there any exclusions from the standard? If yes, report these exclusions in the summary report table.	☐	☒
Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: Pre Submission Q140167

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

[3] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
CLSI guidance document, EP07-A2 (Interference Testing in Clinical Chemistry: Approved Guideline- Second Edition)

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
All		☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *
Did not use the number of replicates calculated per section 7.1.3.4 (<3)

DESCRIPTION
Followed the recommendation of the guideline to design the study necessary to evaluate interference.

JUSTIFICATION
Used 10 replicates per FDA recommendation (Pre-Submission Q140167)

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.

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Food and Drug Administration

STANDARDS DATA REPORT FOR 510(k)s

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

CLSI guidance document, EP09-A2-IR, Vol. 30 No. 17 (Method Comparison and Bias Estimation Using Patient samples; Approved Guideline - Second Edition)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3] #		
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☐	☒
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☒	☐
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☐	☒
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☐	☒
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
Were there any exclusions from the standard? If yes, report these exclusions in the summary report table.	☐	☒
Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: Pre Submission Q140167

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

[3] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE

CLSI guidance document, EP09-A2-IR, Vol. 30 No. 17 (Method Comparison and Bias Estimation Using Patient samples; Approved Guideline - Second Edition)

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
All		☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

Followed the recommendation of the guideline to design the study necessary to evaluate method comparison and bias estimation between test and reference methods

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

CLSI guidance document, EP14-A2, Vol 25, No 4 (Evaluation of Matrix Effect: Approved Guideline-Second Edition)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3]	#7-143	
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☐	☒
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☒	☐
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☐	☒
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☐	☒
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
Were there any exclusions from the standard? If yes, report these exclusions in the summary report table.	☐	☒
Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: Pre Submission Q140167

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

[3] http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm

[4] The summary report should include: any adaptations used to adapt to the device under review (for example, alternative test methods); choices made when options or a selection of methods are described; deviations from the standard; requirements not applicable to the device; and the name and address of the test laboratory or certification body involved in conformance assessment to this standard. The summary report includes information on all standards utilized during the development of the device.

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[6] The online search for CDRH Guidance Documents can be found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm

EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
CLSI guidance document, EP14-A2, Vol 25, No 4 (Evaluation of Matrix Effect: Approved Guideline-Second Edition)

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
All		☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION
Followed the recommendation of the guideline to design the study necessary to evaluate matrix effect.

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED *

DESCRIPTION

JUSTIFICATION

* For completeness list all sections of the standard and indicate whether conformance is met. If a section is not applicable (N/A) an explanation is needed under "justification." Some standards include options, so similar to deviations, the option chosen needs to be described and adequately justified as appropriate for the subject device. Explanation of all deviations or description of options selected when following a standard is required under "type of deviation or option selected," "description" and "justification" on the report. More than one page may be necessary.

* Types of deviations can include an exclusion of a section in the standard, a deviation brought out by the FDA supplemental information sheet (SIS), a deviation to adapt the standard to the device, or any adaptation of a section.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 1 hour per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

(b)(4) Confidential and Proprietary Information - Studies - Section V

(b)(4) Confidential and Proprietary Information - Studies - Section V

(b)(4) Confidential and Proprietary Information - Studies - Section V

(b)(4) Confidential and Proprietary Information - Studies - Section V

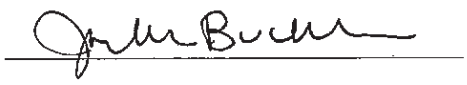
SECTION W:

Truthful and Accurate Statement

Premarket Notification Truthful And Accurate Statement

[As Required by 21 CFR 807.87(k)]

I certify that, in my capacity as Regulatory Affairs Representative of *Bio-Rad Laboratories, Inc.*, I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.



(Signature)

Jackie Buckley

(Typed Name)

15 May 2015

(Date)

Premarket Notification [510(k)] Number:_____

SECTION X:

Medical Device User Fee Cover Sheet

Form Approved: OMB No. 0910-0511 Expiration Date: April 30, 2016. See instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
MEDICAL DEVICE USER FEE COVER SHEET

PAYMENT IDENTIFICATION NUMBER: (b) (4)
Write the Payment Identification number on your check.

A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at: http://www.fda.gov/oc/mdufma/coversheet.html

1. COMPANY NAME AND ADDRESS (Include name, street address, city state, country, and post office code)

BIO RAD LABORATORIES INC
4000 Alfred Nobel Dr
Hercules
CA 94547
US

1.1 EMPLOYER IDENTIFICATION NUMBER (EIN)
*****1833

2. CONTACT NAME
Jackie Buckley
2.1 E-MAIL ADDRESS
jackie_buckley@bio-rad.com
2.2 TELEPHONE NUMBER (Include Area code)
510-7415309
2.3 FACSIMILE (FAX) NUMBER (Include Area code)

3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm345263.htm

Select an application type:
[X] Premarket notification(510(k)); except for third party
[ ] 513(g) Request for Information
[ ] Biologics License Application (BLA)
[ ] Premarket Approval Application (PMA)
[ ] Modular PMA
[ ] Product Development Protocol (PDP)
[ ] Premarket Report (PMR)
[ ] 30-Day Notice

3.1 Select a center
[X] CDRH
[ ] CBER

3.2 Select one of the types below
[X] Original Application

Supplement Types:
[ ] Efficacy (BLA)
[ ] Panel Track (PMA, PMR, PDP)
[ ] Real-Time (PMA, PMR, PDP)
[ ] 180-day (PMA, PMR, PDP)

4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status)
[ ] YES, I meet the small business criteria and have submitted the required qualifying documents to FDA
[X] NO, I am not a small business
4.1 If Yes, please enter your Small Business Decision Number:

5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA?
[X] YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.)
[ ] NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see http://www.fda.gov/cdrh/mdufma for additional information)

6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION.
[ ] This application is the first PMA submitted by a qualified small business, including any affiliates
[ ] The sole purpose of the application is to support conditions of use for a pediatric population
[ ] This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only
[ ] The application is submitted by a state or federal government entity for a device that is not to be distributed commercially

7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA).
[ ] YES [X] NO

PAPERWORK REDUCTION ACT STATEMENT
Public reporting burden for this collection of information is estimated to average 16 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the address below.

Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 8455 Colesville Road, COLE-14-14253 Silver Spring, MD 20993-0002
[Please do NOT return this form to the above address, except as it pertains to comments on the burden estimate.]

8. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION
(b) (4)

22-Jan-2015

Form FDA 3601 (05/13)

"Close Window" Print Cover sheet

Jackie Buckley
22 Jan 2015

SECTION Y:

Certificate of Compliance

Form FDA 3674

Form Approved: OMB No. 0910-0616. Expiration Date: 2/28/2018. See PRA Statement on page 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance

Under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. Name of Sponsor/Applicant/Submitter
Bio-Rad Laboratories, Inc.

2. Date of the Application/Submission Which This Certification Accompanies
April 15, 2015

3. Address

Address 1 *(Street address, P.O. box, company name c/o)*
4000 Alfred Nobel Dr.

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City	State/Province/Region	ZIP or Postal Code
Hercules	CA	94547

Country: USA

4. Telephone and Fax Numbers *(Include country code if applicable and area code)*
(Tel): 510-741-5309
(Fax): 510-741-3954

PRODUCT INFORMATION

5. **For Drugs/Biologics:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s).
For Devices: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)

Trade Name- D-100 HbA1c
Model Numbers - 290-1004 D-100 HbA1c Analytical Cartridge/Calibrator Pack
290-1007 D-100 Prefilters, 290-1009 D-100 Sample Diluent, 290-1010 D-100 Elution Buffer A, 290-1011 D-100 HbA1c Elution B

290-1012 D-100 Wash Solution

Continuation Page for #5

APPLICATION / SUBMISSION INFORMATION

6. Type of Application/Submission Which This Certification Accompanies

☐ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☒ 510(k) ☐ PDP ☐ Other

7. Include IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/ Other Number *(If number previously assigned)*

If BLA was selected in item 6, provide Supplement Number

8. Serial Number Assigned to Application/Submission Which This Certification Accompanies

CERTIFICATION STATEMENT / INFORMATION

9. Check only one of the following boxes *(See instructions for additional information and explanation)*

☒ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

☐ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

☐ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

Certification Statement / Information section continued on page 2

CERTIFICATION STATEMENT / INFORMATION *(Continued)*

10. If you checked box C, in number 9, provide the National Clinical Trial (NCT) Number(s) for any "applicable clinical trial(s)," under 42 U.S.C. § 282(J)(1)(a)(i), section 402(j)(1)(a)(i) of the Public Health Service Act, referenced in the application/ submission which this Certification accompanies. *(Add continuation page as necessary.)*

NCT Number(s): _____ _____ _____ _____ _____

Continuation Page for #10

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.

Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. Name and Title of the Person who Signs Number 15

Name	Title
Jackie Buckley	Regulatory Affairs Rep IV

12. Address

Address 1 *(Street address, P.O. box, company name c/o)*	
4000 Alfred Nobel Dr.	
Address 2 *(Apartment, suite, unit, building, floor, etc.)*	
City: Hercules	State/Province/Region: CA
Country: USA	ZIP or Postal Code: 94547

13. Telephone and Fax Numbers *(Include country code if applicable and area code)*

(Tel): 510-741-5309

(Fax): 510-741-3954

14. Date of Certification

05/06/2015

15. Signature of Sponsor/Applicant/Submitter or an Authorized Representative *(Sign)*

Sign

Jackie Buckley

Digitally signed by Jackie Buckley
DN: dc=com, dc=Bio-Rad, dc=Global, ou=North America, ou=NorCal, ou=CDG, ou=Users, ou=Standard Users, cn=Jackie Buckley
Date: 2015.05.06 17:19:13 -07'00'

This section applies only to requirements of the Paperwork Reduction Act of 1995.

*****DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.*****

The burden time for this collection of information is estimated to average 15 minutes and 45 minutes (depending on the type of application/ submission) per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

SECTION Z:

Refuse to Accept Checklist

Acceptance Checklist for Traditional 510(k)s				
Preliminary Questions	Yes	N/A	No	Further Information
1. Is the product a device or a combination product?	X			Device
2. Is the application with the correct center?	X			CDRH-Chemistry (75)
3. Is a Request for a Designation (RFD) submitted with device?			X	
4. Is this type of device eligible for 510(k) submission?	X			
5. Is there a pending PMA for the same device with the same Indication for Use?			X	
6. If clinical studies were submitted , is the submitter subject to AIP?		X		
Organizational Elements				
a. Submission contains Table of Contents	X			Pages 14- 17
b. Each section is labeled	X			
c. All submission pages are numbered	X			
d. Type of 510(k) is identified?	X			Traditional
Elements of a Complete Submission (RTA Items):				
A. Administrative				
1. All content is written in English	X			
2. 510(k) Cover letter identifies:				
a. Device trade name or proprietary name	X			Page 30
b. Device common name	X			Page 30
c. Device class and panel	X			Page 30
3. Submission contains Indication for Use Statement	X			Page 32
4. Submission contains 510(k) Summary or 510(k) Statement	X			Pages 30- 45
a. Summary contains all elements of 21 CFR 807.92	X			Pages 30 - 45
b. Statement contains all elements per 21 CFR 807.93		X		
5. Submission contains Truthful and Accurate Statement per 21 CFR 807.87(k)	X			Page 167
6. Submission contains Class III Summary and Certification		X		
7. Submission contains clinical data		X		No clinical data used
8. If submission relies upon a national or international standard as part of demonstration of substantial equivalence, submission contains Standard Data Report for 510(k), form 3654	X			Pages 151 - 161

9. The submission identifies prior submissions for the same device for which the FDA provided feedback related to the data or information to support substantial equivalence.		X		
a. If there were prior submissions, the submitter has identified where in the current submission any issue related to a determination of substantial equivalence outlined in prior communications are addressed.		X		
B. Device Description				
10a. If there are requirements regarding the device description, such as special controls, in a device-specific guidance documents that are applicable to the device, the submission includes device description information to establish that the submitter has followed the device-specific requirement.	X			Pages 4 – 5 and page 27
10b. If there is a device-specific guidance document or special controls applicable to this submission, documentation has been provided to establish that the submitter has followed the recommendation in the applicable device-specific guidance documents or special controls regarding the device description or otherwise met the applicable statutory or regulatory criteria through an alternative approach.	X			Pages 4 – 5 and page 27
11. All descriptive information is present and consistent within the submission	X			Pages 18 - 20 Appendix C-1
a. A description of the principle of operation and mechanism of action for achieving the intended therapeutic diagnostic effect.	X			Pages 18 - 20 Appendix C-1
b. A description of proposed conditions for use.	X			Appendix B-1
c. A list and description of each model for which clearance is requested.	X			Appendix B-1, C-1

12. Submission contains illustrations of the device that are clear, labeled, and include dimensions.	X			Appendix C-1
13. If device is intended to be marketed with multiple components.				
a. List of all components and accessories to be marketed with the subject device.	X			Appendices B-1 and C-1
b. A description of all components	X			Appendices B-1 and C-1
c. A 510(K) number is provided for each component or accessory that received a prior 510(K) clearance.		X		
C. Substantial Equivalence Discussion				
14. Submitter has identified a predicate device				
a. Predicate's 510(k) number, trade name, and model number	X			Page 10 and Page 30
b. The identified predicates is consistent throughout submission	X			Page 10 and Page 30
15. Includes a comparison of the following for the predicate and subject device				
a. Indications for use	X			Pages 34 and 35

b. Technology, including features, materials, and principles of operation	X			Page 18 to 20 and Appendix C-1
16. Submission includes an analysis of why any differences between the subject device and predicates do not render the device NSE, affect safety or effectiveness, or raise questions of safety and effectiveness.	X			Page 27 to 28 and pages 34 - 35
D. Proposed Labeling				
17. Submission includes proposed package labels and labeling that include a description of the device, its intended use, and the directions for use.	X			Appendices A, B, C, D and E
a. Indications for use are stated in labeling and are identical to Indications for Use form and 510(k) Summary	X			Appendix B-1
b. Submission includes directions for use that -includes statements of all conditions, purposes for use	X			Appendix B-1, C-1
18. If indicated for prescription use, labeling includes the prescription use statement or "Rx only" symbol	X			Appendices B-1, C-1
19. General labeling provisions				
a. Labeling includes name and place of business of the manufacturer packer, or distributor	X			Appendices B-1, C-1 and E-1
b. Labeling includes common or usual name	X			Appendices B-1 & E-1
20a. If there are requirements regarding labeling, such as special controls, in a device-specific guidance documents that are applicable to the device, the submission includes labeling information to establish that the submitter has followed the device-specific requirement.	X			Appendix B-1
20b. If there is a device-specific guidance document or special controls applicable to this submission, documentation has been provided to establish that the submitter has followed the recommendation in the applicable device-specific guidance documents or special controls regarding the labeling or otherwise met the applicable statutory or regulatory criteria through an alternative approach.	X			Appendix B-1
20c. If there is a special controls applicable to this device, the submission includes labeling to establish that the submitter has complied with particular mitigations.	X			Appendix B-1

20d. Predicate Labeling – VARIANT II TURBO HbA1c Kit – 2.0 IFU	X			Appendix A-1
E. Sterilization				
For in vitro diagnostic devices and sterilization is no applicable, select N/A.		X		
F. Shelf Life				
a. Proposed shelf life/expiration date stated	X			Section S, page 117 to 144
b. For sterile device, summary of methods used for device sterility		X		
c. Summary of methods used to establish that device performance is not adversely affected by aging	X			Section S, page 127 to 130
G. Biocompatibility				
For in vitro diagnostic device, select N/A.		X		
H. Software				
Submission does contain software/firmware.	X			
32. Submission includes a statement of software level of concern and rationale for the software level of concern.	X			Appendix S-1 to S-2
33. All applicable software documentation provided based on level of concern identified by submitter, as described in the Guidance for the Content of Premarket Submissions for Software Contained in a Medical Devices or the submission includes information to establish that the submitter has otherwise met applicable statutory or regulatory criteria through an alternative approach.	X			Appendices S-1 to BB
I. EMC and Electrical Safety				
Submission states that the device does require EMC and Electrical Safety evaluation.				
34. Submission includes evaluation of electrical safety	X			Appendix DD and FF
35. Submission includes evaluation of EMC	X			Appendix EE
J. Performance Data-General				
36. A full test report is provide for each completed test.	X			Sections J to Section S
37a. If there are requirements regarding performance data, such as special controls, in a device specific regulation that are applicable.	X			Sections J, K, L, M, N, O, P, Q, R and S
37b. If there is a device-specific guidance, other than special controls guidance document, applicable to the device, the submission includes performance data to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.		X		
37c. If there is a special control document applicable to device and submitter compiled mitigations.		X		

38. If literature is referenced in the submission, submission includes				
a. Legible reprints or a summary of each article	X			Available upon request
b. Discussion of how each article is applicable to support the substantial equivalence of the subject device to the predicate		X		
39. Nonclinical studies conducted		X		
K. Performance Characteristics- In Vitro Diagnostic Devices				
Submission does indicate that device is an IVD.	X			
40. Submission includes the following studies, as appropriate for the device type:				
a. Precision/reproducibility	X			Section M
b. Accuracy	X			Section N
c. Sensitivity	X			Section P
d. Specificity	X			Sections J, K, L
41a. If there are requirements regarding performance data, such as special controls, in a device-specific guidance documents that are applicable to the device, the submission includes performance data information to establish that the submitter has followed the device-specific requirement.	X			Sections K, L, M and N
41b. If there is a device-specific guidance document or special controls applicable to this submission, documentation has been provided to establish that the submitter has followed the recommendation in the applicable device-specific guidance documents or special controls regarding the performance data or otherwise met the applicable statutory or regulatory criteria through an alternative approach.	X			Sections K, L, M and N
41c. If there is a special controls document applicable to the device, the submission includes performance data to establish that the submitter has complied with the particular mitigation.		X		

TABLE of CONTENTS

SECTION ZZ:

Appendices

Appendix A:

Predicate Device 510(k) Summary

Predicate Device IFU

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K142448

B. Purpose for Submission:

Addition of a diagnostic claim to an existing device

C. Measurand:

Whole Blood Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Ion-exchange high-performance liquid chromatography (HPLC)

E. Applicant:

Bio-Rad Laboratories, Inc

F. Proprietary and Established Names:

VARIANT II TURBO HbA1c Kit – 2.0
Hemoglobin Capillary Collection System

G. Regulatory Information:

Regulatory Description	Classification	Regulation	Product Code	Panel
Hemoglobin a1c Test System	II	21 CFR 862.1373	PDJ	Chemistry, 75
Tubes, vials, systems, serum separators, blood collection	II	21 CFR 862.1675	JKA	Chemistry, 75

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The VARIANT II TURBO HbA1c Kit – 2.0 is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the VARIANT™ II TURBO Hemoglobin Testing System and VARIANT II TURBO Link Hemoglobin Testing System.

   This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.

   The VARIANT™ II TURBO HbA1c Kit – 2.0 is intended for Professional Use Only.

   The Hemoglobin Capillary Collection System (HCCS) is intended for the collection of human whole blood for the percentage determination of hemoglobin A1c using Bio-Rad HPLC methods

3. Special conditions for use statement(s):

   The HbA1c test is not intended for analysis of samples collected from newborns.

   The HbA1c test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.

   The HbA1c test should not be used to diagnose diabetes during pregnancy.

   The HbA1c test should not be used to diagnose diabetes in patients with the following conditions:

   - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis), or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA1c values.

   - Malignancies or severe chronic hepatic and renal disease.

- In cases of rapidly evolving type 1 diabetes the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.

- Hemoglobin A1c should not be used in the diagnosis of gestational diabetes.

For prescription use only.

4. Special instrument requirements:

All performance data was conducted using the Bio-Rad VARIANT II TURBO Hemoglobin Testing System. The VARIANT II TURBO and the VARIANT II TURBO Link Systems are identical with respect to all operational and system components (See Device Description below).

I. Device Description:

The Bio-Rad VARIANT II TURBO HbA1c Kit – 2.0 contains the following supplies for 2500 tests:

Quantity	Description
2 each	Whole Blood Primer. Each vial contains lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 1.0 mL per vial.
5 each	Elution Buffer A. Each bottle contains 2500 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
1 each	Elution Buffer B. Each bottle contains 2000 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
1 each	Calibrator/Diluent Set. One set consisting of 2 vials of Calibrator Level 1, 2 vials of Calibrator Level 2, and 1 bottle of Calibrator Diluent. The calibrator vials contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Calibrator Diluent contains 100 mL of deionized water with <0.05% sodium azide as a preservative.
1 each	CD with VARIANT II TURBO HbA1c Kit - 2.0 test parameters.

1 each	Analytical Cartridge. Cation exchange cartridge (2500 tests), 4.6 mm ID x 27.5 mm. 5 prefilters (500 tests each) are included with the cartridge.
1 each	Sample Vials. 100 polypropylene microvials with pierceable caps, 1.5 mL.

The Calibrators and the Whole Blood Primer contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives.

Each unit of whole blood used in the manufacture of the calibrators and whole blood primer was tested by FDA accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis.

Calibrator Level 1, Calibrator Level 2 were previously cleared in k070452.

The VARIANT II TURBO HbA1c Kit – 2.0 is designed to be used on the standalone VARIANT II TURBO and the VARIANT II TURBO Link Hemoglobin Testing Systems. VARIANT II TURBO and the VARIANT II TURBO Link are identical with respect to all operational and system components. Physically, VARIANT II TURBO Link VSS outer case is modified for compatibility with a track system. In addition, the barcode reader, tube spinner and tube sensor are controlled by the line system in the VARIANT II TURBO Link Hemoglobin Testing System. Functionality on the VARIANT II TURBO Link has not changed, just the physical orientation to accommodate sample tube management.

The hemoglobin Capillary Collection System (HCCS) contains a combination of the following components:

- Sample Preparation Vials – clear microvials with blue pierceable caps, each contains 1.5 mL of HCCS reagent (aqueous solution of EDTA and potassium cyanide (.25mmol/L). The microvials are 11 mm x 40 mm and have a maximum volume of 2.0 mL.
- Capillaries – plastic capillaries (5 µL) in a dispenser
- Capillary holder – holder for manipulating the capillaries
- Labels – to label prepared samples

J. Substantial Equivalence Information:

1. Predicate device name(s):

k121291

2. Predicate 510(k) number(s):

   Roche COBAS INTEGRA 800 Tina-quant HbA1c DX Gen. 2 assay

3. Comparison with predicate:

Similarities		
Item	Candidate Device: VARIANT II TURBO HbA1c Kit – 2.0	Predicate Device: COBAS INTEGRA 800 HbA1c DX Gen. 2
Intended Use	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood. This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.	Same
Measuring Range	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)	4.3 – 24.8% (NGSP) 23 to 258 mmol/mol HbA1c (IFCC)
Traceability	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)	Same

Differences		
Item	Candidate Device: VARIANT II TURBO HbA1c Kit-2.0	Predicate Device: COBAS INTEGRA 800 HbA1c DX Gen.2
Sample Types	K_2-EDTA, K_3-EDTA, Capillary blood in Hemoglobin Capillary Collection System (HCCS)	K_2-EDTA, K_3-EDTA, KF/Na2- EDTA, Na-heparin NF/K-oxalate NF/NA2- EDTA Li-Heparin
Instrument Platform	VARIANT™ II TURBO Hemoglobin Testing System and VARIANT™ II TURBO Link Hemoglobin Testing System	Roche COBAS INTEGRA 800 analyzer
Assay Principal	Ion exchange HPLC	Turbidimetric inhibition immunoassay

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition
CLSI EP9-A2-IR: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition
CLSI EP5-A2: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline
CLSI EP6-A: Evaluation of the Linearity of Quantitative Measuring Procedures: A Statistical Approach; Approved Guideline
CLSI EP14-A2: Evaluation of Matrix Effects; Approved Guideline

L. Test Principle:

The test principal of the device is based on chromatographic separation of HbA1c on a cation exchange cartridge. The various forms of hemoglobin exhibit charge differences (positive) at the acidic pH of the mobile phase, and thus can be separated on a support that is negatively charged (cation exchange). The use of ion-exchange chromatography then allows molecules to be separated based upon a molecule's charge. Separation is optimized to minimize interferences from hemoglobin variants (HbS, HbC, HbD and HbE trait), labile A1c, hemoglobin F and carbamylated hemoglobin.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the VARIANT™ II TURBO HbA1c Kit – 2.0 was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four EDTA whole blood samples at the following targeted HbA1c concentrations of ~5%, ~6.5%, ~8% and ~12% were analyzed in the study. In addition, five whole blood quality control materials (Control 1, Control 2, QC1, QC2, QC3) were also tested. Precision was evaluated using three reagent lots and three VARIANT™ II TURBO Hemoglobin Testing Systems at two different sites. The samples were run in duplicate in 2 runs per day for 20 days. For each sample. there were 720 measurements. Results are shown in the tables below.

Results in NGSP Units:

Instrument 1 (% CV by Sample (NGSP))

Variation Source	Instrument ID: VART15								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5	9.9	5.1	6.7	8.0	12.0	5.5	9.9	15.0
Repeatability	0.5%	0.3%	0.5%	0.6%	1.0%	0.3%	0.6%	0.4%	0.4%
Between-Run	0.4%	0.0%	0.3%	0.0%	0.2%	0.3%	0.3%	0.3%	0.2%
Between-Day	0.8%	0.6%	0.8%	0.7%	0.6%	0.5%	1.2%	0.8%	0.6%
Between-Lot	0.8%	0.6%	1.0%	0.8%	0.6%	0.6%	1.0%	0.5%	0.2%
Total Precision	1.4%	0.9%	1.4%	1.2%	1.3%	0.9%	1.7%	1.0%	0.8%

Instrument 2 (% CV by Sample (NGSP))

Variation Source	Instrument ID: VART17								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.5	9.8	5.1	6.6	7.9	2.0	5.5	9.9	14.9
Repeatability	0.5%	0.3%	0.6%	0.6%	0.5%	0.4%	0.6%	0.5%	0.4%
Between-Run	0.4%	0.2%	0.5%	0.0%	0.3%	0.4%	0.0%	0.2%	0.3%
Between-Day	0.5%	0.3%	0.4%	0.5%	0.7%	0.3%	0.9%	0.7%	0.4%
Between-Lot	0.9%	0.7%	0.9%	0.7%	0.6%	0.4%	1.3%	0.6%	0.3%
Total Precision	1.2%	0.9%	1.3%	1.0%	1.1%	0.8%	1.7%	1.1%	0.8%

Instrument 3 (% CV by Sample (NGSP))

Variation Source	Instrument ID: VartGerm01								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4	9.7	5.1	6.6	8.0	12.1	5.4	9.8	15.0
Repeatability	0.6%	0.5%	0.8%	0.8%	0.5%	0.4%	0.8%	0.4%	0.4%
Between-Run	0.2%	0.0%	0.1%	0.0%	0.0%	0.2%	0.0%	0.2%	0.0%
Between-Day	0.6%	0.3%	0.6%	0.5%	0.5%	0.4%	0.7%	0.4%	0.3%
Between-Lot	2.0%	0.9%	1.6%	1.4%	1.0%	0.7%	2.2%	1.1%	0.7%
Total Precision	2.2%	1.1%	1.9%	1.7%	1.3%	0.9%	2.5%	1.2%	0.9%

Instruments Combined (% CV by Sample (NGSP))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (NGSP%)	5.4	9.8	5.1	6.6	7.9	12.1	5.4	9.9	15.0
Repeatability	0.5%	0.4%	0.7%	0.7%	0.7%	0.4%	0.7%	0.5%	0.4%
Between-Run	0.3%	0.0%	0.4%	0.0%	0.2%	0.3%	0.2%	0.2%	0.2%
Between-Day	0.7%	0.4%	0.6%	0.5%	0.6%	0.4%	1.0%	0.7%	0.5%
Between-Instrument	1.3%	1.1%	0.4%	0.0%	0.4%	0.6%	0.8%	0.4%	0.0%
Between-Lot	1.4%	0.8%	1.2%	1.0%	0.8%	0.6%	1.6%	0.7%	0.5%
Total Precision	2.1%	1.5%	1.6%	1.3%	1.3%	1.1%	2.2%	1.2%	0.8%

Results in IFCC Units:

Instrument 1 (% CV by Sample (IFCC))

Variation Source	Instrument ID: VART15								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC%)	5.5	9.9	5.1	6.7	8.0	12.0	5.5	9.9	15.0
Repeatability	0.5%	0.3%	0.5%	0.6%	1.0%	0.3%	0.6%	0.4%	0.4%
Between-Run	0.4%	0.0%	0.3%	0.0%	0.2%	0.3%	0.4%	0.3%	0.2%
Between-Day	0.8%	0.6%	0.8%	0.7%	0.6%	0.5%	1.2%	0.8%	0.6%
Between-Lot	0.8%	0.6%	1.0%	0.8%	0.6%	0.6%	1.0%	0.5%	0.2%
Total Precision	1.4%	0.9%	1.4%	1.2%	1.3%	0.9%	1.7%	1.0%	0.8%

Instrument 2 (% CV by Sample (IFCC))

Variation Source	Instrument ID: VART17								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC%)	5.5	9.8	5.1	6.6	7.9	2.0	5.5	9.9	14.9
Repeatability	0.5%	0.3%	0.6%	0.6%	0.5%	0.4%	0.6%	0.5%	0.4%
Between-Run	0.4%	0.2%	0.5%	0.0%	0.3%	0.4%	0.0%	0.2%	0.3%
Between-Day	0.5%	0.3%	0.4%	0.5%	0.7%	0.3%	0.9%	0.7%	0.4%
Between-Lot	0.9%	0.7%	0.9%	0.7%	0.6%	0.4%	1.3%	0.6%	0.3%
Total Precision	1.2%	0.9%	1.3%	1.0%	1.1%	0.8%	1.7%	1.1%	0.8%

Instrument 3 (% CV by Sample (IFCC))

Variation Source	Instrument ID: VartGerm01								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC%)	5.4	9.7	5.1	6.6	8.0	12.1	5.4	9.8	15.0
Repeatability	0.6%	0.5%	0.8%	0.8%	0.5%	0.4%	0.8%	0.4%	0.4%
Between-Run	0.2%	0.0%	0.1%	0.0%	0.0%	0.2%	0.0%	0.2%	0.0%
Between-Day	0.6%	0.3%	0.6%	0.5%	0.5%	0.4%	0.7%	0.4%	0.3%
Between-Lot	2.0%	0.9%	1.6%	2.5%	1.0%	0.7%	2.2%	1.1%	0.7%
Total Precision	2.2%	1.1%	1.9%	1.7%	1.3%	0.9%	2.5%	1.2%	0.9%

Instruments Combined (% CV by Sample (IFCC))

Variation Source	All Instruments								
	Control 1	Control 2	Patient 1	Patient 2	Patient 3	Patient 4	QC 1	QC 2	QC 3
Concentration HbA1c (IFCC%)	5.4	9.8	5.1	6.6	7.9	12.1	5.4	9.9	15.0
Repeatability	0.5%	0.4%	0.7%	0.7%	0.7%	0.4%	0.7%	0.5%	0.4%
Between-Run	0.3%	0.0%	0.4%	0.0%	0.2%	0.3%	0.2%	0.2%	0.2%
Between-Day	0.7%	0.4%	0.6%	0.5%	0.6%	0.4%	1.0%	0.7%	0.5%
Between-Instrument	1.3%	1.1%	0.4%	0.0%	0.4%	0.6%	0.8%	0.4%	0.0%
Between-Lot	1.4%	0.8%	1.2%	1.0%	0.8%	0.6%	1.6%	0.7%	0.5%
Total Precision	2.1%	1.5%	1.6%	1.3%	1.3%	1.1%	2.2%	1.2%	0.8%

b. *Linearity/assay reportable range:*

A linearity study was performed per CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; Linearity across the reportable range was performed using altered samples to obtain a low 3.4% HbA1c (14 mmol/mol) and a high 20.65% HbA1c (203 mmol/mol) EDTA whole blood patient samples. These samples were mixed together in varying ratios to obtain the 9 intermediate samples levels. The measured values were compared to the expected values. Polynomial regression analysis (for first, second, and third order polynomials) were performed to determine the statistical significance of non-linearity. The higher order coefficients were found not to be significant and linearity was demonstrated using the second order regression analysis. The regression parameters (slope, intercept, and R^2) were the following:

NGSP:

Slope	Intercept	R^2	Sample range tested
1.033	-0.269	0.998	3.4-20.65% HbA1c

IFCC:

Slope	Intercept	R^2	Sample range tested
1.033	-2.171	0.998	14-203 mmol/mol HbA1c

The linearity study supports the device's claimed assay measuring range of 3.4 to 20.6% HbA1c (NGSP) and 14 to 203 mmol/mol HbA1c (IFCC).

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The assigned HbA1c values of the VARIANT™ II TURBO HbA1c Kit -2.0 are certified with the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org.

The final reportable result is traceable to both the International Federation of Clinical Chemistry (IFCC) and the Diabetes Control and Complications Trial (DCCT). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Calibrator and Control Materials:

Value assignment for calibrators (VARIANT™ II TURBO HbA1c Kit – 2.0 Calibrator/Diluent Set) which are recommended for use with this device, were previously reviewed under 510(k) submission k070452. Bio-Rad's commercially available control materials were previously reviewed in submission k070546 and k052838.

Reagent Stability:

Reagent stability was previously reviewed in k070452. The Elution Buffers and Wash/Diluent Solution are stable until the expiration date when stored unopened at 15–30 °C. After opening the bottles, Elution Buffer A is stable for 30 days, Elution Buffer B is stable for 90 days, and Wash/Diluent Solution is stable for 60 days, when stored at 15–30 °C. The reconstituted Calibrators are stable for 24 hours when stored capped at 2–8 °C. The labeling indicates "Do not use the reconstituted Calibrators after 24 hours."

Sample Stability:

A study was conducted to show the stability of frozen samples collected in K_2 and K_3EDTA. Samples with concentration values spanning 3.50% to 20.60% HbA1c were collected in K_2-EDTA tubes, K_3-EDTA tubes and the aliquots of the whole blood samples were placed at -70°C for four months. Study protocols were reviewed and found to be acceptable. The data supports the use of K_2-EDTA tubes and K_3-EDTA tubes with frozen whole blood aliquoted and stored at -70°C for up to for 4 months when using the VARIANT II TURBO HbA1c Kit 2.0.

d. Detection limit:

Not Applicable

e. Analytical specificity:

i.) Endogenous Interference:

Studies were performed to assess common or known substances that could interfere with the VARIANT II TURBO HbA1c Kit-2.0. Whole blood samples with HbA1c values of ~6.5% HbA1c and ~8% HbA1c were analyzed by spiking the interfering substance into each of the two whole blood samples and then preparing serial dilutions to achieve 10 concentrations. Ten replicates of each of the ten varying concentrations were analyzed and compared to the reference sample (sample containing no interferent). Significant interference was defined by the sponsor as % recovery $\geq$ +/-7% of the expected 100% recovery.

The following substances showed no significant interference at the concentrations described below:

Endogenous Interference Study Results

	Concentration
Endogenous Substance	**Conventional Units**
Lipemia (Intralipid)	6000 mg/dL
Conjugated bilirubin	60 mg/dL
Unconjugated bilirubin	60 mg/dL
Glucose	2000 mg/dL
Rheumatoid factor	750 IU/mL
Total protein	21 g/dL

ii.) Drug Interference:

A Drug Interference study was performed based per CLSI EP07-A2, Interference Testing in Clinical Chemistry. Two EDTA whole blood sample pools were evaluated using a whole blood sample with a concentration ~6.5%HbA1c and whole blood sample with a concentration of ~8.0%HbA1c. Test samples were prepared by spiking each drug at the interferent concentration shown in the Table below. Ten replicates of each drug prepared with the test and control samples were analyzed using the VARIANT™ II TURBO HbA1c Kit-2.0 on the VARIANT™ II TURBO Hemoglobin Testing System.

Significant interference was defined as a more than ± 7% change in %HbA1c value from the control. No significant interference was observed at therapeutic levels up to the stated concentrations in the table below.

Drug Interference Study Results

Potential Drug Interferent	Highest Level Tested showing no Significant Interference
	Conventional (US) units
Acetylcysteine	166 mg/dL
Ampicillin-Na	1000 mg/dL
Ascorbic acid	300 mg/dL
Cefoxitin	2500 mg/dL
Heparin	5000 U/L
Levodopa	20 mg/dL
Methyldopa	20 mg/dL
Metronidazole	200 mg/dL
Doxycyclin	50 mg/dL
Acetylsalicylic acid	1000 mg/dL
Rifampicin	64 mg/L
Cyclosporine	5 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L
Phenylbutazone	400 mg/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

A Hemoglobin Derivatives Interference study was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. Potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c were evaluated using a whole blood sample with a concentration ~6.5%HbA1c and a whole blood sample with a concentration of ~8.0% HbA1c. The potentially interfering hemoglobin derivatives were spiked into the whole blood samples and each sample was analyzed using ten replicates each in the same analytical run on the VARIANT™ II TURBO Hemoglobin Testing System with the VARIANT™ II TURBO HbA1c Kit – 2.0.

Significant interference was defined as more than a ±7% change in HbA1c value from the control. The test result conclusions are as follows:

- Acetylated Hb- (up to 50 mg/dL) does not interfere with this assay.
- Carbamylated Hb - (up to 21 mg/dL) does not interfere with this assay.
- Labile A1c- (up to 1000mg/dL) glucose does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels.

iv.) Hemoglobin Variant Interference:

A Hemoglobin Variant Interference study was performed using a total of 147 samples known to contain hemoglobin variants S, C, E, D, A2 and F. Testing of the samples was performed using the VARIANT™ II TURBO HbA1c Kit – 2.0 on the VARIANT™ II TURBO Hemoglobin Testing System and compared to results obtained by a reference method that has been demonstrated to be free from the hemoglobin interferent being tested. The following is a table of the samples that were measured.

Variant samples used in Hemoglobin Variant Interference Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbC	25	33.3 – 42.4	4.50 - 10.70
HbD	21	30.2 – 41.4	5.5 - 11.4
HbE	24	24.7 - 31.4	5.0 - 8.3
HbS	26	26.8 - 41.6	4.70 - 13.3
HbA2	22	5.0 – 10.2	5.0 – 14.5
HbF	29	3.5 – 29.4	4.4 – 14.4

The following results were obtained and are represented as a mean measurement with the standard deviation in parenthesis for the samples near 6.5 % HbA1c and 8.0 % HbA1c.

Hemoglobin Variant Results Summary

Hemoglobin Variant	Relative % Bias from Reference Method at Low and High Concentrations of HbA1c	
	Relative % Bias (StDev) for HbA1c ~6.5%	Relative % Bias (StDev) for HbA1c ~8.0%
HbC	-0.3 (+/- 3.5)	-2.5 (+/- 2.5)
HbD	-1.1 (+/- 1.7)	-1.2 (+/- 1.0)
HbE	0.7 (+/- 3.0)	2.2 (+/- 1.4)
HbS	1.9 (+/- 2.8)	2.8 (+/- 1.8)
HbA2	1.4 (+/- 2.3)	2.0 (+/-4.1)
HbF	-1.9 (+/- 3.1)	-0.1 (+/-2.1)

Non-significant interference was defined at ±7% from the control. No significant interference was observed for HbC (≤42.4%), HbD (≤41.4%), HbE (≤31.4%), HbS (≤41.6%), HbA2 (≤10.2%) and HbF (≤29.4%) variants at the concentrations tested in this Study.

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

A Method comparison study was performed per CLSI EP09-A2 IR, Method Comparison and Bias Estimation Using Patient Samples. 130 variant-free whole blood EDTA samples, including 10 spiked samples, ranging from 3.4% to 20.0% HbA1c were evaluated using the VARIANT™ II TURBO HbA1c Kit- 2.0 on the VARIANT™ II TURBO Hemoglobin Testing System. Samples were tested in singlicate over several days using one lot of reagents. The results were compared to testing performed at a NGSP Secondary Reference Laboratory using a previously cleared HPLC HbA1c assay method (Trinity Bio-Tech Ultra2). To support the diagnostic claim, the distribution of samples spanned around the clinical decision point as follows in the table below.

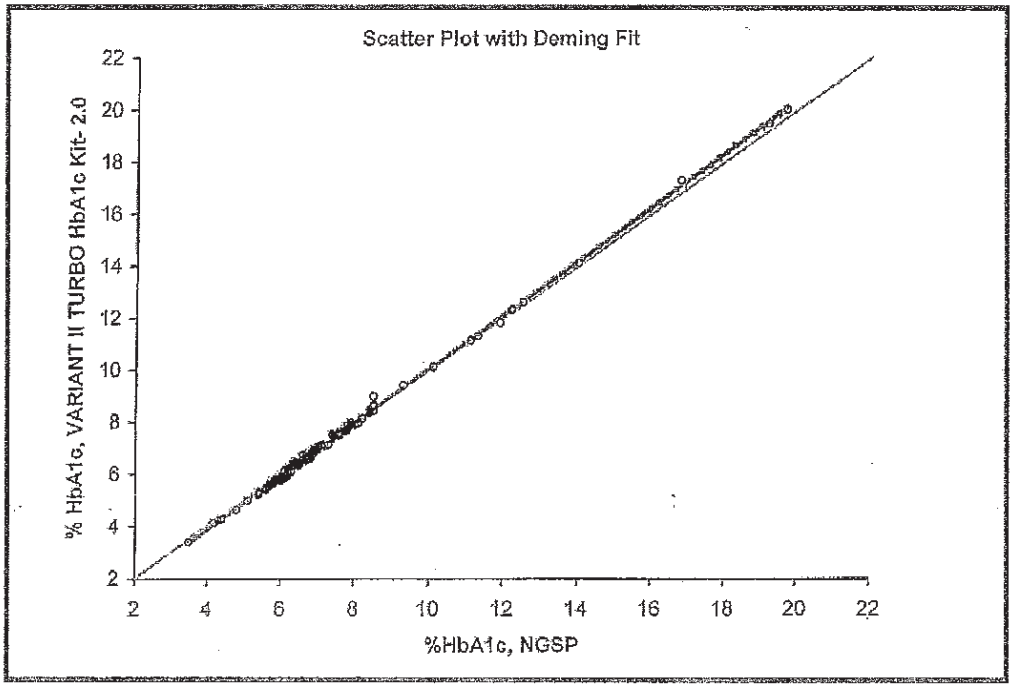
Distribution of samples

Hemoglobin A1c level	n	% Samples tested
≤ 5%	6	4.6
5 – 6%	17	13.1
6 – 6.5%	33	25.4
6.5 – 7%	31	23.8
7 – 8%	21	16.2
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	130	100

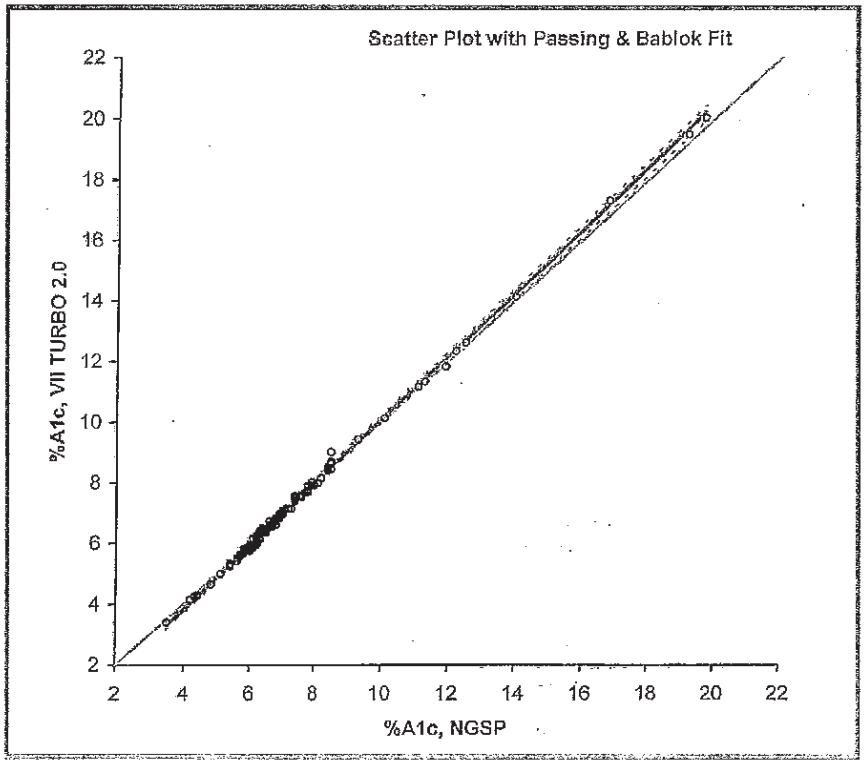
Deming (weighted) and Passing-Bablok regression analyses were performed for the VARIANT™ II TURBO HbA1c Kit – 2.0 versus the NGSP SRL reference method.

Summary of Method Comparison Results

	y-Intercept	95% CI	Slope	95% CI
Deming	-0.275	-0.342 to -0.208	1.033	1.023 to 1.043
Passing-Bablok	-0.331	-0.419 to -0.255	1.041	1.029 to 1.054



Scatter Plot using Deming Fit, %HbA1c, NGSP SRL vs. VARIANT II TURBO HbA1c Kit – 2.0



Scatter Plot using Passing & Bablok Fit, %HbA1c, NGSP SRL vs. VARIANT II TURBO HbA1c Kit – 2.0

The following biases between VARIANT™ II TURBO HbA1c Kit – 2.0 versus the Reference method were observed:

Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0	-0.106	-2.11
6.5	-0.057	-0.87
8.0	-0.008	-0.09
12.0	0.123	1.03

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) at four concentrations: (5.0 %, 6.5%, 8.0% and 12.0%) were calculated as follows: %TE=|%Bias| + 1.96 *CV* (1 + %Bias). The results are presented in the Table below.

Total Error Estimation

%A1c	% Bias	% CV	% TE
5.0	-2.11	1.6	5.2
6.5	-0.87	1.3	3.4
8.0	-0.09	1.3	2.6
12.0	1.03	1.1	3.2

b. *Matrix comparison:*

A matrix study was performed to determine the suitability of K_2-EDTA and K_3-EDTA anticoagulants used with fresh whole blood for use in the VARIANT II TURBO HbA1c Kit – 2.0. An additional study was performed to determine the suitability of Capillary blood in Hemoglobin Capillary Collection System (HCCS) for use in the VARIANT II TURBO HbA1c Kit – 2.0. Specimens with concentration values spanning 3.5 to 20.6% HbA1c were collected from a minimum of 44 paired samples collected from different donors in K_2-EDTA tubes, K_3-EDTA tubes, and in the Capillary blood in Hemoglobin Capillary Collection System (HCCS).

In this Matrix Comparison study, the following tube types under evaluation:

- K_2-EDTA tubes with fresh whole blood
- K_3-EDTA tubes with fresh whole blood
- Hemoglobin Capillary Collection System (HCCS) with fresh capillary blood

The regression results are as follows:

	Matrix Comparison Results			
	Linear Fit			
Collection Device and Sample Type	**Sample Range**	**Slope (95% CI)**	**Intercept (95% CI)**	**r^2 value**
K_3-EDTA versus K_2-EDTA	3.5 – 20.6	0.997	0.031	0.9995
		0.991 to 1.004	-0.023 to 0.084	
Capillary blood in Hemoglobin Capillary Collection System (HCCS) versus K_3-EDTA whole blood.	3.5 – 20.6	0.992	0.057	0.9993
		0.984 to 0.999	-0.007 to 0.121	

The data support the use of the following blood collection tubes and sample types with the VARIANT II TURBO HbA1c Kit – 2.0:

- K3-EDTA tubes with fresh whole blood
- K2-EDTA tubes with fresh whole blood
- Hemoglobin Capillary Collection System (HCCS) with fresh capillary blood

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

Hemoglobin A1c Expected Values

Hemoglobin A1c		
NGSP%	**IFCC mmol/mol**	
≥ 6.5	> 48	Diabetic[1-3]
5.7 — 6.4	39 — 47	Pre-Diabetic[1]
< 5.7	< 39	Non-Diabetic

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010, 33 (Suppl. 1), S62–S69.

2. International Expert Committee. Report on the Role of the A1c Assay in the Diagnosis of Diabetes. Diabetes Care 2009, 32 (7). 1327–1334.

3. World Health Organization. Use of Glycated Hemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. http://www.who.int/diabetes/publications/diagnosis_diabetes2011/en/ (accessed July 2014).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

BIO-RAD

REF 12000447

VARIANT™ II TURBO HbA$_{1c}$ Kit - 2.0

Instructions For Use

Σ 2500

CE

IVD

US: Rx Only

March 2015
16000117revA

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
USE For use with	 Contains Latex	 Number of Tests
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	<0.05 % <0 05%
ANLT CRTR Analytical Cartridge	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL 1 Calibrator Level 1	CAL 2 Calibrator Level 2	CAL DIL Calibrator Diluent
CAL DIL SET Calibrator/Diluent Set	CAL SET Calibrator Set	CD CD-ROM
DI H_2O Deionized Water	PRE FIL Prefilter	RECON Reconstitute with
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH DIL SOLN Wash/Diluent Solution
WSH DIL SOLN SET Wash/Diluent Solution Set	WB PRM Whole Blood Primer	

PRODUCT SAFETY INFORMATION

HbA_{1c} Calibrator 1 and 2, and Whole Blood Primer

WARNING: These products contain a chemical known to the State of California to cause birth defects or other reproductive harm. Contains <0.1% Gentamicin Sulfate and <0.1% Tobramycin.

Table of Contents

INTENDED USE

The VARIANT™ II TURBO HbA_{1c} Kit - 2.0 is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the VARIANT II TURBO Hemoglobin Testing System and VARIANT II TURBO Link Hemoglobin Testing System.

This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.

Measurement of hemoglobin A_{1c} is effective in monitoring long-term glycemic control in individuals with diabetes mellitus.

The VARIANT II TURBO HbA_{1c} Kit - 2.0 is intended for Professional Use Only.

The Hemoglobin Capillary Collection System (HCCS) is intended for the collection of human whole blood for the percent determination of hemoglobin A_{1c} using Bio-Rad HPLC methods.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The VARIANT II TURBO HbA_{1c} Kit - 2.0 is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to the *Limitations of the Procedure* for more information. The VARIANT II TURBO HbA_{1c} Kit - 2.0 also offers automatic sampling from a primary whole blood tube, followed by sample dilution.

PRINCIPLE OF THE PROCEDURE

The Bio-Rad VARIANT II TURBO HbA_{1c} Kit - 2.0 utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the VARIANT II TURBO Sampling Station (VSS) and injected into the analytical cartridge. The VARIANT II TURBO Chromatographic Station (VCS) dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. An additional filter at 690 nm corrects for background absorbance.

The VARIANT II TURBO Clinical Data Management (CDM™) software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. A sample report, including retention times of detected peaks and a chromatogram, is generated by CDM for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm.

The VARIANT II TURBO HbA_{1c} Kit - 2.0 is for use only with the Bio-Rad VARIANT II TURBO Hemoglobin Testing System and VARIANT II TURBO Link Hemoglobin Testing System.

TEST COMPONENTS

REF 12000447, VARIANT II TURBO HbA_{1c} Kit - 2.0

The kit contains supplies for 2500 tests:

REF	*Quantity*	*Description*
270-0350*	2 each	**Whole Blood Primer.** Each vial contains lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 1.0 mL per vial.
270-2456	5 each	**Elution Buffer A.** Each bottle contains 2500 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
270-2457	1 each	**Elution Buffer B.** Each bottle contains 2000 mL of a succinate/sodium perchlorate buffer. Contains <0.05% sodium azide as a preservative.
270-2458	1 each	**Calibrator/Diluent Set.** One set consisting of 2 vials of Calibrator Level 1, 2 vials of Calibrator Level 2, and 1 bottle of Calibrator Diluent. The calibrator vials contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Calibrator Diluent contains 100 mL of deionized water with <0.05% sodium azide as a preservative.
270-2461*	1 each	**CD** with VARIANT II TURBO HbA_{1c} Kit - 2.0 test parameters.
270-2462*	1 each	**Analytical Cartridge.** Cation exchange cartridge (2500 tests), 4.6 mm ID x 27.5 mm. 5 prefilters (500 tests each) are included with the cartridge.
270-2149	1 each	**Sample Vials.** 100 polypropylene microvials with pierceable caps, 1.5 mL.

** Components are not available for individual sale.*

NOTE: *The kit supports a usage scenario of as low as 800 tests/month when 1–2 runs/day are performed 5–6 days/week. Other usage patterns involving smaller average run sizes may require additional buffers.*

REF 270-2730, Wash/Diluent Solution Set

One set consisting of 4 bottles of Wash/Diluent Solution. Each bottle contains 2500 mL of deionized water with <0.05% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
196-2051†	**Hemoglobin Capillary Collection System (HCCS),** 5 Tests
196-2052†	**Hemoglobin Capillary Collection System (HCCS),** 100 Tests
196-2053†	**Hemoglobin Capillary Collection System (HCCS),** 5000 Tests
270-0351	**Whole Blood Primer,** 10 x 1.0 mL
270-0352	**Whole Blood Primer,** 6 x 1.0 mL
270-2464	**Prefilters,** package of 2
12000396‡	**Prediluted Sample Vials,** package of 500 vials and 500 caps
740	**Lyphochek® Diabetes Control Bilevel,** 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak,** 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels),** 6 x 0.5 mL
171	**Liquichek™ Diabetes Control, Level 1,** 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2,** 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3,** 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak,** 3 x 1.0 mL

† *For use with specific systems only; see the VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual for information.*

‡ *For use with the VARIANT II TURBO Link Hemoglobin Testing System integrated with the Inpeco FlexLab® track system only.*

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL, 7 mL

Deionized Water

Disposable Gloves

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Each unit of whole blood used in the manufacture of the calibrators and whole blood primer was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and whole blood primer with the same precautions used with patient specimens.
- Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.

- Dispose of all waste in accordance with applicable national and/or local regulations.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The calibrator vial stoppers contain dry natural rubber.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood and capillary blood.

Specimen Additives, Preservatives

- The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA or K3-EDTA.
- Capillary blood should be collected in HCCS (Hemoglobin Capillary Collection System).

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/ Preservative	Slope	Intercept	R^2
K2-EDTA	0.9974	0.0306	0.9995
HCCS	0.9915	0.0573	0.9993

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

Whole blood specimens may be stored as follows:

- 1 day at room temperature (15–30 °C)
- Up to 7 days at 2–8 °C
- Up to at least 2 months at –70 °C

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the sample is in an abnormal size/type tube, or if the height of the sample in the tube appears to be less than 25 mm, then the sample must be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Wash/Diluent Solution into a labeled predilution vial, followed by 5 µL of the whole blood sample.
  3. Cap the sample vial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

To install or change Elution Buffers and Wash/Diluent Solution, follow the procedure described in the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual*.

Elution Buffers and Wash/Diluent Solution

- Allow the Elution Buffers and Wash/Diluent Solution to reach room temperature (15–30 °C) before performing the test. Mix each bottle by gently inverting prior to installation.
- The Elution Buffers and Wash/Diluent Solution are stable until the expiration date when stored unopened at 15–30 °C. After opening the bottles, Elution Buffer A is stable for 30 days, Elution Buffer B is stable for 90 days, and Wash/Diluent Solution is stable for 60 days, when stored at 15–30 °C.
- With a new kit, install one bottle of each reagent and follow the procedure for *Installing a New Kit Lot* in the *Procedure* section.
- Buffers are compatible within a resin lot. Buffer and cartridge labels are coded using alphabetical letters to indicate compatibility. A compatible set of buffers and cartridge will have the same letter code on each label. Do not use combinations of cartridges and buffers with different letter codes.
- The Wash/Diluent Solution is interchangeable between kit lots.

Whole Blood Primer

- Use fresh aliquots of Whole Blood Primer when installing a new analytical cartridge.
- The Whole Blood Primer is stable until the expiration date when stored unopened at 2–8 °C.
- The Whole Blood Primer is provided in lyophilized form for increased stability.
  1. Reconstitute each Whole Blood Primer by adding 1.0 mL of deionized water to each vial.
  2. Replace the vial stoppers and allow vials to stand for 10 minutes at 15–30 °C.
  3. Swirl gently to dissolve and ensure complete mixing.
- The reconstituted Whole Blood Primer is stable for 1 day when stored at 2–8 °C.
- The Whole Blood Primer is interchangeable between lots.

Calibrator/Diluent Set

- The Calibrator/Diluent Set is stable until the expiration date when stored unopened at 2–8 °C.
- The Calibrator Diluent is ready to use. The Diluent is stable for 60 days, after opening the bottle, when stored at 2–8 °C. The Diluent is interchangeable between lots.
- The Calibrators are provided in lyophilized form for increased stability.
  1. Using a volumetric pipette, reconstitute each Calibrator by adding 7 mL of cold Calibrator Diluent to each vial.
  2. Replace the vial stoppers and allow vials to stand 2 minutes.
  3. Swirl gently to dissolve.
- The reconstituted Calibrators are stable for 24 hours when stored capped at 2–8 °C. Do not use the reconstituted Calibrators after 24 hours.
- Do not freeze the reconstituted Calibrators.
- See the *value card* included with the current lot of calibrators for value assignment. Values are entered automatically using the Update Kit CD. Values must be entered manually if a different lot is being used; see the *CDM Software Operation Manual* to manually enter values in the **Setup/Sample Types/Calibrator** screen.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert. Also see insert for value ranges.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Wash/Diluent Solution into a labeled predilution vial, followed by 5 µL of the reconstituted control. Cap each control vial and mix thoroughly.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Wash/Diluent Solution into a labeled predilution vial, followed by 5 µL of the control. Cap each control vial and mix thoroughly.

Analytical Cartridge and Prefilters

- The Analytical Cartridge should be stored at 2–8 °C. The Analytical Cartridge is stable for 90 days or 2500 tests when installed on the instrument.
- The Prefilters should be stored at 2–30 °C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If reagents were frozen during shipment, mix each bottle by gently inverting before installing on instrument.
- Do not use any reagents which have any indications of discoloration, cloudiness, or precipitation.
- Do not use any reagents that show any signs of leakage.
- Do not use the calibrator or whole blood primer if the pellet is brown or the vial is broken. If the lyophilized material contains insoluble matter, discard the material and reconstitute a new vial.
- If the system overpressures due to excessive particulates (e.g., sample clots or precipitates), the cartridge prefilter should be replaced. Continue to replace the prefilter every 500 tests until the cartridge lifetime (2500 tests or 90 days) is completed. If the prefilter replacement does not resolve the overpressure, then the cartridge may require replacement; contact Bio-Rad Technical Service for troubleshooting assistance.

PROCEDURE

Installing a New Kit Lot (Update Kit CD)

- When changing to a different lot of reagents and/or cartridge, the parameters from the matching CD must be installed to ensure optimum performance of the program.
- The Reagent Set number that appears in the **Setup/Test** screen on CDM is the same as the CD LOT Check that number to verify that you are using the correct CD.

From the **Setup/Test** screen:

1. Insert Update Kit CD into CD drive.
2. Click **Update Kit**.
3. Select drive e:\.
4. Select test to be updated.
5. Click **OK**.

Installing a New Analytical Cartridge

Priming and calibration must be performed before first analysis with a new analytical cartridge.

1. Replace cartridge (install with arrow pointing up). See the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual* for instructions.
2. Go to the **Maintain/Instruments** screen. Select **Do Startup Actions** from the Execute Commands list. Click **Start**.
3. Check for leaks and gradually tighten loose fittings as needed.
4. After the startup actions are completed, return the instrument to Ready state by clicking **Return to READY state**.
5. The cartridge is now ready for priming.
6. See the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual* or applicable *Quick Guide* for run setup and sample order.

Installing a New Prefilter

Replace the prefilter at 500 injections. See the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual* for instructions.

Update the prefilter injection counter in CDM:

1. Go to the **Setup/Test/Cartridges** screen.
2. Change the **In Use** column entry from **Yes** to **No** for the used prefilter; a new line is generated for the new prefilter.
3. In the new prefilter line, enter "**N/A**" in the **Lot #** column and **500** in the **Inj. Limit** column.
4. In the **In Use** column, select **Yes**.

Calibration

Calibration must be performed after priming a new analytical cartridge. See the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual* or applicable *Quick Guide* for run setup and sample order.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Run

Once calibration is completed, use the routine run configuration. See the *VARIANT II TURBO and VARIANT II TURBO Link Hemoglobin Testing Systems Operation Manual* or applicable *Quick Guide* for run setup and sample order.

Certification/Traceability to Reference Material and Method

The VARIANT II TURBO HbA_{1c} Kit - 2.0 is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The VARIANT II TURBO HbA_{1c} Kit - 2.0 is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 x IFCC) + 2.152
IFCC = (10.93 x NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results:

1. The system must pass calibration.
2. Total area of each analysis should range from 1.0 million to 3.5 million units. Results should not be reported if the area is outside this range.
3. The peaks A1c and A0 must be correctly identified.
4. Quality Control values should be in range.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.4–20.6
IFCC mmol/mol HbA_{1c}	14–203

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the Variant and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 and P4 peak area. The following peak concentrations do not interfere with the test:
   - P3 peak ≤5% for hemoglobin variant samples (i.e., HbS-, HbC-, HbD-, and HbE-trait)
   - P3 peak ≤10% for non-variant samples
   - P4 peak ≤10%

   If either peak exceeds the cutoff, the HbA_{1c} result should not be reported; a fresh sample should be obtained for analysis.
9. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. See Figure 11 for a typical example of the integration and reporting of minor "Unknown" peaks. The two largest minor components of hemoglobin A are given designated peak windows P3 and P4.[19] In all cases, all components of hemoglobin A (e.g., P3, P4, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}.

LIMITATIONS OF THE PROCEDURE

Sample Dilution

Normal total hemoglobin concentration corresponds to a total area of approximately 2.5 million units. The required total area range for the VARIANT II TURBO HbA_{1c} Kit - 2.0 is 1.0 million to 3.5 million units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 1.0 million to 3.5 million total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes.
- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,20–22]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test. Typical chromatograms for these variants are provided in Figures 5–8.

NOTE: *Hemoglobins E, D, and S elute in the Variant Window.*

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias from Comparative Method observed at Low and High Concentrations of HbA_{1c}	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ~8.0%
HbS	1.9 (± 2.8)	2.8 (± 1.8)
HbC	–0.3 (± 3.5)	–2.5 (± 2.5)
HbD	–1.1 (± 1.7)	–1.2 (± 1.0)
HbE	0.7 (± 3.0)	2.2 (± 1.4)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the VARIANT II TURBO HbA_{1c} Kit - 2.0. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 25% do not interfere with the test.
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias from Reference Method observed at Low and High Concentrations of HbA_{1c}	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ≥8.0%
HbF	−1.9 (± 3.1)	−0.1 (± 2.1)
HbA_2	1.4 (± 2.3)	2.0 (± 4.1)

- At physiologically occurring concentrations, there is no interference from labile A_{1c}, carbamylated hemoglobin, or acetylated hemoglobin.[23]
- Common drugs at therapeutic concentrations do not interfere with the test.[23]
- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

Diagnosis of Diabetes

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

Monitoring HbA_{1c} in Diabetic Patients

The following HbA_{1c} ranges may be used for interpretation of results; however, factors such as duration of diabetes, adherence to therapy, and the age of the patient should also be considered in assessing the degree of blood glucose control. These values are for nonpregnant adults.

Hemoglobin A_{1c}		Glycemic Goal[22]
NGSP %	IFCC mmol/mol	
<8	<64	Less Stringent Goal*
<7	<53	General Goal†
<6.5	<48	More Stringent Goal‡

* *May be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, and those with longstanding diabetes in whom the general goal is difficult to attain.*

† *Shown to reduce microvascular and neuropathic complications and, if implemented soon after diagnosis of diabetes, is associated with long-term reduction in macrovascular disease.*

‡ *May be appropriate for selected patients (e.g., those with short duration of diabetes, long life expectancy, and no significant cardiovascular disease) if this can be achieved without significant hypoglycemia or other adverse effects of treatment.*

PERFORMANCE CHARACTERISTICS

Precision

The precision of the VARIANT II TURBO HbA_{1c} Kit - 2.0 was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different kit lots, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.7%)	Patient 3 (8.0%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.9%)
Repeatability	0.5	0.6	1.0	0.3	0.5	0.3
Between-Run	0.3	0.0	0.2	0.3	0.4	0.0
Between-Day	0.8	0.7	0.6	0.5	0.8	0.6
Between-Lot	1.0	0.8	0.6	0.6	0.8	0.6
Total Precision	1.4	1.2	1.3	0.9	1.4	0.9
Variation Source	**Instrument 2 % CV by Sample**					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (7.9%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.8%)
Repeatability	0.6	0.6	0.5	0.4	0.5	0.3
Between-Run	0.5	0.0	0.3	0.4	0.4	0.2
Between-Day	0.4	0.5	0.7	0.3	0.5	0.3
Between-Lot	0.9	0.7	0.6	0.4	0.9	0.7
Total Precision	1.3	1.0	1.1	0.8	1.2	0.9
Variation Source	**Instrument 3 % CV by Sample**					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.0%)	Patient 4 (12.1%)	Control 1 (5.4%)	Control 2 (9.7%)
Repeatability	0.8	0.8	0.5	0.4	0.6	0.5
Between-Run	0.1	0.0	0.0	0.2	0.2	0.0
Between-Day	0.6	0.5	0.5	0.4	0.6	0.3
Between-Lot	1.6	1.4	1.0	0.7	2.0	0.9
Total Precision	1.9	1.7	1.3	0.9	2.2	1.1
Variation Source	**Combined % CV by Sample**					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (7.9%)	Patient 4 (12.1%)	Control 1 (5.4%)	Control 2 (9.8%)
Repeatability	0.7	0.7	0.7	0.4	0.5	0.4
Between-Run	0.4	0.0	0.2	0.3	0.3	0.0
Between-Day	0.6	0.5	0.6	0.4	0.7	0.4
Between-Instrument	0.4	0.0	0.4	0.6	1.3	1.1
Between-Lot	1.2	1.0	0.8	0.6	1.4	0.8
Total Precision	1.6	1.3	1.3	1.1	2.1	1.5

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (64 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (85 mmol/mol)
Repeatability	0.8	1.0	1.3	0.4	0.9	0.4
Between-Run	0.5	0.0	0.3	0.4	0.6	0.0
Between-Day	1.4	1.0	0.9	0.6	1.4	0.7
Between-Lot	1.7	1.1	0.8	0.7	1.4	0.8
Total Precision	2.4	1.8	1.8	1.1	2.2	1.2
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (32 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (63 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (84 mmol/mol)
Repeatability	1.1	1.0	0.7	0.4	0.8	0.4
Between-Run	0.9	0.0	0.5	0.5	0.6	0.2
Between-Day	0.6	0.7	1.0	0.4	0.9	0.4
Between-Lot	1.6	1.0	0.9	0.5	1.5	1.0
Total Precision	2.2	1.5	1.6	0.9	2.0	1.1
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (32 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (64 mmol/mol)	Patient 4 (109 mmol/mol)	Control 1 (35 mmol/mol)	Control 2 (83 mmol/mol)
Repeatability	1.4	1.2	0.7	0.5	1.1	0.6
Between-Run	0.2	0.0	0.0	0.3	0.3	0.0
Between-Day	1.0	0.7	0.6	0.4	1.0	0.4
Between-Lot	2.8	2.1	1.4	0.9	3.4	1.2
Total Precision	3.3	2.5	1.7	1.1	3.7	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (32 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (63 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (84 mmol/mol)
Repeatability	1.1	1.0	1.0	0.5	0.9	0.5
Between-Run	0.6	0.0	0.3	0.4	0.5	0.0
Between-Day	1.1	0.8	0.8	0.5	1.1	0.6
Between-Instrument	0.8	0.0	0.5	0.7	2.1	1.5
Between-Lot	2.1	1.5	1.1	0.7	2.2	1.0
Total Precision	2.8	2.0	1.8	1.3	3.4	1.9

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The VARIANT II TURBO HbA_{1c} Kit - 2.0 was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2-IR guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the VARIANT II TURBO HbA_{1c} Kit - 2.0 was 3.4–20.0% (14–195 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The VARIANT II TURBO HbA_{1c} Kit - 2.0 estimated bias compared to the NGSP SRL Method is presented in Table 4.

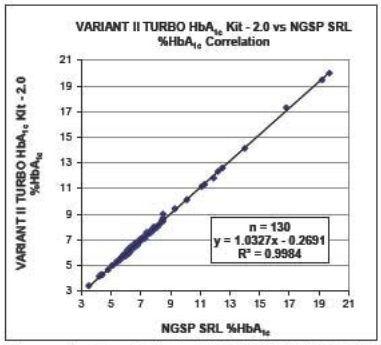


Figure 1a: Correlation of VARIANT II TURBO HbA_{1c} Kit - 2.0 vs NGSP SRL Method (NGSP %)

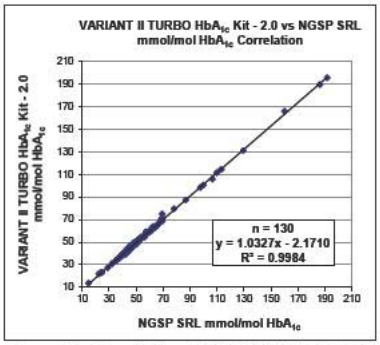


Figure 1b: Correlation of VARIANT II TURBO HbA_{1c} Kit - 2.0 vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.11	–2.11
6.5	–0.06	–0.87
8.0	–0.01	–0.09
12.0	0.12	1.03

n	130
Mean Difference	–0.03%
Lower 95% CI	–0.32%
Upper 95% CI	0.22%

Table 4: VARIANT II TURBO HbA_{1c} Kit - 2.0 Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the VARIANT II TURBO HbA_{1c} Kit - 2.0. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.4–20.6% (14–203 mmol/mol) within a maximum measured difference of ± 0.03% (or ± 0.38 mmol/mol) in this interval.

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data
Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/27/2012 09:54:59
Injection Number: 29
Run Number: 3
Rack ID: 0008
Tube Number: 10
Report Generated: 04/27/2012 11:01:16
Operator ID: .

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	---	0.4	0.106	7100
A1a	---	---	1.1	0.157	20292
A1b	---	---	1.0	0.226	19103
F	---	---	1.0	0.287	17500
LA1c	---	---	1.7	0.412	31307
A1c	36	5.5	---	0.519	80385
P3	---	---	4.3	0.770	78666
P4	---	---	1.2	0.868	22526
Ao	---	---	84.9	1.011	1559008

Total Area: 1,835,886

HbA1c (IFCC) = 36 mmol/mol HbA1c (NGSP) = 5.5 %

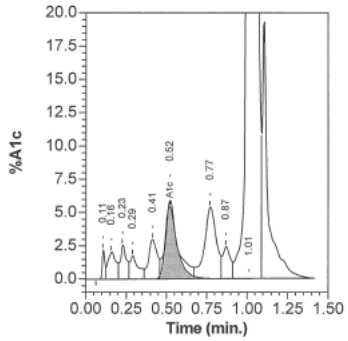


Figure 2: *Non-Diabetic (Normal) Result*

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data
Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/27/2012 09:56:35
Injection Number: 30
Run Number: 3
Rack ID: 18
Tube Number: 1
Report Generated: 04/27/2012 10:19:09
Operator ID: .

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.3	0.106	7352
A1a	---	1.4	0.159	31416
A1b	---	1.5	0.224	32933
Unknown	---	0.9	0.252	18891
F	---	1.1	0.286	23364
LA1c	---	3.3	0.405	72673
A1c	10.6*	---	0.510	198496
P3	---	5.8	0.770	128081
P4	---	1.6	0.869	34443
Ao	---	75.1	1.012	1649275

*Values outside of expected ranges Total Area: 2,196,923

HbA1c (NGSP) = 10.6* %

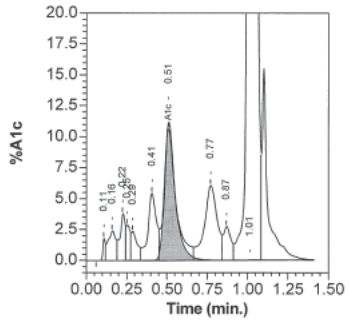


Figure 3: Diabetic Result with an Elevated HbA_{1c} Level

SAMPLE REPORT FORMAT

Bio-Rad CDM System

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data

Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:

Comments:

Analysis Data

Analysis Performed: 04/27/2012 09:58:11
Injection Number: 31
Run Number: 3
Rack ID: 18
Tube Number: 2
Report Generated: 04/27/2012 10:19:09
Operator ID: .

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.6	0.106	10775
A1a	---	7.5	0.170	144352
F	---	19.8	0.287	378923
LA1c	---	1.1	0.419	20366
A1c	4.9	---	0.520	59727
P3	---	4.1	0.770	78383
P4	---	0.9	0.869	17481
Ao	---	62.9	1.017	1203823

Total Area: 1,913,830

HbA1c (NGSP) = 4.9 %

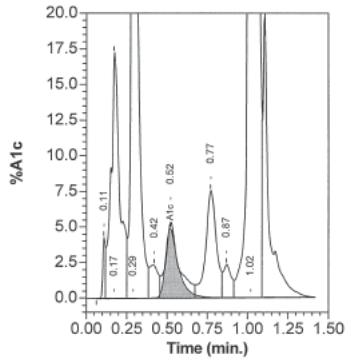


Figure 4: *Non-Diabetic Result with Elevated HbF*

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data
Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/27/2012 10:07:49
Injection Number: 37
Run Number: 3
Rack ID: 18
Tube Number: 9
Report Generated: 04/27/2012 11:01:44
Operator ID: .

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	---	0.5	0.106	11278
A1a	---	---	1.2	0.157	27595
A1b	---	---	0.8	0.226	17922
F	---	---	1.2	0.269	27764
Unknown	---	---	0.3	0.336	7647
LA1c	---	---	0.9	0.422	20023
A1c	82*	9.6*	---	0.516	115711
P3	---	---	4.6	0.768	102976
P4	---	---	1.3	0.867	27856
Ao	---	---	48.1	1.015	1070947
Variant Window	---	---	35.8	1.136	795806

*Values outside of expected ranges Total Area: 2,225,524

HbA1c (IFCC) = 82* mmol/mol HbA1c (NGSP) = 9.6* %

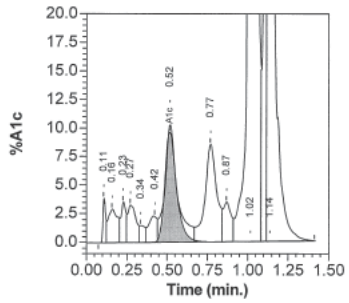


Figure 5: Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data
Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 04/27/2012 10:12:38
Injection Number: 40
Run Number: 3
Rack ID: 0010
Tube Number: 2
Report Generated: 04/27/2012 10:20:07
Operator ID: .

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.4	0.106	7883
A1a	---	0.7	0.161	15696
A1b	---	0.9	0.225	18841
F	---	0.8	0.287	18072
LA1c	---	1.0	0.415	22352
A1c	7.7*	---	0.519	87407
P3	---	2.6	0.773	58400
P4	---	1.0	0.868	22735
Ao	---	50.3	1.017	1114842
C	---	38.3	1.187	848550

*Values outside of expected ranges Total Area: 2,214,778

HbA1c (NGSP) = 7.7* %

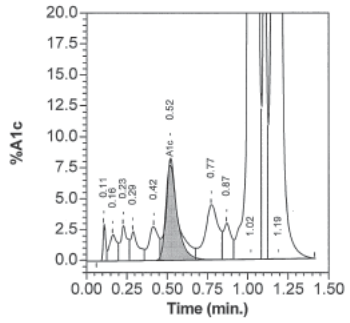


Figure 6: *Diabetic Result with Hemoglobin C Trait (AC)*

SAMPLE REPORT FORMAT

Bio-Rad CDM System

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data		Analysis Data	
Sample ID:	Unknown	Analysis Performed:	04/27/2012 10:06:12
Patient ID:		Injection Number:	36
Name:		Run Number:	3
Physician:		Rack ID:	18
Sex:		Tube Number:	8
DOB:		Report Generated:	04/27/2012 11:01:44
		Operator ID:	.

Comments:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	---	0.4	0.106	8712
A1a	---	---	1.1	0.163	26360
A1b	---	---	0.9	0.225	20013
F	---	---	1.3	0.287	30700
LA1c	---	---	1.0	0.424	23213
A1c	62*	7.8*	---	0.519	92907
P3	---	---	2.5	0.773	58079
P4	---	---	0.9	0.869	20568
Ao	---	---	49.3	1.018	1157140
Variant Window	---	---	38.7	1.126	907967

*Values outside of expected ranges Total Area: 2,345,658

HbA1c (IFCC) = 62* mmol/mol HbA1c (NGSP) = 7.8* %

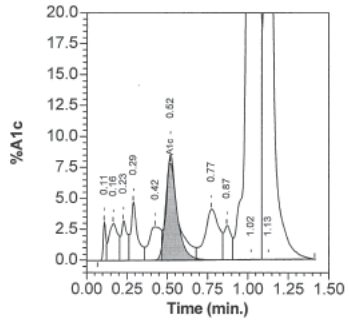


Figure 7: *Diabetic Result with Hemoglobin D Trait (AD)*

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data		Analysis Data	
Sample ID:	Unknown	Analysis Performed:	04/27/2012 10:04:36
Patient ID:		Injection Number:	35
Name:		Run Number:	3
Physician:		Rack ID:	18
Sex:		Tube Number:	7
DOB:		Report Generated:	04/27/2012 10:19:36
		Operator ID:	.

Comments:

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.3	0.105	7799
A1a	---	0.9	0.162	25308
A1b	---	0.6	0.224	17208
F	---	1.5	0.288	42003
LA1c	---	1.0	0.415	27787
A1c	6.1*	---	0.516	94443
P3	---	2.7	0.773	74128
P4	---	2.6	0.849	70572
Unknown	---	1.5	0.953	40575
Ao	---	55.7	1.012	1510888
Variant Window	---	29.6	1.097	804264

*Values outside of expected ranges Total Area: 2,714,976

HbA1c (NGSP) = 6.1* %

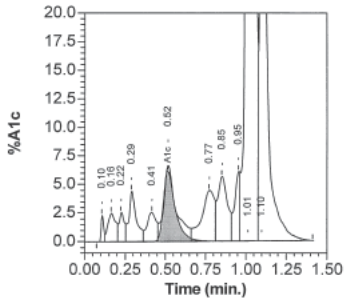


Figure 8: *Result with Hemoglobin E Trait (AE)*

SAMPLE REPORT FORMAT

Bio-Rad CDM System

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data

Sample ID: Unknown
Patient ID:
Name:
Physician:
Sex:
DOB:

Comments:

Analysis Data

Analysis Performed: 04/27/2012 09:53:23
Injection Number: 28
Run Number: 3
Rack ID: 0008
Tube Number: 9
Report Generated: 04/27/2012 10:19:09
Operator ID:

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.4	0.105	7482
A1a	---	1.4	0.156	25217
A1b	---	3.4	0.223	61098
LA1c	---	5.9	0.402	108462
A1c	9.4*	---	0.513	145763
P3	---	5.3	0.773	97237
P4	---	1.5	0.870	27130
Ao	---	74.1	1.015	1350620

*Values outside of expected ranges Total Area: 1,823,011

HbA1c (NGSP) = 9.4* %

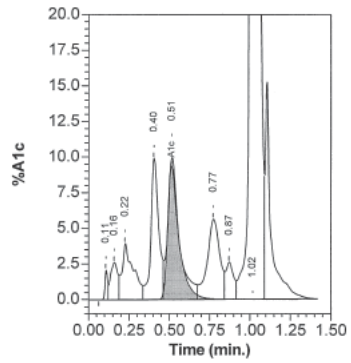


Figure 9: *Diabetic Result with Elevated Labile A_{1c} (LA1c)*

SAMPLE REPORT FORMAT

Bio-Rad CDM System **PATIENT REPORT**
V2TURBO_A1c_2.0

Patient Data		Analysis Data	
Sample ID:	Unknown	Analysis Performed:	05/04/2012 14:37:34
Patient ID:		Injection Number:	20
Name:		Run Number:	2
Physician:		Rack ID:	14
Sex:		Tube Number:	1
DOB:		Report Generated:	05/04/2012 14:39:33
		Operator ID:	

Comments:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	---	0.3	0.107	3982
A1a	---	---	1.6	0.155	23137
Unknown	---	---	0.6	0.198	9052
A1b	---	---	1.5	0.226	21635
F	---	---	1.3	0.292	19161
LA1c	---	---	3.4	0.425	48360
A1c	38	5.6	---	0.515	64946
P3	---	---	6.0	0.799	86534
P4	---	---	1.2	0.863	17544
Ao	---	---	79.6	1.017	1146394

Total Area: 1,440,744

HbA1c (IFCC) = 38 mmol/mol HbA1c (NGSP) = 5.6 %

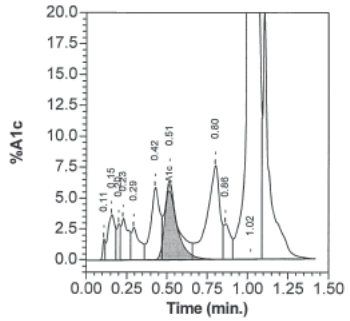


Figure 10: *Non-Diabetic Result with Elevated Carbamylated Hemoglobin*
NOTE: *Carbamylated hemoglobin elutes in the LA1c window.*

SAMPLE REPORT FORMAT

Bio-Rad CDM System

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data		Analysis Data	
Sample ID:	Unknown	Analysis Performed:	05/04/2012 15:00:32
Patient ID:		Injection Number:	24
Name:		Run Number:	3
Physician:		Rack ID:	14
Sex:		Tube Number:	10
DOB:		Report Generated:	05/04/2012 15:02:43
		Operator ID:	

Comments:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
Unknown	---	---	0.4	0.107	4178
A1a	---	---	1.9	0.163	21754
A1b	---	---	0.9	0.229	10884
F	---	---	2.6	0.293	29905
LA1c	---	---	1.3	0.417	14731
A1c	81*	9.6*	---	0.518	67706
P3	---	---	3.9	0.771	45553
P4	---	---	3.9	0.850	44928
Unknown	---	---	2.3	0.954	26186
Ao	---	---	50.2	1.029	578742
Variant Window	---	---	26.8	1.100	309325

*Values outside of expected ranges Total Area: 1,153,890

HbA1c (IFCC) = 81* mmol/mol HbA1c (NGSP) = 9.6* %

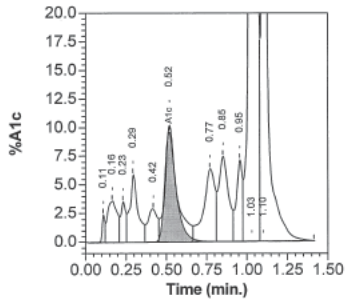


Figure 11: *Diabetic Result with Multiple Minor Components (Unknown Peaks) Integrated*

TRADEMARK INFORMATION

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NOTES:

TECHNICAL ASSISTANCE

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

Outside the USA: Contact your regional Bio-Rad office.
Go to www.bio-rad.com for contact information.

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Appendix B:

DRAFT Labeling

D-100™ HbA1c IFU

BIO-RAD

D-100™ HbA_{1c}

Instructions For Use

USE REF

290-1004
290-1006
290-1007
290-1008
290-1009
290-1010
290-1011
290-1012

Σ 10000

CE

IVD

US: Rx Only

May 2015
16000328revB

UNITED STATES, *Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000*

EC REP **FRANCE**, *Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1) **Danger**	
H314	Causes severe skin burns and eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P311	If exposed or if you feel unwell: call a poison center or doctor/physician.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.

Measurement of hemoglobin A_{1c} is effective in monitoring long-term glycemic control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

Hemoglobin Capillary Collection System

The Hemoglobin Capillary Collection System (HCCS) is intended for the collection of human whole blood for the percent determination of hemoglobin A_{1c} using Bio-Rad HPLC methods.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL
196-2051	**Hemoglobin Capillary Collection System (HCCS)**. 5 Tests
196-2052	**Hemoglobin Capillary Collection System (HCCS)**. 100 Tests
196-2053	**Hemoglobin Capillary Collection System (HCCS)**. 5000 Tests

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human

source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.

- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood and capillary blood.

Specimen Additives, Preservatives

- The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.
- Capillary blood should be collected in HCCS (Hemoglobin Capillary Collection System).

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997
HCCS	1.0109	–0.1059	0.9996

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).
- HCCS samples are stable for 4 days at 42 °C, 14 days at 15–35 °C, or 28 days at 2–8 °C.

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.
5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.

- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes.
- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	~6.5% HbA_{1c}	~9.0% HbA_{1c}
HbS	–0.6	–1.5
HbC	–1.3	–3.9
HbD	–4.7	–4.4
HbE	–2.7	–1.3

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	~6.5% HbA_{1c}	~9.0% HbA_{1c}
HbF	–2.3	–3.5
HbA_2	–1.3	3.4

- At physiologically occurring concentrations, there is no interference from labile A_{1c}, carbamylated hemoglobin, or acetylated hemoglobin.[25]
- Common drugs at therapeutic concentrations do not interfere with the test.[25]

- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

Diagnosis of Diabetes

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

Monitoring HbA_{1c} in Diabetic Patients

The following HbA_{1c} ranges may be used for interpretation of results; however, factors such as duration of diabetes, adherence to therapy, and the age of the patient should also be considered in assessing the degree of blood glucose control. These values are for nonpregnant adults.

Hemoglobin A_{1c}		Glycemic Goal[23]
NGSP %	IFCC mmol/mol	
<8	<64	Less Stringent Goal*
<7	<53	General Goal†
<6.5	<48	More Stringent Goal‡

* *May be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, and those with longstanding diabetes in whom the general goal is difficult to attain.*

† *Shown to reduce microvascular and neuropathic complications and, if implemented soon after diagnosis of diabetes, is associated with long-term reduction in macrovascular disease.*

‡ *May be appropriate for selected patients (e.g., those with short duration of diabetes, long life expectancy, and no significant cardiovascular disease) if this can be achieved without significant hypoglycemia or other adverse effects of treatment.*

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

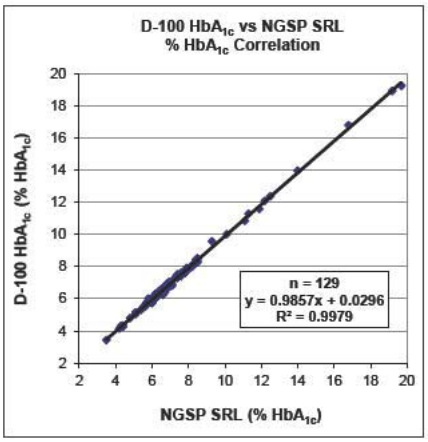


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

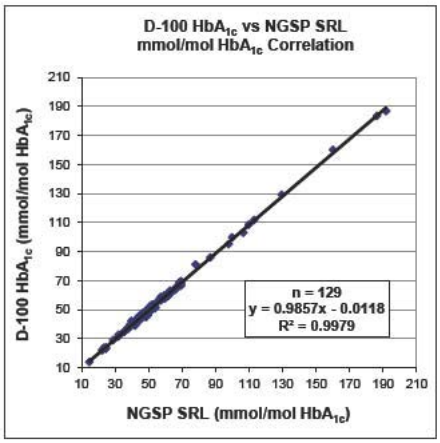


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.04	–0.84
6.5	–0.06	–0.97
8.0	–0.09	–1.06
12.0	–0.14	–1.18

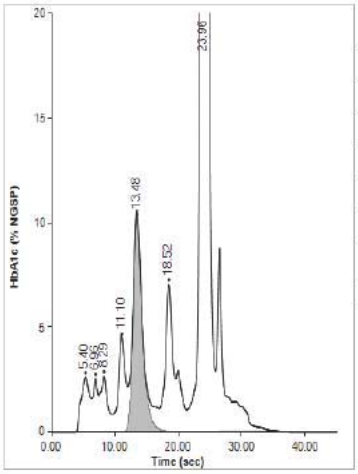
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



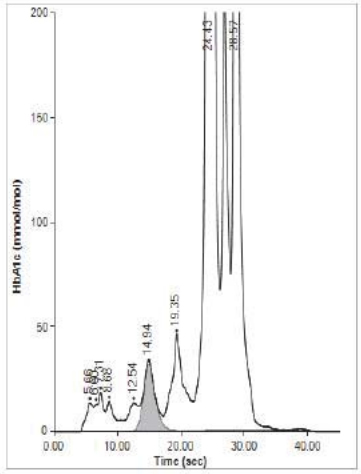
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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NOTES:

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TECHNICAL ASSISTANCE

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

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Appendix C:

DRAFT Labeling

Operation Manual

D-100™ Hemoglobin Testing System

D-100™ Hemoglobin Testing System

REF 290-1000

BIO-RAD

D-100™ Hemoglobin Testing System

Operation Manual

USE D-100™ Software Version 1.0

CE

IVD

US: Rx Only

May 2015
LB000341revB

UNITED STATES Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000

EC REP FRANCE Bio Rad, boulevard Raymond Poincaré, 92430 Marnes-la Coquette, 33-1-4795-6000

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	EC REP Authorized Representative in the European Union	Manufacturer	Consult Instructions for Use
IVD For In Vitro Diagnostic Use	REF Catalog Number	Date of Manufacture	Temperature Limit
SN Serial Number	USE For use with	Rx Only Prescription Use Only	UDI-DI Unique Device Identifica ion-Device Identifier
Biohazard	Caution	Fuse	Sharp Biohazard
Protective Conductor Terminal	Alternating Current (AC)	Ground Terminal	Power On
Power Off	Humidity Range	Moving Parts	Waste from Electrical and Electronic Equipment
ANLT CRTR Analy ical Cartridge	PRE FIL Prefilter		

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Lyphochek and Unity Real Time are registered trademarks of Bio-Rad Laboratories, Inc.

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Naming Conventions

Abbreviated text is used in place of full names or descriptions. The following is a list of naming conventions used in this manual and/or in the user interface.

CONVENTION	DESCRIPTION
D-100	D-100 Hemoglobin Testing System
A1c	Hemoglobin A_{1c}

Graphic Conventions

Throughout the text, icons and signal words appear where the information warrants special attention. The following conventions are used in this manual.

CONVENTION	DESCRIPTION
NOTE:	Note statements alert you to important information relevant to the current subject matter.
	**Warning:** This icon directs you to follow specified instructions where safety is involved.
	**Caution:** This icon directs you to follow specified instructions to prevent electrical shock.
	**Biohazard:** This icon alerts you to a potentially biohazardous condition.
	**Sharp Biohazard:** This icon alerts you to a potentially biohazardous sharp condition.

GENERAL SAFETY INFORMATION

- The D-100 System was designed, tested, and certified to meet various safety standards.
- These safety certifications do not extend to other equipment or accessories not similarly certified, even when connected to the D-100 System.
- This system is safe to use when operated in accordance with the instructions in this operation manual.
- Unauthorized modification or alteration of this system voids the warranty, voids the certifications, and creates a potential safety hazard for the operator.
- Read through and familiarize yourself with the contents of this operation manual before using the system for the first time.

HAZARDS

- The D-100 System is designed to operate safely and effectively when used in the manner prescribed by the manufacturer.
- If the D-100 or any of its associated components are used in a manner not specified by the manufacturer, the inherent protection provided by the equipment may be impaired.
- Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by the use of this system for purposes other than for which it is intended or by unauthorized modifications of the system.
- Service of the D-100 System should be performed only by qualified Bio-Rad personnel.
- The D-100 System should be moved only by qualified Bio-Rad personnel.
- Although the D-100 System provides some inherent protection against hazards, special precautions should be taken to avoid harm to the operator or equipment.

Biohazards

The following activities may expose the operator to biohazardous conditions:

- Handling samples, calibrator packs, and controls
- Cleaning spills
- Handling and disposing of solid and liquid waste
- Performing maintenance procedures
- Replacing system parts

Biohazard General Precautions

To protect yourself from potentially biohazardous materials, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location:

- Always wear laboratory gloves, coat, and goggles or safety glasses with side shields.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials. Seal wounds with waterproof bandages under protective clothing (e.g., gloves, sleeves). Individuals with cuts or abrasions that cannot be completely sealed under protective clothing should not handle any potentially infectious materials.

- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Remove wristwatches and jewelry before working at the bench to facilitate hand washing and prevent puncturing of gloves.
- Store all potentially infectious materials in unbreakable, leakproof containers.
- Before leaving the laboratory or clean-up room for non-laboratory areas, remove protective clothing and leave it in the laboratory or clean-up room; wash your hands thoroughly.
- Do not use a gloved hand to write, answer the telephone, turn on a light, or touch anything that other people may touch without gloves.
- Synthetic gloves, such as nitrile, neoprene, and vinyl, are recommended because they are effective and contain no natural latex ingredients associated with latex glove allergic reaction.
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.
- Keep only materials needed for the day's procedures in the work area to prevent contamination of non-laboratory materials.
- Materials that cannot be properly decontaminated should not be exposed to potentially infectious material.
- Upon completion of the operations involving biohazardous materials, decontaminate the work area with an appropriate disinfectant (e.g., 1:10 dilution of household bleach).
- If material becomes contaminated with dried blood or other potential biohazard, decontaminate it and clean up any solid material before completing decontamination. The dried blood should be wetted and softened with diluted bleach (1:10 dilution) or detergent disinfectant. Carefully remove to prevent scattering potentially infectious material. After removal, disinfect the cleaned surfaces.

Biohazard Specific Precautions

The following precautions are necessary when handling and disposing of potentially biohazardous material:

- Solid and liquid waste from the D-100 System should always be considered potentially biohazardous and should be handled accordingly.
- All patient samples are potentially biohazardous and should be handled accordingly, using Universal Precautions.
- Liquid waste should be decontaminated on site if possible. It is recommended to mix liquid waste with household bleach (5% sodium hypochlorite) at a ratio of 1 part bleach to 10 parts waste. Let the mixture stand for at least 30 minutes before emptying.
- Treat all calibrator packs and controls as potentially biohazardous materials and handle accordingly.

Disposal of Biohazardous Materials

Dispose of the following potentially contaminated materials in accordance with local, regional, and national laboratory regulations:

- Clinical samples
- Reagents
- Calibrator packs
- Controls
- Used sample vials and other consumables (e.g., cartridges, prefilters) that may be contaminated

Sharp Biohazard

The sample probe is very sharp. Use caution when handling to avoid injury. The used sample probe should be considered potentially biohazardous; discard according to the laboratory standard operating procedures for biohazardous sharps.

Chemical Hazards

D-100 assay components may contain potentially harmful chemical materials. Follow all instructions for handling, storage, and disposal as described in the applicable assay Instructions For Use.

- Consult the Safety Data Sheets (SDS) for specific safety information.
- Do not smoke, eat, or drink in areas where reagents are handled.
- Wear personal protective equipment while handling all reagents.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Use caution when handling the following reagent:

CLN **TUBE** **Cleaning Tube**

Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1)

Danger

H314	Causes severe skin burns and eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P311	If exposed or if you feel unwell: call a poison center or doctor/physician.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Electrical Hazards

NOTE: *The main power cord at the rear of the instrument serves as the primary power disconnect. Do not position the system where it is difficult to disconnect the main power cord.*

- Do not remove instrument covers. There are no user-serviceable parts inside. Refer all servicing to Bio-Rad service personnel.
- Always follow basic safety precautions when using this instrument to reduce the risk of injury, fire, or electrical shock.
- Do not perform Hipot Testing (i.e., Dielectric Withstand Testing) on the instrument without contacting Bio-Rad Technical Service for assistance. The instrument power supply requires a temporary modification for this test to prevent damage to the unit.

Electrical and Electronic Waste Hazards

The Waste Electrical and Electronic Equipment (WEEE) Regulations implement provisions of the European Parliament and Council Directive 2002/96/EC aimed at reducing the amount of EEE waste going for final disposal. As the producer, Bio-Rad Laboratories, Inc. has specific instructions for the recovery of this instrument at the time of end of use. Please refer to the **EU Recycle Program** at www.bio-rad.com for the process applicable to your region.

Environmental Hazards

In the event of a flood or other natural disaster, contact Bio-Rad Technical Service before resuming use of the instrument.

Warning Regarding Cybersecurity on the D-100 System

Please observe the following precautions to protect the integrity of your D-100 System:

1. Do not attempt to install any third-party software.
2. Do not attempt to access the World Wide Web.
3. Before using any removable media (e.g., USB flash drive), ensure the media is free of malware.
4. If your D-100 System is connected to a network, it is your laboratory's responsibility to monitor and protect the network.

EQUIPMENT AUTHORIZATION AND REGULATORY COMPLIANCE

Federal Communications Commission (FCC) Compliance

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

This device complies with FCC RF radiation exposure limits set forth for general population (uncontrolled exposure). This device must be installed to provide a separation distance of at least 20 cm from all persons and must not be colocated or operating in conjunction with any other antenna or transmitter.

CAUTION: This equipment may not be modified, altered, or changed in any way without signed written permission from BIO-RAD Laboratories. Unauthorized modification may void the equipment authorization from the FCC and will void the BIO-RAD Laboratories warranty.

Industry Canada (IC) Compliance

WARNING TO USERS IN CANADA

This device complies with Industry Canada license-exempt RSS standard(s), and with ICES-003 (Class B) for its non-RF parts. Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.

Under Industry Canada regulations, this radio transmitter may only operate using an antenna of a type and maximum (or lesser) gain approved for the transmitter by Industry Canada. To reduce potential radio interference to other users, the antenna type and its gain should be so chosen that the equivalent isotropically radiated power (e.i.r.p.) is not more than that necessary for successful communication.

This device complies with Industry Canada RF radiation exposure limits set forth for general population (uncontrolled exposure). This device must be installed to provide a separation distance of at least 20 cm from all persons and must not be colocated or operating in conjunction with any other antenna or transmitter.

Contents

1 Introduction

1.1 General Description

The Bio-Rad D-100™ Hemoglobin Testing System performs automated analysis of whole blood samples within the clinical laboratory. With advanced automation and high-throughput operation, the D-100 generates high-quality results using high-performance liquid chromatography (HPLC) technology with an efficient and flexible testing workflow.

The D-100 System consists of a fully-integrated standalone workstation of automated HPLC instrumentation that includes an onboard computer. The software operating the instrumentation is controlled through a graphical user interface via an onboard touchscreen.

The D-100 is intended for use only with Bio-Rad D-100 assays. It must be operated by trained laboratory staff within a conventional laboratory environment.

Figure 1-1: *D-100 Hemoglobin Testing System*

Figure 1-2: *D-100 with Optional Internal Printer*

1.2 Principles of Operation

The D-100 uses the principles of high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative area percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

1 System Description

2.1 D-100 System Components

Figure 2-1: *D-100 Front View*

Figure 2-2: *D-100 Right Side View*

No.	Name	Function
❶	Touchscreen	The liquid crystal display (LCD) detects human touch to interact with the instrument software. To optimize visibility, the angle of the touchscreen can be adjusted by tilting the top of the screen forward or back. See Chapter 4 for a detailed description of the user interface.
❷	Internal Printer	The optional thermal printer prints sample results and reports. See Section 2.8.
❸	Soft Power Button 	The soft power button activates the instrument from the standby power mode to full operating power. The button LED is green when activated.
❹	Cartridge/Prefilter Compartment	This compartment houses the sample analysis components. See Section 2.7.
❺	Rack Handler	The rack handler consists of the Sysmex® rack processing components. See Section 2.4.
❻	Stat Area	The Stat Area includes components to process the Calibrator Pack and other special samples. See Section 2.2.
❼	Reagent Compartment	This compartment houses the assay reagents. See Section 2.3.

No.	Name	Function
❽	USB Ports	2 ports accommodate USB storage devices for exporting/storing data.
❾	Probe Compartment	This compartment houses the sample probe and supporting components. See Section 2.5.
❿	Low-Pressure Filter Compartment (not shown)	This compartment houses the sample dilution components and low-pressure filter. See Section 2.6.

2.2 Stat Area

Figure 2-3: *Stat Area with Stat Rack in the Loading Position*

Figure 2-4: *Stat Rack with Controls and Calibrator Pack in the Loading Position*

The Stat Area is used to run the Calibrator Pack, Controls, urgent patient samples, and the Cleaning Tube via the Stat rack. The Stat rack holds the Calibrator Pack and/or up to 3 samples in microvials or primary tubes. The Stat rack is anchored to the instrument, but can be removed for cleaning. See Section 3.6 for more information regarding the Stat rack.

The Stat Area has its own barcode reader to scan the barcodes on the adapters/tubes in the Stat rack for identification purposes. See Section 3.5 for information regarding barcode labels.

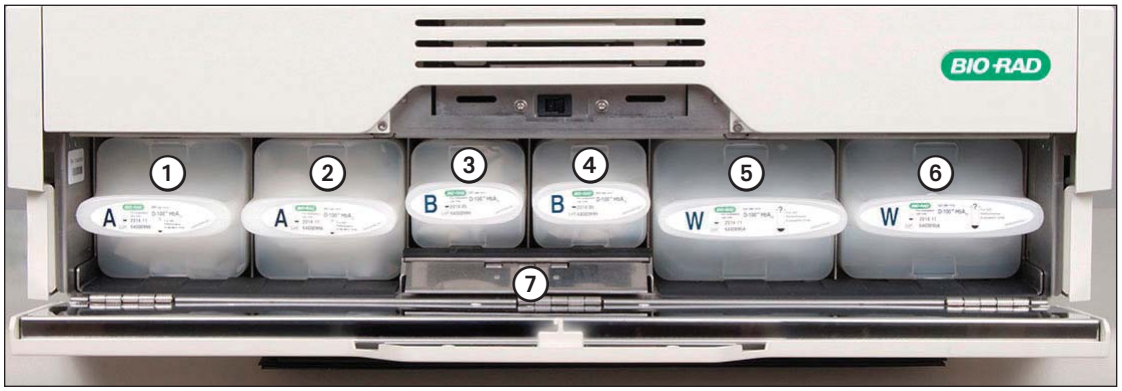
See Chapter 5 for instructions on running the Calibrator Pack (Section 5.3), Controls (Section 5.4), and Stat samples (Section 5.6). See Section 7.8 for instructions regarding running the Cleaning Tube.

2.3 Reagent Compartment

The reagent compartment houses the assay buffers (used to form the elution gradient) and wash solution (used for diluting samples and rinsing the sample probe). The system uses positive pressure to fill each bottle with air, squeezing the reagent from the interior bag.

The D-100 holds 2 bottles of each reagent; however, only one bottle is in use at a time. The system automatically switches to the second bottle when the first is empty, allowing the empty bottle to be removed without stopping the run.

Figure 2-5: *Reagent Compartment (Door Open)*

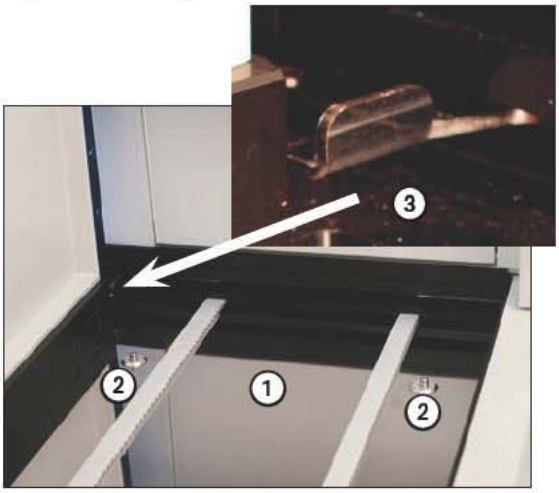


No.	Name	Function
1	Buffer A Bottle 1 Compartment	Holds 1 bottle of Elution Buffer A.
2	Buffer A Bottle 2 Compartment	Holds 1 bottle of Elution Buffer A.
3	Buffer B Bottle 1 Compartment	Holds 1 bottle of Elution Buffer B.
4	Buffer B Bottle 2 Compartment	Holds 1 bottle of Elution Buffer B.
5	Wash Bottle 1 Compartment	Holds 1 bottle of Wash Solution.
6	Wash Bottle 2 Compartment	Holds 1 bottle of Wash Solution.
7	Leak Tray Compartment	Holds tray to collect any fluid leaking from the system.

2.4 Rack Handler

Figure 2-6: *Rack Input and Output Areas*

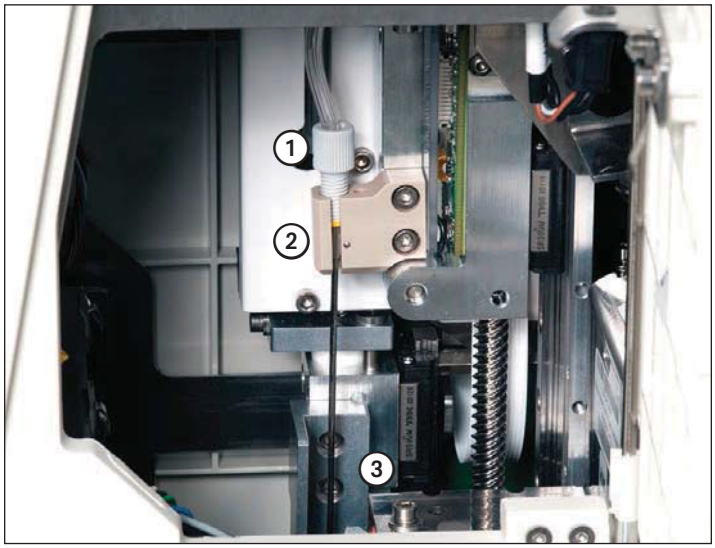
Figure 2-7: *Stopper Pins and Shuttle*



No.	Name	Function
❶	Rack Input Area	Sysmex racks are placed in the right rack input area; the conveying belts move the racks to the sampling area.
❷	Stopper Pins	Racks are placed anywhere between the pins and the front of the instrument. The stopper pins retract to release the rack into the shuttle.
❸	Shuttle	The shuttle moves the rack into and out of the sampling area.
❹	Tube Spinner (not shown)	The tube spinner rotates sample tubes and microvials in the rack to check for barcodes.
❺	Barcode Reader (not shown)	The barcode reader scans the barcodes from the racks, sample tubes, microvials, and/or microvial adapters. The information is included with the sample result for identification purposes. See Section 3.5 for information regarding barcode labels.
❻	Rack Output Area	The forward pusher moves the Sysmex racks away from the sampling area to the left rack output area after processing is complete.

2.5 Probe Compartment

Figure 2-8: *Probe Compartment (Door Open)*

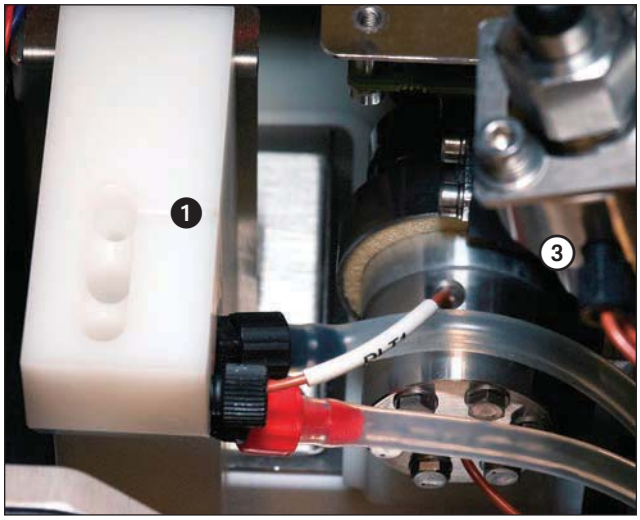


NOTE: *The probe door is interlocked. When open, the interlock switch shuts off power to the probe assembly.*

No.	Name	Function
❶	Sample Probe	The sample probe pierces each primary tube to release the vacuum and to aspirate sample from the tube. The probe then dispenses the sample with a measured amount of diluent (Wash Solution) into the dilution well. The sample probe and dilution well are rinsed between samples to prevent cross-contamination. The sample probe also pierces the microvials to aspirate sample.
❷	Probe Carrier	The probe carrier secures and moves the probe.
❸	Tube Holder	The tube holder detects the sample tube/adapter in the rack, aligns it, and stabilizes the tube/microvial during probe piercing.

2.6 Low-Pressure Filter Compartment

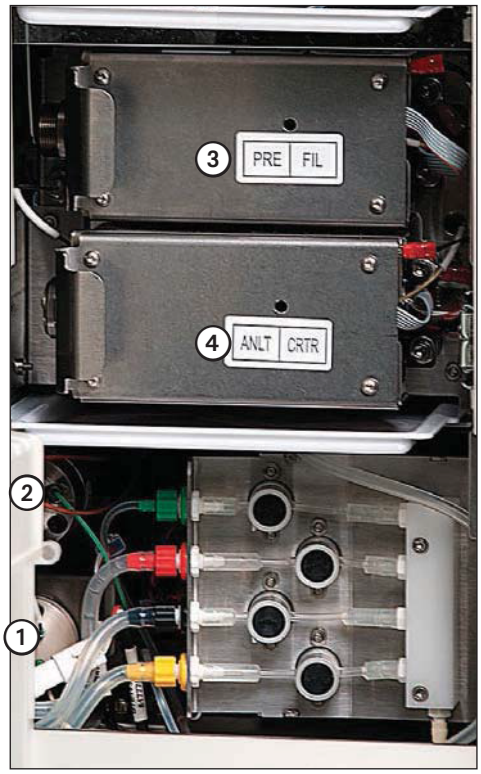
Figure 2-9: *Low-Pressure Filter Compartment (instrument cover removed for visibility)*



No.	Name	Function
❶	Dilution Manifold	The dilution manifold includes the dilution well (front), overflow well (center), and wash well (rear). The dilution well is where the sample is diluted before introduction into the analytical path. The wash well is where the sample probe is rinsed. The dilution manifold is not user-accessible.
❷	Dilution Pump (not shown)	The dilution pump consists of 2 syringes. The sample is aspirated from the tube/microvial via the sample probe using the first syringe. The sample is dispensed into the dilution well where it is diluted to the required concentration.* The second syringe introduces the diluted sample into the fluid circuit through the low-pressure filter. ***NOTE:** *The dilution step is omitted for prediluted samples in microvials.*
❸	Low-Pressure Filter	The low-pressure filter protects the downstream valves from sample particulates. This filter is replaced only by Bio-Rad service personnel.

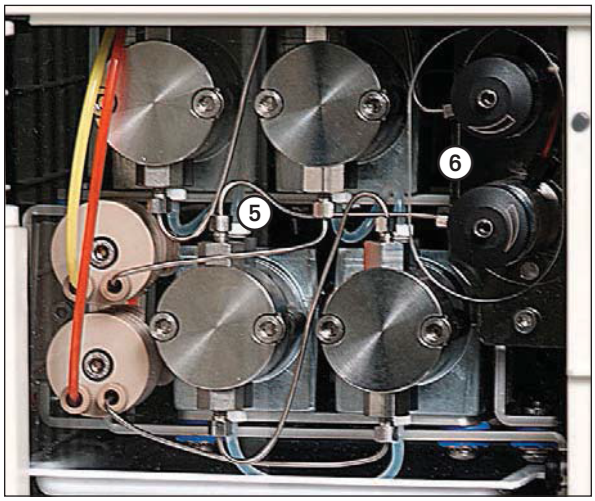
2.7 Cartridge/Prefilter Compartment

Figure 2-10: *Cartridge/Prefilter Compartment (Door Open)*



No.	Name	Function
❶	Rotary Valve	The rotary valve directs the flow of the sample into the fluid circuit and into the sample loop. It has two possible positions: • The first position creates a fluidic path between the second syringe and the dilution well through the low-pressure filter. • The second position creates a fluidic path between the second syringe and the injection valve.
❷	Injection Valve	The injection valve contains two 5-µL internal sample loops and switches from load position to inject position. The diluted sample is loaded into one sample loop and then injected into the analytical flow path. While the sample is being injected, the second loop is flushed with Wash Solution and loaded with the next sample.
❸	Prefilter Holder	The prefilter holder houses the prefilter, which protects the analytical cartridge from sample particulates. The LED indicates whether the prefilter holder door is open (green) or closed (red).
❹	Cartridge Holder	The cartridge holder houses the analytical cartridge, which is responsible for the hemoglobin separation. An attached Peltier device provides temperature control. The LED indicates whether the cartridge holder door is open (green) or closed (red).

Figure 2-11: *High-Pressure Pumps*



No.	Name	Function
❺	High-Pressure Pumps	There are two dual-piston reciprocating pumps that generate the buffer gradient. • Pump A (upper) delivers Elution Buffer A. • Pump B (lower) delivers Elution Buffer B.
❻	Purge Valves	Each pump has a purge valve that allows manual priming of the buffer inlet line. • Purge valve A (upper) is used to prime Elution Buffer A. • Purge valve B (lower) is used to prime Elution Buffer B.
❼	Detector (not shown)	The detector measures the absorbance of the sample constituents at 415 nm.

2.8 Internal Printer

Figure 2-12: *Printer (Door Open)*

No.	Name	Function
1	Paper Feed Button	Press this button to advance the paper.
2	Printer Settings Button	For Service use only.
3	Printer Power LED	Green light indicates the printer is on.
4	Printer Door LED	Red light indicates the printer paper door is open.
5	Printer Error LED	Red light indicates a printer problem. • 1 flash = Printer memory is full and cannot receive additional data. • 2 flashes = Printer head temperature too high. • 3 flashes = Entering configuration mode. • Permanent blink = No paper or paper door is open. • 1 long flash and 1 short flash = Printer head failure.
6	Printer Paper Door Latch	Push down the latch to open the printer paper door.
7	Printer Paper Input Well	The paper roll is inserted in the input well.
8	Printer Paper Output Well	The printout rolls up inside the output well.

2.9 D-100 Rear Components

Figure 2-13: *D-100 Left Rear View*

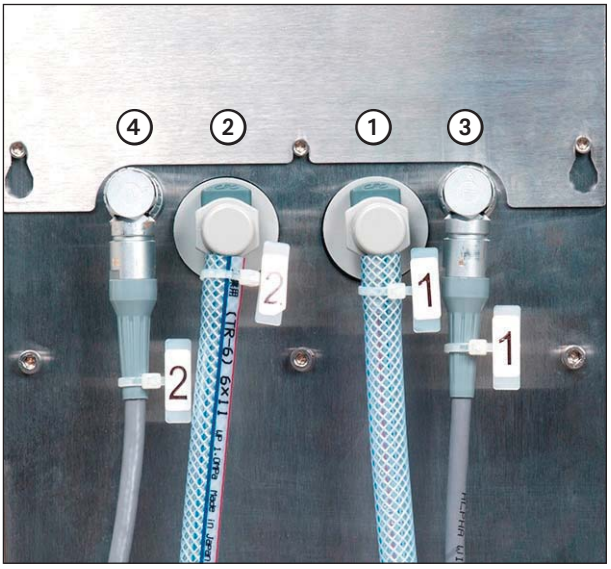
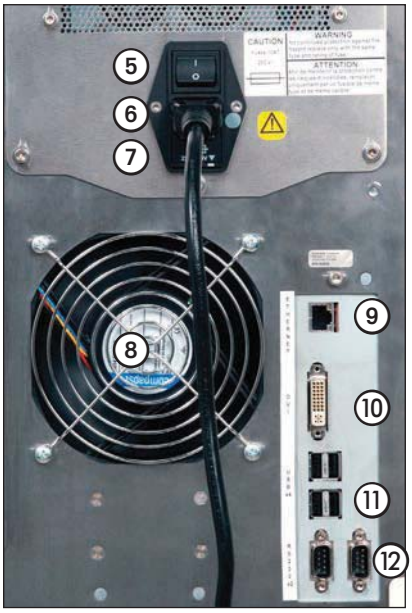


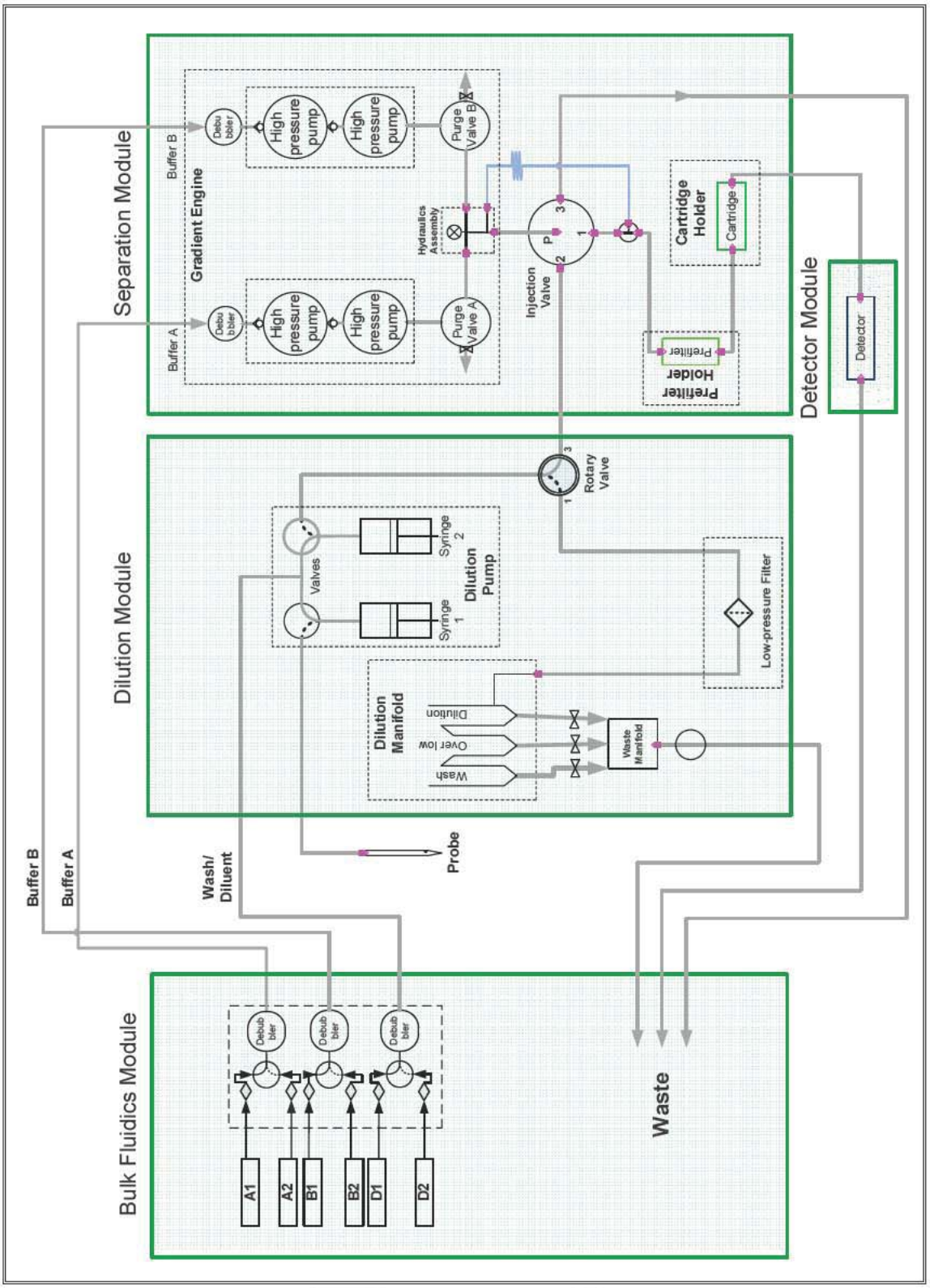
Figure 2-14: *D-100 Right Rear View*



No.	Name	Function
❶	Waste Port 1	This port is for connection of the external waste tubing to Waste Bottle 1 or to a drain.
❷	Waste Port 2	This port is for connection of the external waste tubing to Waste Bottle 2 or to a drain.
❸	Waste Sensor Port 1	This port is for connection of the level sensor cable to Waste Bottle 1, or a waste sensor connector when the lab drain is used.
❹	Waste Sensor Port 2	This port is for connection of the level sensor cable to Waste Bottle 2, or a waste sensor connector when the lab drain is used.
❺	Power Switch	The power switch controls power to all system components.
❻	AC Power Input	The power input allows connection of a 3-conductor modular cord with ground to a suitable power source (110 VAC or 220 VAC).
❼	Fuse Holder	The 2 main power fuses provide over-current protection.
❽	Main Fan	The fan expels warm air away from the components.
❾	LAN Port	This port is for connection to a Local Area Network.
❿	DVI Port	Digital visual interface (DVI) port for connection to an external display.
⓫	USB Ports	These 4 ports are for connection of USB storage devices, printer, keyboard, or mouse.
⓬	Serial Ports	These 2 ports are for connection of an RS-232 cable to an LIS interface.

2.10 D-100 Fluid System

Figure 2-15: *D-100 Fluidics Diagram*



1 Installation

NOTE:

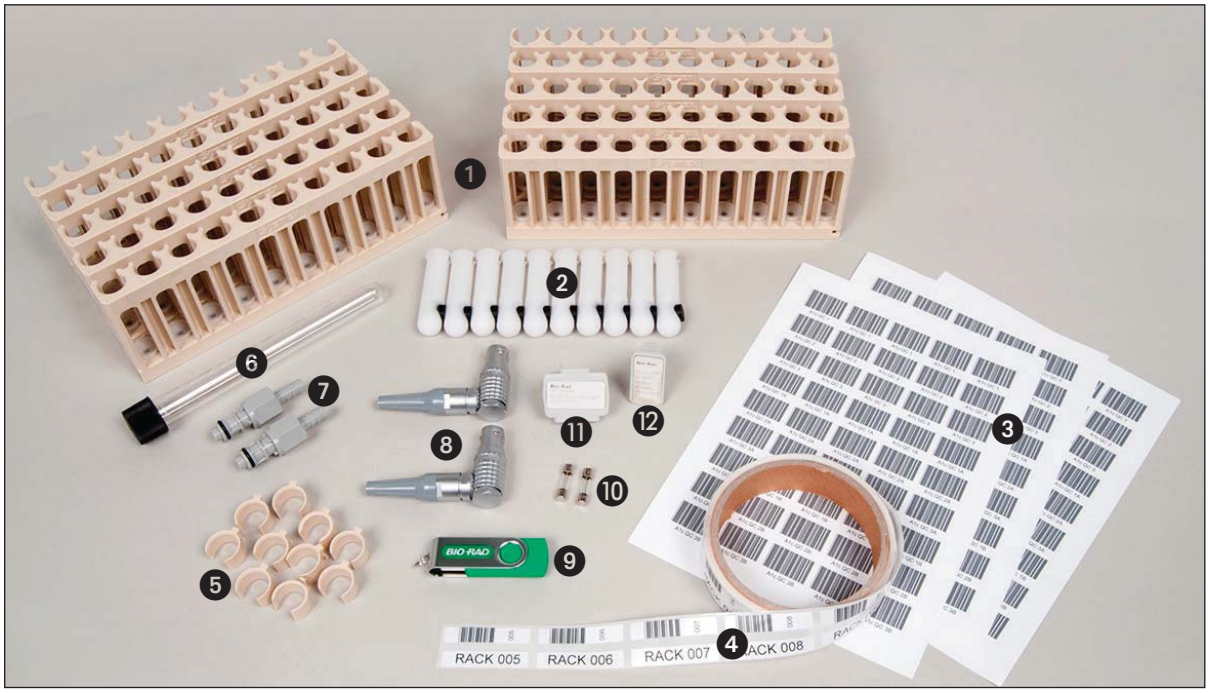
- *Installation of the D-100 System should be performed only by an authorized Bio-Rad representative; installation by any other person invalidates the system warranty.*
- *To move the D-100 System, contact your local Bio-Rad office for assistance.*

3.1 Installation Requirements

1. Choose a location for the system away from direct sunlight and relatively dust-free.
2. Room temperature should be 15–35 °C.
3. The bench or table should have a flat, level surface free from vibrations and capable of supporting 121 kg (266 lb).
4. The system requires a minimum benchtop space (W x D x H) of 66 x 65 x 73 cm (26 x 26 x 29 in.).
5. Maintain a minimum clearance of 13 cm (5 in.) on the left side, right side, and rear of the instrument.
6. A grounded electrical outlet should be within 1.8 m (6 ft) of the system. The maximum power consumption of the system is 1100 VA. See Appendix B for specifications.

3.2 Accessories

Figure 3-1: *D-100 Accessories*



No.	Description	Qty
1	Sysmex Racks	10
2	Microvial Adapters	10
3	Microvial Adapter QC Barcode Labels (3 sheets of 3 levels)	3
4	Sysmex Rack Barcode Labels (1 roll of 100)	1
5	13-mm Sysmex Rack Inserts, preinstalled in racks	100
6	Sample Probe	1
7	Waste Tubing Bottle Connector	2

No.	Description	Qty
8	Waste Sensor Connector	2
9	8GB USB Flash Drive	1
10	Spare Fuses	2
11	Utility Cartridge, shipped installed on instrument	1
12	Utility Prefilter, shipped installed on instrument	1
13	D-100 Operation Manual with Multi-Language CD (not shown)	1

3.3 Waste Bottles

There are 2 external waste bottles included with the D-100 to collect the liquid waste from the instrument. The external waste bottle tubings and level sensor cables are connected to the rear of the instrument (see Figure 2-13). See Section 7.1 for instructions on emptying the waste bottles.

NOTE: *Alternatively, the external waste tubing can be connected to a laboratory drain.*

Figure 3-2: *Waste Bottle*

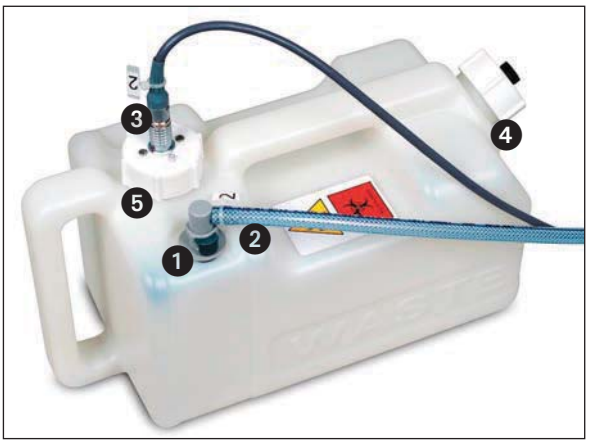
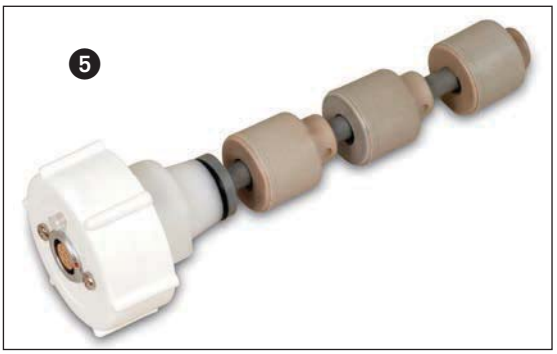


Figure 3-3: *Waste Level Sensor*



1. External Waste Quick-Disconnect
2. External Waste Tubing
3. Level Sensor Connector
4. Waste Bottle Cap
5. Level Sensor

3.4 Sysmex® Racks

Sysmex racks are used to hold all routine samples to be tested by the D-100. The racks include rotary bottoms in each tube position to allow rotation of primary sample tubes for barcode reading. A maximum of 10 samples can be loaded into each rack. Sysmex racks are placed in the rack input area with the Sysmex logo facing you.

Figure 3-4: *Sysmex Rack, Front View*

NOTE: *If you use sample racks from another system that do not include rotary bottoms, tube spinning must be disabled. See Section 4.7.6.*

3.4.1 Sysmex Rack Inserts

The Sysmex racks are designed to hold 16-mm sample tubes. For proper alignment of smaller diameter tubes (i.e., 12 mm, 13 mm, and 14 mm), plastic inserts must be inserted into the racks. 13-mm inserts are included in the D-100 Accessories Kit; other insert sizes can be purchased from Bio-Rad.

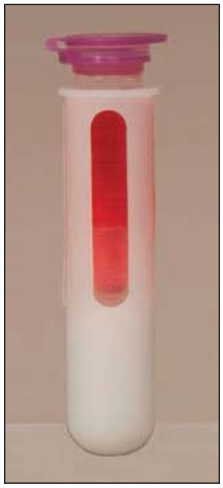
Figure 3-5: *Insert for Small Diameter Tubes*

3.4.2 Microvial Adapters

If the sample is in an abnormal size/type tube, or if the height of the sample in the tube appears to be ≤1 cm, then the sample must be prediluted in a 1.5-mL microvial prior to analysis. See the assay Instructions For Use for Specimen Preparation instructions.

For proper alignment of 1.5-mL microvials, microvial adapters must be used in the rack. See Figure 3-6.

Figure 3-6: *Microvial Adapter with Prediluted Sample*



The D-100 recognizes the microvial adapter by sensing the adapter's attached magnet and bypasses the sample dilution process. The microvial adapter must be positioned in the Sysmex rack so that the magnet faces the back of the rack.

Figure 3-7: *Adapters Inserted in Sysmex Rack, Rear View*

3.5 Barcode Labels

To correctly identify the Sysmex racks and samples, barcode labels are required in the proper locations.

NOTE: *A starter set of Sysmex rack and QC microvial adapter barcodes is provided with the system. Additional barcodes can be purchased from Bio-Rad.*

- Sysmex rack barcodes must be affixed on the rear of the rack, between the first and second sample tube positions, higher than 18 mm from the bottom of the rack; see Figure 3-8 for correct label placement. The corresponding human-readable rack number label should be affixed on the front of the rack (see Figure 3-4).

  Figure 3-8: *Rack Barcode Label on Rear of Sysmex Rack*

- QC barcodes must be affixed to the microvial adapters above the magnet with the barcode lines running horizontally, so that when the adapter is in the Sysmex rack, the barcode faces the back of the rack. See Figure 3-7.
- Patient samples may be labeled with your site-specific barcode labels. See Section B.7 for supported barcode symbologies. Barcodes on the primary sample tubes and prediluted sample microvials should be checked for quality to ensure correct identification by the barcode reader; there is a risk of sample misidentification if the barcode print quality is poor. Barcoded primary sample tubes and prediluted sample microvials do not require particular orientation in the Sysmex rack; they are rotated by the tube spinner for barcode reading.

  NOTE: *If you use sample racks from another system that do not include rotary bottoms, tube spinning must be disabled. See Section 4.7.6. The primary sample tubes must be positioned in these racks so that the barcodes face the back of the rack, toward the instrument.*

3.6 Stat Rack

A maximum of 3 samples (in primary tubes or microvials) and/or the Calibrator Pack can be loaded into the Stat rack. Samples can be loaded in positions 1–3 only; the Calibrator Pack can be loaded in positions 4–6 (dedicated Calibrator Pack position) only.

NOTE: *Samples are not rotated in the Stat Area. You must align the barcode labels for proper scanning when loading the Stat rack. Barcode labels must face you so they are visibly displayed through the rack slots.*

Figure 3-9: *Stat Rack with Controls, Patient Sample, and Calibrator Pack*

3.7 Test Component Identification

Radio-Frequency Identification (RFID) technology is used to automatically identify and track most test components. The following test components have an RFID tag attached that is encoded with information specific to the component.

Figure 3-10: *Test Component RFID Tag*

Test Component	RFID Information
Elution Buffers A and B	• Catalog number • Lot number • Expiration date • Open stability • Capacity (volume or number of tests) • Date of first use • Remaining volume or number of tests
Wash Solution	
Prefilter	
Analytical Cartridge	Includes all information listed above, plus the following: • Serial number • Test parameters • Buffer and Calibrator Pack compatibility information

Other test components have barcode labels with information specific to the component:

Test Component	Barcode Information
Calibrator Pack	• Lot number • Expiration date • Assigned calibrator values

NOTE: *Some component labels include an SN (Serial Number) or T# (Tracking Number). These numbers are for internal tracking purposes only.*

3.8 Replacing the Prefilter

NOTE: *When the prefilter is removed, a small amount of liquid may drip. Use a paper towel to absorb any drips. Never leave the instrument without a prefilter installed.*

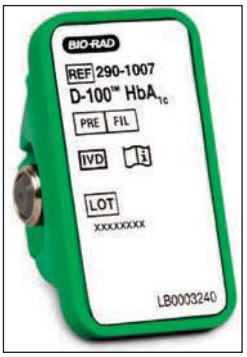
1. The instrument must be in Sleeping state.
2. Open the cartridge/prefilter compartment door to access the prefilter holder.

Figure 3-11: *Prefilter Holder*

3. Grasp the handle of the prefilter holder door and pull it open.

Figure 3-12: *Prefilter Holder (Door Open)*

Figure 3-13: *Prefilter*



4. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
5. Remove a new prefilter from its package; remove the black caps.
6. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.

   NOTE: *The prefilter information is automatically updated when the RFID is read.*
7. Close the prefilter holder door.
8. Close the cartridge/prefilter compartment door.

3.9 Replacing the Analytical Cartridge

NOTE: *When the cartridge is removed, a small amount of liquid may drip. Use a paper towel to absorb any drips. Never leave the instrument without a cartridge installed.*

1. The instrument must be in Sleeping state.
2. Open the cartridge/prefilter compartment door to access the cartridge holder.

Figure 3-14: *Cartridge Holder*

3. Grasp the handle of the cartridge holder door and pull it open.

Figure 3-15: *Cartridge Holder (Door Open)*

Figure 3-16: *Analytical Cartridge*

4. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
5. Remove a new cartridge from its package; remove the black caps.
6. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.

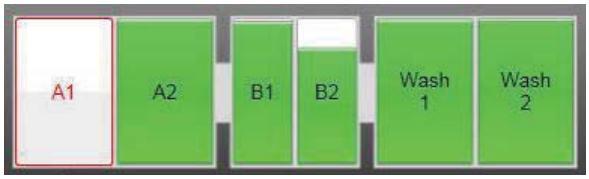
   NOTE: *Test parameters are automatically updated when the RFID is read.*
7. Close the cartridge holder door.
8. Close the cartridge/prefilter compartment door.

3.10 Replacing Reagents

A reagent bottle can be removed and replaced at any time, except when the bottle is "In Use" while the instrument is in Running state (i.e., the Remove button is disabled).

3.10.1 Replacing an Empty Reagent Bottle

Figure 3-17: *Consumables Panel, Bottle A1 Empty*



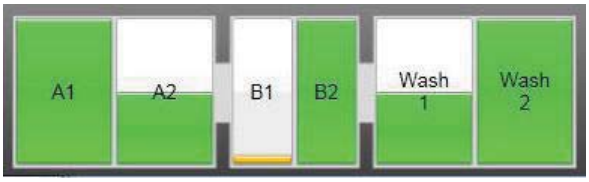
1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.

   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

NOTE: *If you install a bottle of reagent that is not full while the instrument is in Running state, the message panel displays a white message "Partial bottle will be loaded after the run", the reagent indicator appears blank, and the reagent dialog box indicates "Not Ready". When the instrument transitions to Standby after the run, the bottle will be pressurized, and both the indicator and dialog will update.*

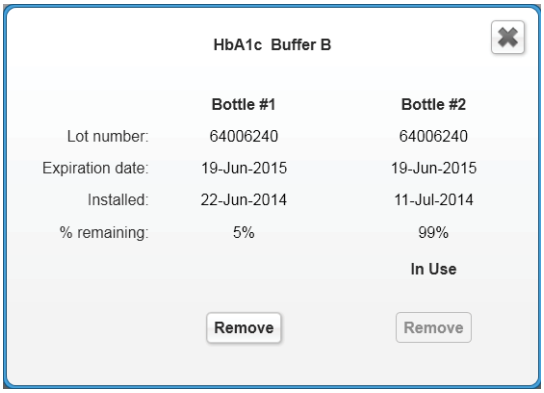
3.10.2 Replacing a Non-Empty Reagent Bottle

Figure 3-18: *Consumables Panel, Bottle B1 Low*



1. Touch the reagent indicator in the consumables panel for the bottle you want to replace.

Figure 3-19: *HbA1c Buffer B Dialog Box, Bottle B1 5% Remaining*



2. In the reagent dialog box, touch Remove to depressurize the bottle.

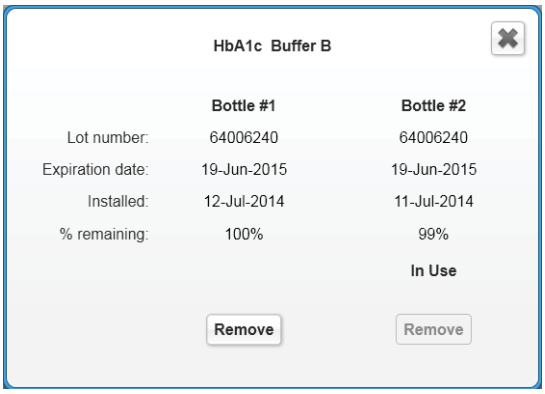
Figure 3-20: *HbA1c Buffer B Dialog Box, Bottle B1 Depressurized*

3. After depressurization, the dialog box indicates "Please Remove".
4. Open the reagent compartment door.
5. Remove the bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.

Figure 3-21: *HbA1c Buffer B Dialog Box, Bottle B1 Removed*

6. After removing the bottle, the reagent information is blank.

Figure 3-22: *HbA1c Buffer B Dialog Box, New Bottle B1 Installed*



7. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.

   NOTE: *The reagent information is automatically updated when the RFID is read.*
8. Close the compartment door.
9. Touch ✖ to close the dialog box.

3.11 Replacing Internal Printer Paper

1. Open the green printer door.
2. Push down the latch to open the printer paper door.
3. Remove the remaining paper roll and/or core. Ensure the paper well is free from debris, which may cause the printer to jam.
4. Remove the adhesive strip from a new roll of thermal paper.
5. Position the roll in the input well so that the paper exits the top of the roll towards you.

Figure 3-23: *Positioning the Roll of Paper*

6. Pull the paper towards you and close the printer paper door, with the paper exiting through the slot above the door.
7. Press the paper feed button  to feed the paper through the printer.
8. Place the starting edge of the paper in the paper output well so that the printout rolls up neatly inside the well.
9. Close the green printer door.

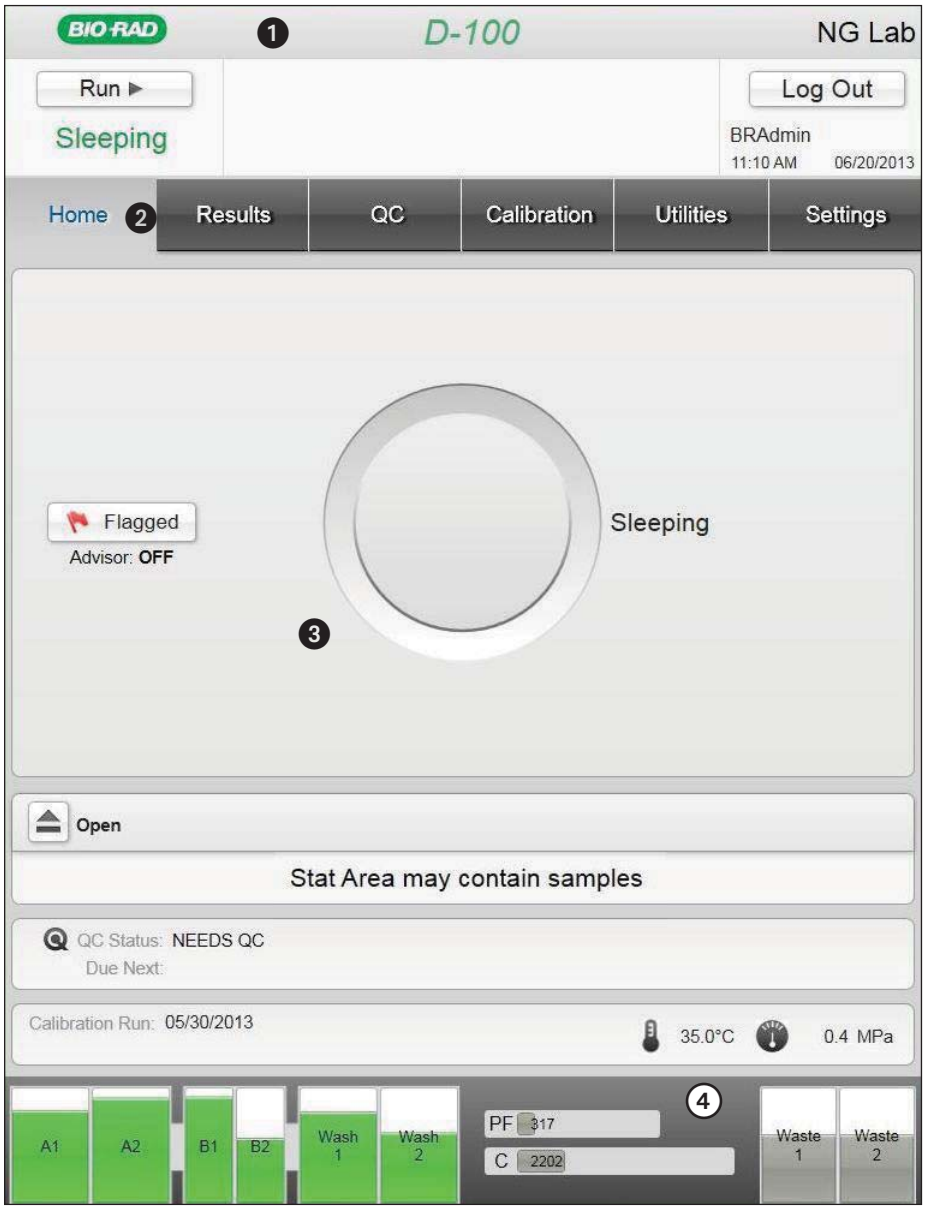
1 Software Overview

4.1 User Interface Layout

The user interface has 4 main areas:

1. Banner
2. Navigation Controls
3. Workspace
4. Consumables Panel

Figure 4-1: *User Interface Layout, Home Tab*



4.1.1 Banner

Figure 4-2: *Banner*

1	System Name (See Section 4.7.1)
2	**Run/Stop/Resume/Reset** button: This toggle button is used to start, stop, and resume runs and to reset the system.
3	Instrument State (See Section 4.1.1.1)
4	***Figure 4-3:*** *Message Panel, Information-Only Message*

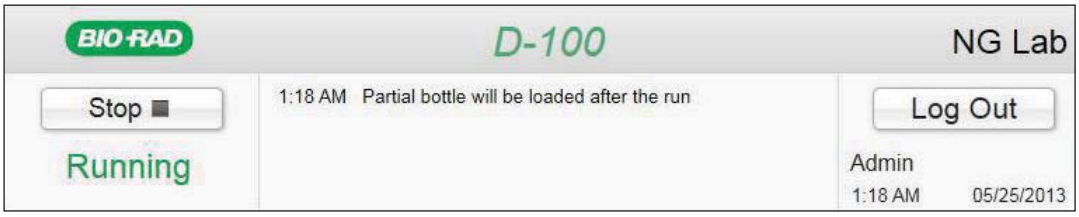


Figure 4-4: *Message Panel, Warning Messages*

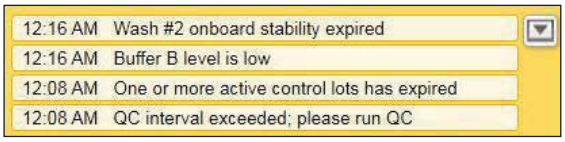
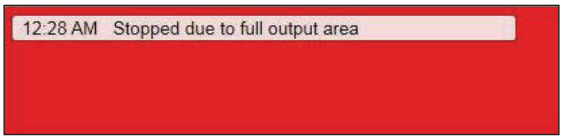


Figure 4-5: *Message Panel, Critical Message*

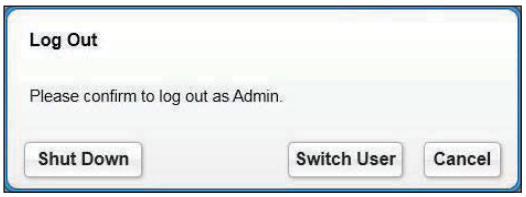


Message Panel: The instrument generates messages to alert you to important conditions. These messages are displayed in this area of the banner, which is color-coded based on the urgency of the message.

- For information-only messages (e.g., Waiting for cartridge information, Partial bottle will be loaded after the run, etc.), the message panel is white. Information-only messages do not need to be acknowledged.
- For warning messages (e.g., Buffer level is low, lot expired, printer error, etc.), the message panel is yellow. Warning messages require user action soon to remain running, or indicate that an important function is not working. When warning messages require user action, the instrument senses when the action is taken and clears the message.
- For critical messages (e.g., Buffer is empty, Stopped due to full output area, Calibration required-new cartridge installed, etc.), the message panel is red and the run is disabled or stopped. Urgent messages require user action to start or resume the run.

NOTE: *When the message panel contains more messages than fit in the display area, touch* ▼ *to extend the panel to view all messages; touch* ▲ *to condense the panel.*

<table>
<tr><td>❺</td><td colspan="2">Log In/Log Out button: This toggle button is used to log in, log out, switch users, and shut down the system.</td></tr>
<tr><td></td><td>Figure 4-6: Log In Dialog Box
 

NOTE: Touch Cancel to close the dialog box without taking action.</td><td><u>To log in:</u>
1. Touch Log In.
2. In the Log In dialog box, touch the User Name field or drop-down arrow to enter your user name.
3. Touch the Password field to type your password.
NOTE: User names are not case-sensitive; passwords are case-sensitive.
4. Touch Log In.</td></tr>
<tr><td></td><td>Figure 4-7a: Log Out Dialog Box (when user is logged in)
 
</td><td><u>To log out:</u>
1. Touch Log Out.
2. In the Log Out dialog box, touch Log Out.</td></tr>
<tr><td></td><td>Figure 4-7b: Log Out Dialog Box (when Default user is enabled)
 
</td><td><u>To switch the user:</u>
If the Default user feature is enabled (see Section 4.7.3), but you want to log in as the user, you can switch the user.
1. Touch Log Out.
2. In the Log Out dialog box, touch Switch User.
3. The Log In dialog box appears. Continue to log in as instructed above.</td></tr>
<tr><td></td><td colspan="2"><u>To shut down the D-100 System:</u>
1. Touch Log Out.
2. In the Log Out dialog box, touch Shut Down.</td></tr>
<tr><td>❻</td><td colspan="2">User Name: The user currently logged in to the system.</td></tr>
<tr><td>❼</td><td colspan="2">Current time and date (See Section 4.7.1 to select format.)</td></tr>
</table>

4.1.1.1 Instrument States

Instrument State	Description
Sleeping	The HPLC core (i.e., cartridge heater and detector) is cold and the system is in the low-fluidic mode. The system enters Sleeping state if a run is not initiated within 2 hours. You can access and use features of the user interface while in Sleeping state. Touch Run ► to go to Warming Up state (or Running state if samples are loaded).
Warming Up	This is a transitional state between Sleeping and Standby. During this state, buffer is flushed through the fluid path, the detector LED and cartridge heater warm up, the buffer and waste levels are checked, and the calibration of the system is confirmed.
Standby	The system enters Standby (i.e., ready-to-run) state under the following conditions: • The system has completed an automatic warm-up. • The system is finished warming up but there are no samples available to run. • The system has finished running samples and there are no other samples available to run. The HPLC core is hot and the system is in the low-fluidic mode. Touch Run ► to go to Running state.
Running	The system is processing samples or actively waiting for samples.
Paused	The system enters Paused state under the following conditions: • The system is out of reagents, waste space, or output space. The system remains in this state until you replenish the resource and touch Resume ►.
Stopping	This transitional state occurs under the following conditions: • The user has touched Stop ■ and the system is processing the last samples that have already been queued. Touch Run ► to return to Running state. • The system has encountered an error and is attempting to process samples already aspirated and ejecting all racks from the shuttle. The **Run** button is briefly disabled (appears dimmed) until the shuttle is empty.
Stopped	The system has stopped processing samples and the output area is full. During this state, the **Run** button is briefly disabled (appears dimmed) until the shuttle is empty.
Cooling Down	The system components are turned off and in the process of cooling down before transitioning to Sleeping state.
Maintenance	The system is performing a maintenance operation. After the maintenance operation is completed, the system transitions to Sleeping state.
Fault	The system has encountered a critical error and has stopped all processing. The system remains in this state until you resolve the error and touch Reset ►. The system then transitions to Initialization state.

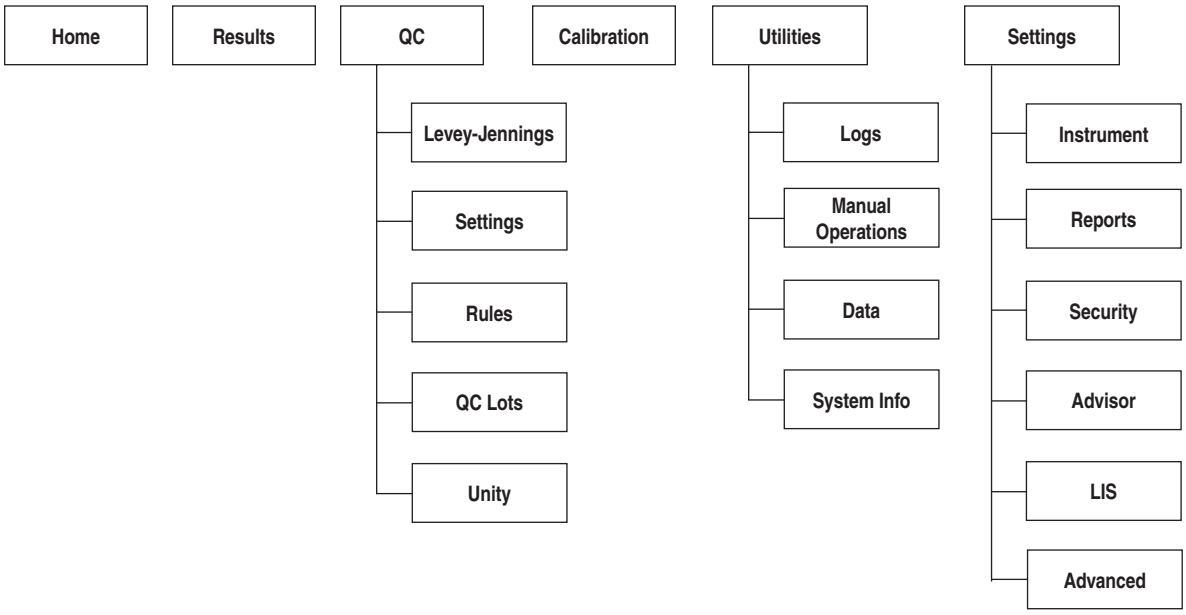
Instrument State	Description
Initialization	This transitional state occurs between powering on the instrument and Sleeping, and between Fault and Sleeping. The system initializes all hardware and software to a functional state.

4.1.2 Navigation Controls

Figure 4-8: *Navigation Controls, QC/Levey-Jennings Selected*

❶	Primary navigation tabs: The **Home, Results, QC, Calibration, Utilities,** and **Settings** tabs are used to move between the main function screens.
❷	Secondary navigation buttons: The **QC, Utilities,** and **Settings** tabs include secondary buttons that are used to move between the subfunction screens.

Figure 4-9: *Screen Navigation Diagram*



For ease of reference in this manual, subscreen names are written with the name of the main screen first, followed by the subscreen name, separated by a forward slash (e.g., **QC/Levey-Jennings**).

The selected tab and button are highlighted to indicate the screen currently displayed.

4.1.3 Workspace

The selected primary tab and secondary button determine what information is displayed in the workspace. This area is where data is entered, displayed, and managed.

Figure 4-10: *Workspace, Home Tab*

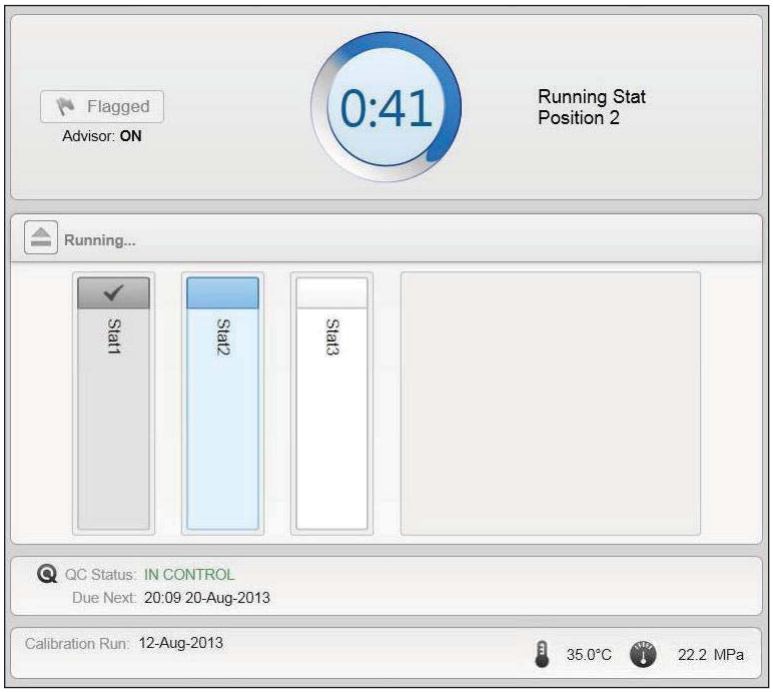


Figure 4-11: *Keyboard*

NOTE: *Touch* ✖ *to close the keyboard without saving the entry.*

The user interface provides on-screen character input devices when needed.

When you touch a text field, a keyboard displays at the bottom of the screen to allow alphanumeric data entry. The text field is replicated above the keyboard so you can see the text entered as you touch the keys.

- Use the **Shift** key to switch from lower case and numbers to upper case and symbols.
- Touch **Enter** to save the entry and close the keyboard.

Figure 4-12: *Number Pad*

NOTE: *Touch* ✖ *to close the number pad without saving the entry.*

When you touch a value field, a number pad displays at the bottom of the screen to allow numeric data entry. The value field is replicated above the number pad so you can see the number entered as you touch the keys.

- Touch **Enter** to save the entry and close the number pad.

Figure 4-13: *Month/Day Calendar*

Figure 4-14: *Year/Month Calendar*

Figure 4-15: *Year Calendar*

When you touch a date field, a calendar displays on the screen to allow selection of a date.

1. To change the month, use the arrows to scroll backward or forward.
2. To change the year, touch the month/year in the header to open the year/month calendar.
   - Touch the year in the header to open the year calendar and use the arrows to scroll through decades to find the year.
   - Touch the appropriate year, then touch the appropriate month.
3. Touch the appropriate date.

NOTE: *The selected month/year/date are highlighted blue.*

4. Touch **OK** to save the entry and close the calendar.

NOTE: *Touch* ***Cancel*** *to close the calendar without saving the entry.*

4.1.4 Consumables Panel

The consumables panel displays the status of the test consumables.

Figure 4-16: *Consumables Panel*

Remaining level in Buffer A1/A2 bottles.	Remaining level in Buffer B1/B2 bottles.	Remaining level in Wash 1/2 bottles.	Number of tests completed on the Prefilter (PF) and Analytical Cartridge(C).	Level in Waste 1/2 bottles.

NOTE:

- *If a reagent is not installed, the indicator appears blank.*
- *If an empty reagent bottle is installed, the indicator appears blank.*
- *If the Waste tubings are connected to a drain, both Waste indicators appear blank.*

Indicator color-coding helps alert you to the status of each consumable:

Consumable	Color Code			
	Green	Gray	Yellow	Red
Buffer A, Buffer B, Wash	>10–100% remaining	NA	≤10% remaining	Empty
Prefilter	NA	Tests remain	Exceeded recommended number of tests	No Prefilter installed
Cartridge	NA	Tests remain	Exceeded recommended number of tests	No Cartridge installed
Waste	NA	0–90% full	>90% full	Full

In addition to the consumables panel indicators, warning messages will appear in the message panel at the top of the screen, alerting you when the total volume (i.e., bottle 1 + 2) of each reagent is low (yellow message) and empty (red message), when the cartridge or prefilter is past the recommended number of tests (yellow message), and when the waste level is full (red message). See Section 4.1.1.

Figure 4-17: *Consumables Panel, Reagent Dialog Box*

Touching a reagent icon opens a dialog box indicating the lot number, expiration date, date installed, and % remaining. It also indicates if the reagent bottle is "In Use" or "Empty".

See Section 3.10 for more information.

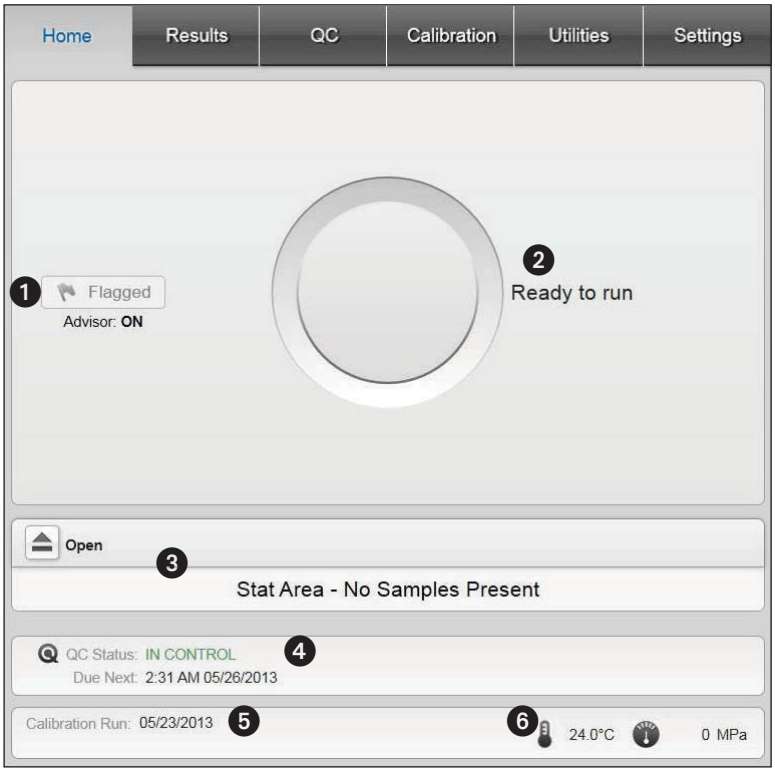
NOTE:

- *If there is no bottle installed, there is no reagent information displayed.*
- *A bottle cannot be removed if it is "In Use" while the instrument is in Running state (i.e., the* Remove *button is disabled).*

4.2 Home Screen

The **Home** screen indicates the status of the calibration, QC, and samples being processed. It also indicates the cartridge temperature and pump pressure. The Stat Area is controlled and displayed on this screen. There are 6 main areas:

Figure 4-18: *Home Screen*



NOTE: *The upper pane of the **Home** screen (which includes the Advisor Status and Instrument Status) expands when the Stat Area contains no samples; the upper pane contracts when the Stat Area is open (i.e., Stat rack is ejected) or contains samples.*

1. Advisor Status
2. Instrument Status
3. Stat Area
4. QC Status
5. Calibration Status
6. Temperature and Pressure

4.2.1 Advisor Status

1	**Flagged** button: This button is enabled (flag is red) when one or more sample results meet the **Flagged** filter criteria defined in the **Results** screen. Touching the button takes you to the **Results** screen, where results have been filtered to display only flagged samples. See Section 4.3.1 for information regarding the **Flagged** filter.
2	Advisor: **ON/OFF**: Indicates whether the Advisor rules processing function is on or off. See Section 4.7.4 for information regarding Advisor.

4.2.2 Instrument Status

The Activity Indicator (large circle) becomes animated when the instrument is active.

Figure 4-19: *Instrument Status: Sleeping State* 	❶	When the instrument is in Sleeping state, the Activity Indicator is gray with no animation; the status is indicated to the right.
Figure 4-20: *Instrument Status: Warming Up State*  ***NOTE:** In specific instances (e.g., after instrument shutdown or after a fault occurs) a longer warm-up (approximately 6 minutes) is performed to ensure the pumps are adequately primed.*	❷	During an automatic warm-up, the instrument transitions to Warming Up state. During this period, the Activity Indicator remains gray, but becomes animated; the status is "Performing Warm-Up". After warming up (approximately 3 minutes*), the instrument transitions to Standby state.
	❸	If there are no samples loaded and the **Run** button is touched in Sleeping state, the instrument transitions to Warming Up state. During this period, the Activity Indicator remains gray, but becomes animated; the status is "Performing Warm-Up". After warming up (approximately 3 minutes*), the instrument transitions to Standby state or, if samples were loaded during warm-up, transitions to Running state.
Figure 4-21: *Instrument Status: Preparing System*  ***Figure 4-22:*** *Instrument Status: Flushing System* 	❹	If there are samples loaded and the **Run** button is touched in Sleeping state, the instrument transitions to Running state. The Activity Indicator turns blue and becomes animated. The status first indicates "Preparing system" (approximately 1 minute*) and then transitions to "Flushing System" (approximately 1 minute). **NOTE:** *When samples are loaded and the **Run** button is touched in Standby state, the status immediately transitions to "Flushing System".*

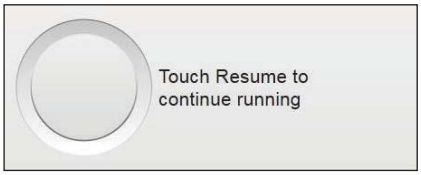
Figure 4-23: *Instrument Status: Rack R1, Tube Position 3 being processed* 	❺	After sample processing begins, a countdown timer appears, indicating the sample's remaining processing time (seconds). As rack samples are processed, the rack number, tube position number, and accession number of the sample in process are displayed. The 10 small circles represent the tube positions in the sample rack; the tube position being processed is colored blue.
Figure 4-24: *Instrument Status: Stat Position 1 being processed* 	❻	When samples in the Stat Area are being processed, the Stat position is displayed.
Figure 4-25: *Instrument Status: Calibrator Pack being processed* 	❼	When the Calibrator Pack is being processed, the status is "Calibrating"; there is no countdown timer. Calibration takes approximately 30 minutes.
Figure 4-26: *Instrument Status: Paused State*  ***Figure 4-27:*** *Instrument Status: Paused State after resource error resolved* 	❽	If the system runs out of a reagent, waste space, or rack output space during a run, it transitions to Paused state. The Activity Indicator is gray with no animation; the status is "Resolve error to continue running". After resolving the resource error, the status changes to "Touch Resume to continue running".

Figure 4-28: *Instrument Status: Standby State* 	❾	After the last sample is processed, the instrument performs end-of-run operations (approximately 5 minutes), then transitions to Standby state. The Activity Indicator is gray with no animation; the status is “Ready to run”. If a run is not started during the Standby period (approximately 2 hours), the instrument transitions to Sleeping state.
Figure 4-29: *Instrument Status: Fault State* 	❿	If the system encounters a critical error, it stops all processing and transitions to Fault state. The Activity Indicator is gray with no animation; the status is “Resolve error to continue running”. After resolving the error, touch **Reset**. The instrument then transitions to Sleeping state.
Figure 4-30: *Instrument Status: Maintenance State* 	⓫	When a maintenance procedure is being performed on the instrument, the instrument transitions to Maintenance state. The Activity Indicator is gray with no animation; the status is “Performing Maintenance…”

4.2.3 Stat Area

This area displays the status and contents of the Stat rack.

NOTE: *The Stat Area display expands when the Stat rack is loaded (i.e., inside the instrument) and contains at least one sample or when the Stat rack is ejected; the Stat Area display contracts when the Stat rack is loaded but empty.*

Figure 4-31: *Stat Area: Stat Rack Loaded but Empty*

Figure 4-32: *Stat Area: Stat Rack Ejected*

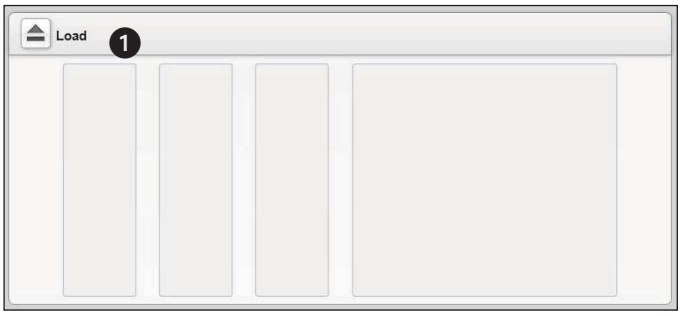
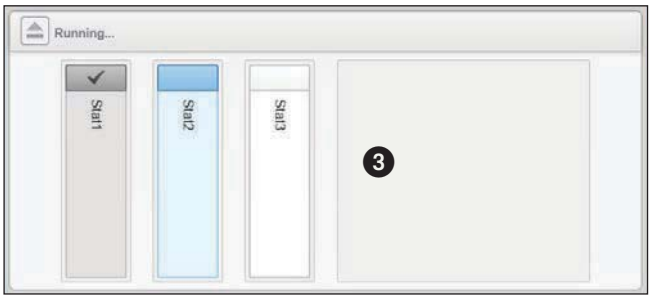


Figure 4-33: *Stat Area: Stat Rack Loaded with Samples in Positions 1–3*



1	**Open/Load** button: This toggle button is used to eject the Stat rack from the Stat Area and load the Stat rack into the Stat Area. • The Stat Rack cannot be ejected when a Stat sample is being processed; the button is disabled and indicates "Running". See Figure 4-33. • When the Stat rack is ejected, the Stat Area display is empty and the **Load** button is enabled. See Figure 4-32.
2	The Stat Area indicates whether or not there are samples present in the Stat rack. • If the Stat rack is empty, it indicates "No Samples Present". • If the Stat rack is loaded and contains samples when the system is shut down, the Stat Area indicates "Stat area may contain samples" when the system is restarted. • The rectangles represent the positions in the rack. • An empty rectangle indicates there is no sample present in that position. • The sample ID is shown for each Stat position that is occupied.
3	Color-coding indicates the status of each Stat position: • Stat positions that have not been processed are white. • The Stat position currently being processed is blue. • Stat positions that are finished being processed are gray and have a checkmark at the top.

The following are examples of the Stat Area when the Stat rack contains the Calibrator Pack, QC samples, and a patient sample. See Section 5.3 for information regarding running the Calibrator Pack.

Figure 4-34a: *Stat Samples Not Processed Yet*

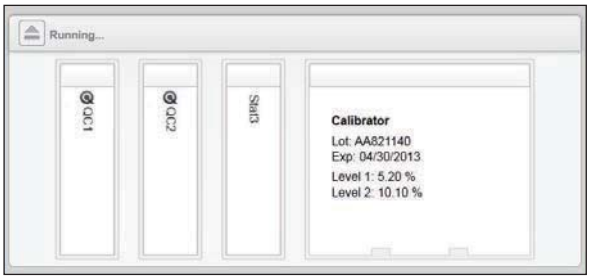


Figure 4-34b: *Calibrator Pack Being Processed*

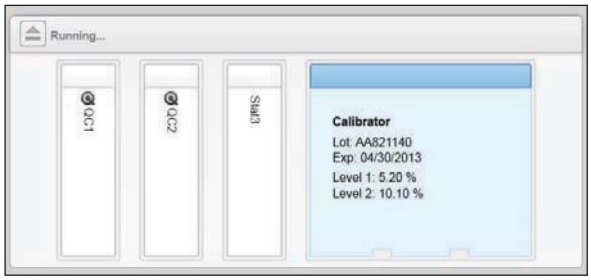


Figure 4-34c: *Calibrator Pack Finished, QC1 Being Processed*

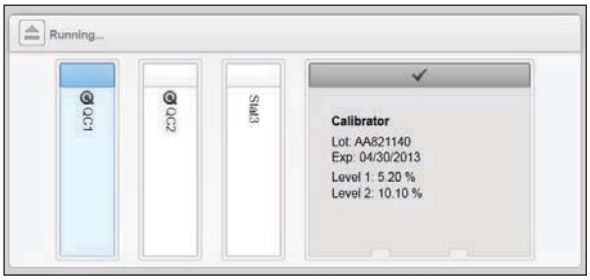
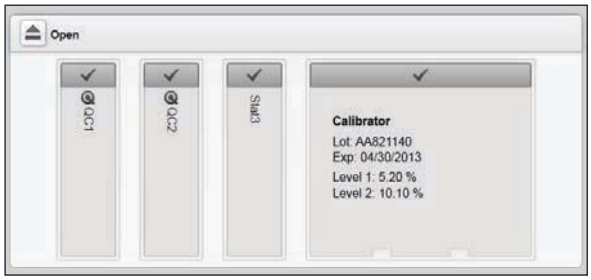
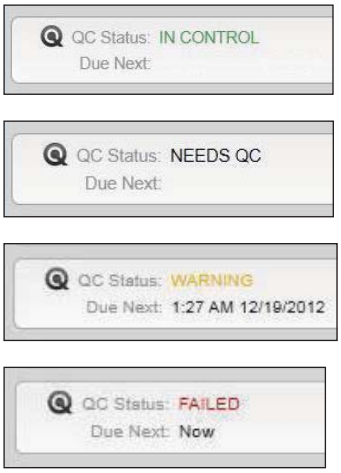


Figure 4-34d: *All Stat Samples Finished*



4.2.4 QC Status

This area displays the status of the active controls. The active lot numbers are indicated in the **QC/QC Lots** screen (see Section 4.4.4).

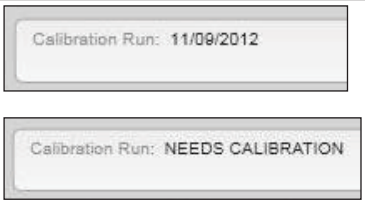


QC Status: There are 4 possible statuses for Quality Control (QC):

- **IN CONTROL:** QC samples have been run within the required time interval and the results are within the acceptable range.
- **NEEDS QC:** QC samples must be run because the system has been recalibrated.
- **WARNING:** The results of one or more QC samples have violated a QC rule.
- **FAILED:** The results of one or more QC samples have failed (i.e., are outside the fixed control range or failed a QC rule).

	Due Next: Indicates the next time QC must be run, either in number of samples or time and date. Once the interval is reached, it will indicate "Now". **NOTE:** *See Section 4.4.2 for information regarding setting the QC Interval. If the QC Interval option is not selected, the Due Next field will be blank.* In addition to the QC Status indicator, a yellow message appears in the message panel at the top of the screen, alerting you that QC is due.

4.2.5 Calibration Status

	Calibration Run: The date the cartridge was last calibrated. "NEEDS CALIBRATION" is displayed after a new cartridge is installed; no samples can be run (i.e., the **Run** button is disabled) until the Calibrator Pack is run. In addition to the Calibration indicator, a red message appears in the message panel at the top of the screen, alerting you that calibration is required.
NOTE: *If a calibrated cartridge is removed from the instrument and later reinstalled, the "Calibration Run" date will display the date that cartridge was last calibrated. The message panel displays a yellow message "Cartridge reloaded – Calibration recommended".* *If the cartridge was stored properly (i.e., tightly capped and refrigerated at 2–8 °C), it can be reinstalled without recalibration; ensure the first run begins with QC samples and that the results are acceptable. If you choose to run without recalibrating, a pop-up message alerts you that the cartridge has not been recalibrated and asks if you want to run anyway. If you select **Yes**, the last calibration of that cartridge will be used to calibrate the run.*	

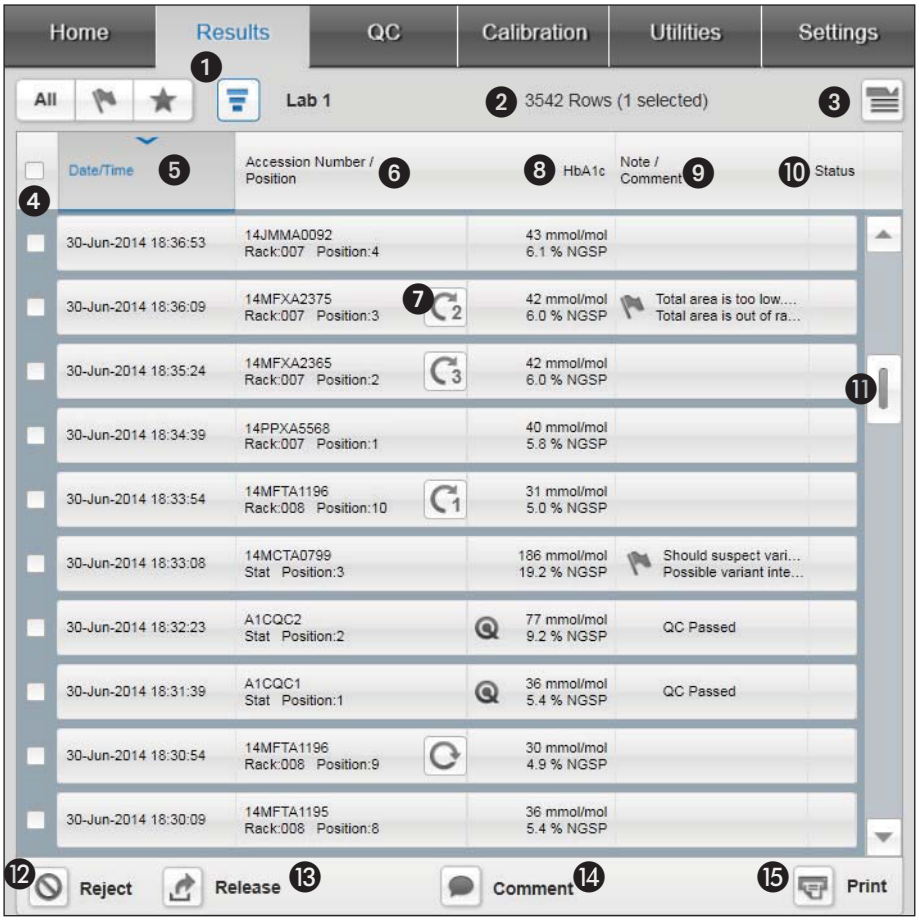
4.2.6 Temperature and Pressure

	❶ The cartridge holder temperature (°C) is displayed. ❷ The system pressure (MPa=megapascal) is displayed. **NOTE:** *If the temperature or pressure is outside the acceptable range, a message appears in the message panel to alert you.*

4.3 Results Screen

The **Results** screen provides access to all sample results in a table format. The table configuration can be customized to meet your laboratory's needs. The results can be filtered for display and searching purposes. The **Results** screen can be accessed at any time with the instrument in any state. Sample results can be released or rejected in this screen.

Figure 4-35: *Results Screen, Default Table Configuration*



1	Filter buttons: These buttons are used to filter the results in the table. • All button: Displays results for All processed samples, or as configured. • button: Displays results for Flagged samples only, or as configured. • button: Displays results for samples meeting the Favorite filter criteria only, or as configured. • button: Opens the Filter dialog box. See Section 4.3.1. The filter currently in use is indicated to the right of the button.
2	The number of rows in the table and the number selected are indicated. Each row represents a sample result.
3	Results Menu button: See Section 4.3.3.

❹	Row checkbox: Each sample result has a checkbox for selection purposes. To take the same action on one or more results, select the corresponding checkbox(es) for the desired sample(s). Selected results are highlighted blue. **NOTE:** *To select all results, touch the checkbox in the column header.*
❺	Date/Time: The date and time the sample result was generated.
❻	Accession Number/Position: The sample ID (i.e., barcode) appears on the first line. The Sysmex rack ID and tube position, or the position in the Stat rack, appears on the second line.
❼	Repeat icon: Displayed when a sample has been processed multiple times (i.e., repeated) within a defined interval. See Section 4.7.1 to set the interval. For example:  indicates the first result of a sample that has been repeated.  indicates the first repeat (i.e., second result) of the sample.  indicates the second repeat (i.e., third result) of the sample. **NOTE:** *When there are >9 repeats, an ellipsis appears instead of a number (i.e.,*  *).*
❽	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s).
❾	Note/Comment: Any notes (associated with rules or QC), or comments entered regarding the sample, appear in this field. •  indicates the sample result has been flagged for violating a rule or failing QC. • A Note provides instruction to the user regarding a flag. Notes are not transmitted to the LIS. • A Comment provides information regarding the sample. Comments can be predefined for Advisor rules or can be entered by the user. Comments can be transmitted to the LIS.
❿	Status: The status of the sample result (i.e., Released  or Rejected ). Results with no icon in the Status field are Held.
⓫	Scroll bar: Use the scroll bar to move up/down the table.
⓬	 **Reject** button: Touch to reject the selected sample result(s).
⓭	 **Release** button: Touch to release the selected sample result(s).
⓮	 **Comment** button: Touch to enter a comment for the selected sample result(s) (maximum: 40 characters).
NOTE: *The **Reject, Release,** and **Comment** buttons are disabled (appear dimmed) if no sample result is selected in the table.*	
⓯	 **Print** button: Touch to access the Print Results dialog box. See Section 4.3.4.
NOTE: • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To view the sample result in detail, touch the result row. See Section 4.3.2.*	

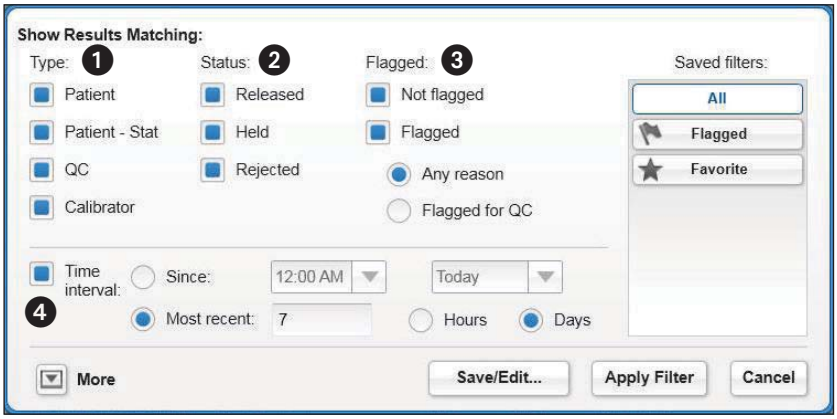
4.3.1 Filtering Results

From the **Results** screen, touch  . The Filter dialog box appears. The saved filters are listed in the right-hand column; the selected filter appears in blue.

There are 3 default filters predefined in the software:

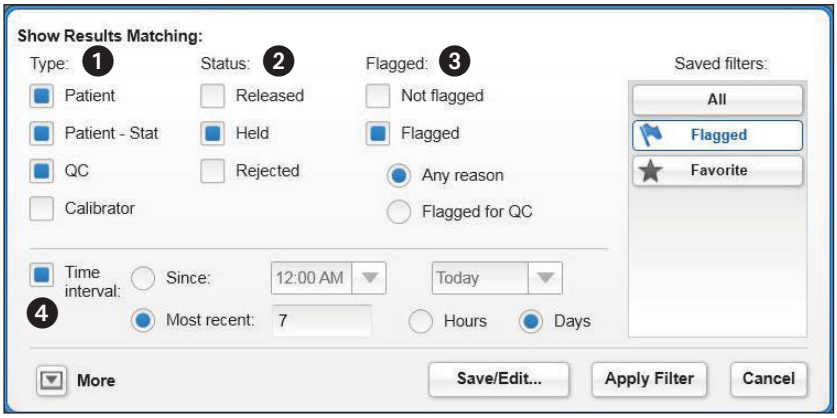
- **All** (See Figure 4-36)
- **Flagged** (See Figure 4-37)
- **Favorite** (See Figure 4-38)

Figure 4-36: *Results Screen, Filter Dialog Box, Default All Filter*



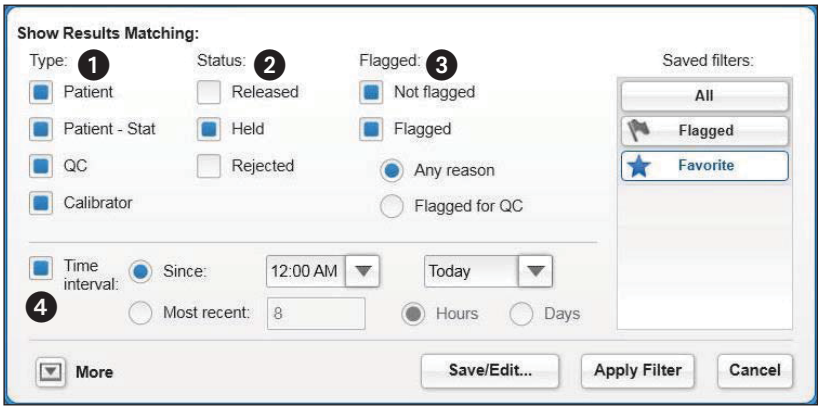
The default **All** filter uses the following sample criteria:	
1	Type: **Patient, Patient-Stat, QC**, and **Calibrator**
2	Status: **Released, Held,** and **Rejected**
3	Flagged: **Not flagged** and **Flagged** for **Any reason**
4	Time interval: **Most recent 7 Days**

Figure 4-37: *Results Screen, Filter Dialog Box, Default Flagged Filter*



The default **Flagged** filter uses the following sample criteria:	
1	Type: **Patient, Patient-Stat** and **QC**
2	Status: **Held**
3	Flagged: **Flagged** for **Any reason**
4	Time interval: **Most recent 7 Days**

Figure 4-38: *Results Screen, Filter Dialog Box, Default Favorite Filter*



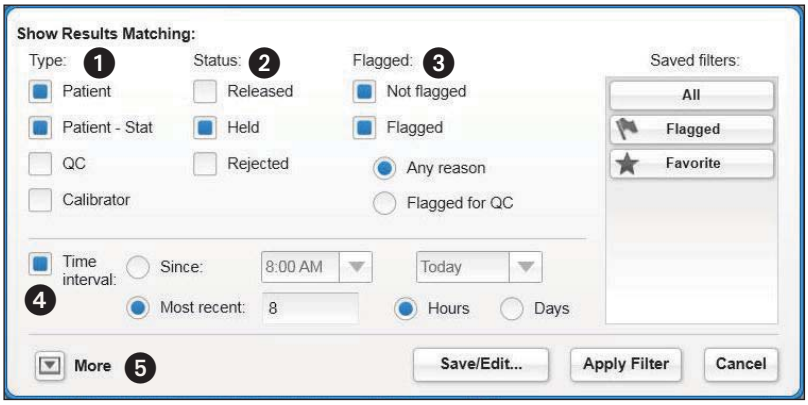
The default **Favorite** filter uses the following sample criteria:

❶	Type: **Patient, Patient-Stat, QC**, and **Calibrator**
❷	Status: **Held**
❸	Flagged: **Not flagged** and **Flagged** for **Any reason**
❹	Time interval: **Since 12:00 AM (or 00:00) Today**

4.3.1.1 Creating Custom Filters

The default filters can be customized for laboratory preferences and workflows. From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-39: *Results Screen, Filter Dialog Box (Condensed)*

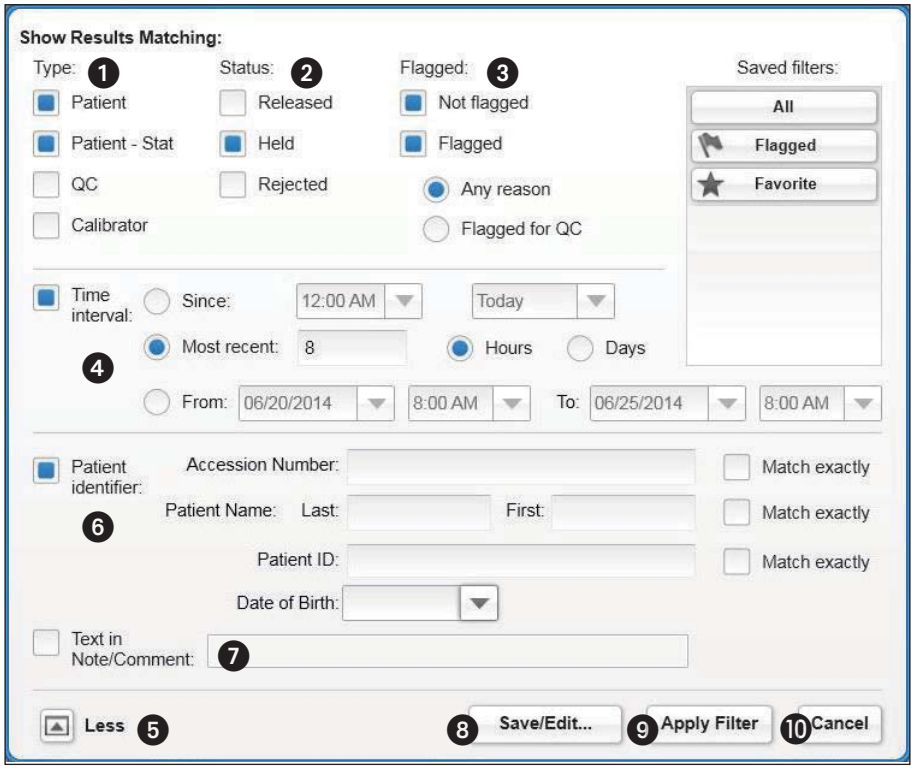


To create a custom filter for your lab, select or enter the applicable criteria as follows:

❶	Type: Select **Patient, Patient-Stat, QC**, and/or **Calibrator** **NOTE:** ***Patient-Stat*** *results are those that have a "Stat" (S) priority code in the LIS order.*
❷	Status: Select **Released, Held,** and/or **Rejected**
❸	Flagged: Select **Not flagged** and/or **Flagged**; if **Flagged** is selected, must select **Any reason** or **Flagged for QC**.
❹	To filter by an interval of time, select **Time interval** and then select one of the following options: • **Since**: enter a time and select a day (**Today** or **Yesterday**) from the drop-down list. • **Most recent**: enter a number and select **Hours** or **Days**. • **From/To**: enter a "From" date and time plus a "To" date and time. **NOTE:** *The date format and time format are set in the* ***Settings/Instrument*** *screen. See Section 4.7.1.*

❺ Touch the **More** button to extend the dialog box to view all remaining filter criteria; touch the **Less** button to condense the dialog box.

Figure 4-40: *Results Screen, Filter Dialog Box (Extended)*



❻ To filter by a patient identifier, select **Patient identifier** and then enter one or more of the following identifiers:

- **Accession Number** (i.e., sample ID or barcode)
- **Patient Name** (**Last** and/or **First**)
- **Patient ID** (i.e., healthcare system's patient identification)
- **Date of Birth**

If you want to search for the exact number/name/ID entered, select the corresponding **Match exactly** checkbox for the identifier; otherwise, if you enter only part of an identifier, any sample containing that partial information will be included in the filter.

NOTE: *None of the Patient identifier information is retained when the new filter is saved.*

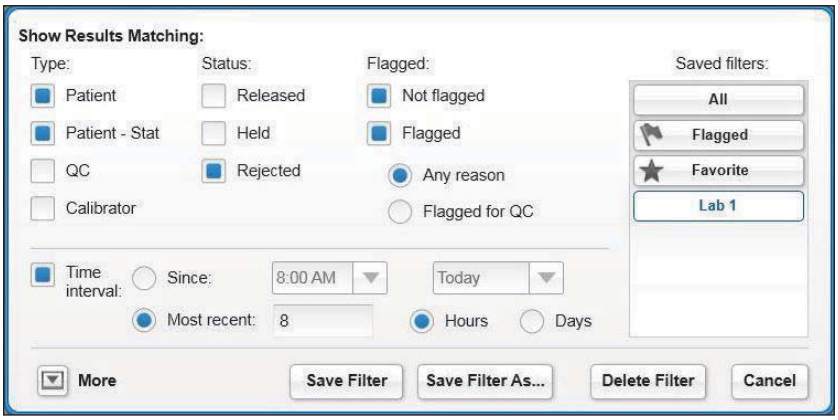
❼ To filter by the text in the Note/Comment field, select **Text in Note/Comment** and then enter the applicable text.

❽	**Save/Edit** button: After selecting filter criteria, touch this button to save the filter. The **Save Filter As** button appears. Touch this button to open the Save Filter As dialog box. Enter a name (maximum 15 characters) for the filter and touch **Save**. ***Figure 4-41:*** *Save Filter As Dialog Box* 
❾	**Apply Filter** button: To apply the filter without saving it, touch this button. The results table will display the filtered results (under the name "Custom Filter") until you log out of the system or select a new filter.
❿	**Cancel** button: Closes the dialog box without saving filter selections.

4.3.1.2 Editing a Filter

From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-42: *Filter Dialog Box, Saving Edited Filter*



1. In the Saved filters column, ensure the filter you want to edit is selected (appears in blue). If it is not selected, touch the desired filter button (which will display the filtered results), then touch  again.
2. Touch **Save/Edit**.
3. Make your desired edits to the filter criteria.
4. To save the changes under the existing filter name, touch **Save Filter**.
   - To save the changes under a new filter name, touch **Save Filter As**, enter a new name (maximum 15 characters), and touch **Save**.

4.3.1.3 Deleting a Filter

From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-43: *Filter Dialog Box, Deleting a Filter*

1. In the Saved filters column, ensure the filter you want to delete is selected (appears in blue).
2. Touch **Save/Edit**.
3. Touch **Delete Filter**.
4. A pop-up message appears, prompting you to confirm the deletion. Touch **Yes** to confirm.

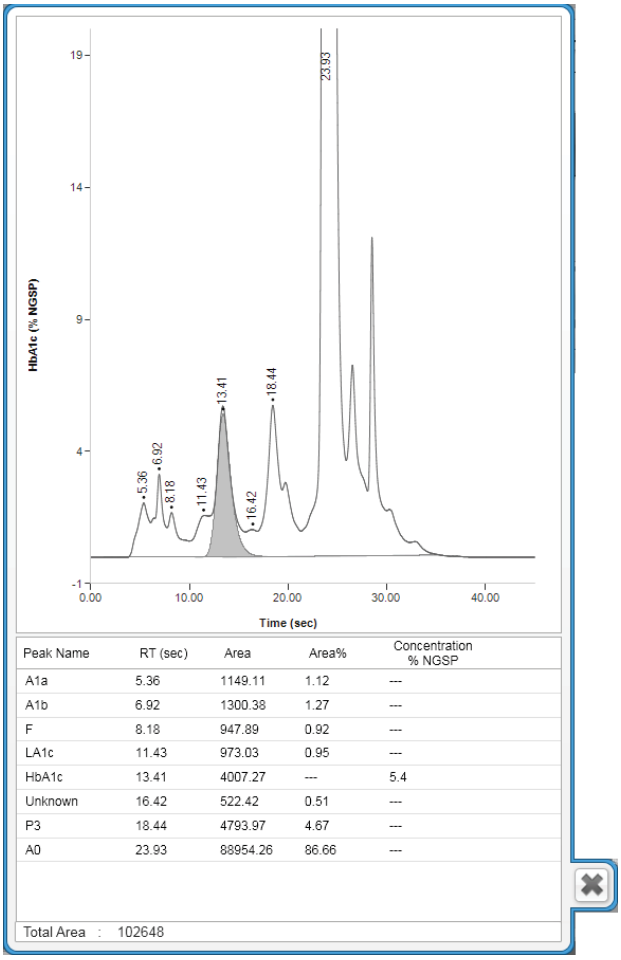
4.3.2 Viewing Result Details

From the **Results** screen, touch the desired sample result row in the table. The Result Details screen appears.

Figure 4-44: *Result Details Screen*

In addition to the sample information already provided in the **Results** screen (described in Section 4.3), the Result Details screen displays the following:

❶	Accession Number: The sample ID (i.e., barcode). This field becomes editable if the barcode is missing (e.g., prediluted patient sample) or unread. See Section 5.7.1.
❷	Patient ID: The healthcare system's patient identification (optional).
❸	Physician: The name of the physician (optional).
❹	DOB: The patient's date of birth (optional).
❺	Gender: The patient's gender (optional).
❻	If the sample has been repeated, all results for the sample will be displayed in table format, exactly as shown in the **Results** screen. The result details displayed are for the result highlighted blue. Touch another result in this table to display the details for that result. **NOTE:** *If there are more than 2 results for the sample, a scroll bar is provided to move up/down the table.*

❼	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s). The primary reporting unit appears first and is bolded.
❽	Note: Any notes associated with rules or QC. •  indicates the sample result has been flagged for violating a rule or failing QC. • Long notes appear truncated; view complete note in the **More Details** window (see Figure 4-46). • Notes are not transmitted to the LIS.
❾	Comment: To enter a comment, edit an existing comment, or view a truncated comment in its entirety, touch this field (no limit to number of characters).
❿	***Figure 4-45:*** *Enlarged Chromatogram and Peak Table*  Chromatogram: graph of detector output vs time. For an enlarged view of the chromatogram and peak table, touch the chromatogram. See Figure 4-45. Touch  to close the enlarged view.
⓫	Peak Table: This table includes the list of detected peaks, retention time (RT) in seconds, area, area % of non-calibrated peaks, and concentration of HbA1c peak. The HbA_{1c} result appears in the primary reporting unit.
⓬	Total Area: The sum of all detected analyte peak areas.

Peak Name	RT (sec)	Area	Area%	Concentration % NGSP
A1a	5.36	1149.11	1.12	---
A1b	6.92	1300.38	1.27	---
F	8.18	947.89	0.92	---
LA1c	11.43	973.03	0.95	---
HbA1c	13.41	4007.27	---	5.4
Unknown	16.42	522.42	0.51	---
P3	18.44	4793.97	4.67	---
A0	23.93	88954.26	86.66	---

Total Area : 102648

⓭ ***Figure 4-46:*** *More Details Pop-Up Window*

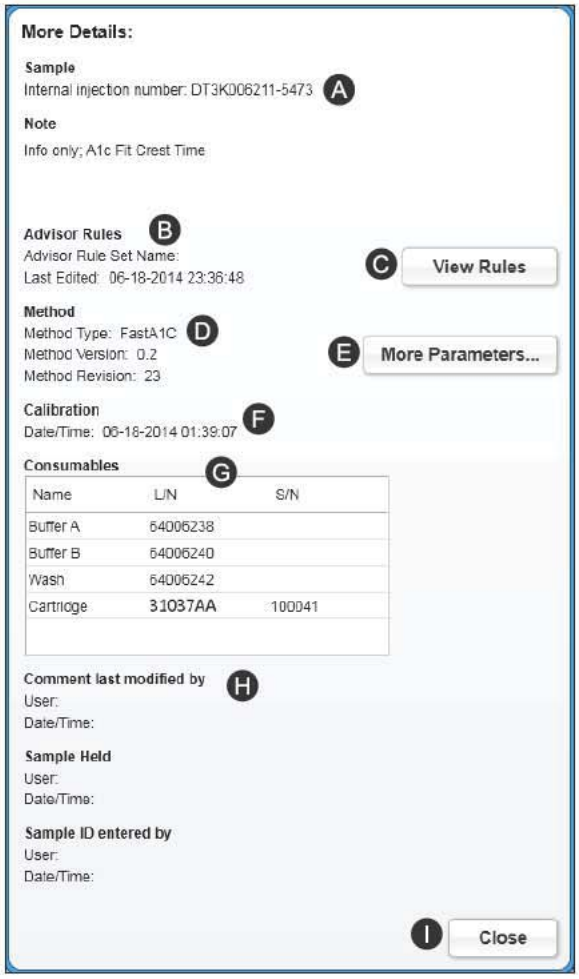


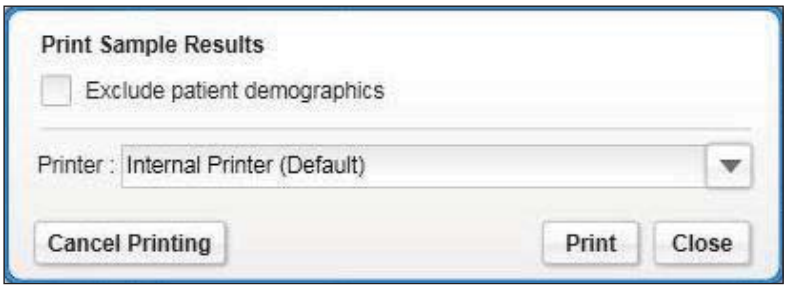
Figure 4-47: *More Details/More Parameters Pop-Up Window*

More Details button: to view additional details regarding the sample analysis, touch this button. A pop-up window displays the following:

- Ⓐ Internal injection number
- Ⓑ Advisor Rule Set name and date last edited
- Ⓒ **View Rules** button: To view the complete set of Advisor rules used to process the sample, touch this button.
- Ⓓ Method name, version, and revision.
- Ⓔ **More Parameters** button: To view additional analysis parameters for the sample, touch this button. See Figure 4-47. For more information about these parameters, see Appendix C, rules 29–37.
- Ⓕ Calibration date and time
- Ⓖ Consumables lot numbers and serial numbers
- Ⓗ User name, date, and time of specific user actions.
- Ⓘ Touch **Close** to close the window.

⓮ **Print** button: Touch to print the sample result report.

Figure 4-48: *Print Sample Results Dialog Box*



- **Exclude patient demographics**: Select this checkbox to exclude demographic information from the printed report; if not selected, patient demographics will be included in the report.
- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).

  NOTE: *The default printer is set in the **Settings/Reports** screen. See Section 4.7.2.*

  The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the sample result report (i.e., Patient Report) is "Accession Number-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print results.

  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Result Details screen, then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

⓯ **Reject** button: Touch to reject the sample result.

⓰ **Release** button: Touch to release the sample result. See Section 6.2 for more information regarding releasing results.

NOTE: *After rejecting or releasing a sample result in the Result Details screen, the software immediately displays the next sample result.*

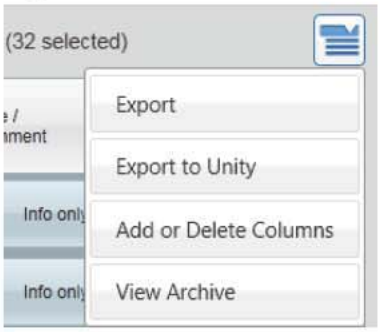
⓱ Touch ▶ to display the next sample result or ◀ to display the previous sample result.

NOTE: *To exit the Result Details screen, touch any primary navigation tab (e.g., touch **Results** to return to the **Results** screen).*

4.3.3 Results Menu

From the **Results** screen, touch . The Results Menu appears. There are 4 functions available:

Figure 4-49: *Results Menu*



- **Export** (See Section 4.3.3.1)
- **Export to Unity** (See Section 4.3.3.2)
- **Add or Delete Columns** (See Section 4.3.3.3)
- **View Archive** (See Section 4.3.3.4)

4.3.3.1 Exporting Results

1. From the **Results** screen, select the corresponding checkbox(es) for the desired sample(s). Selected results are highlighted blue.
2. Touch .
3. From the Results Menu, touch **Export**.

Figure 4-50: *Export Results Dialog Box*

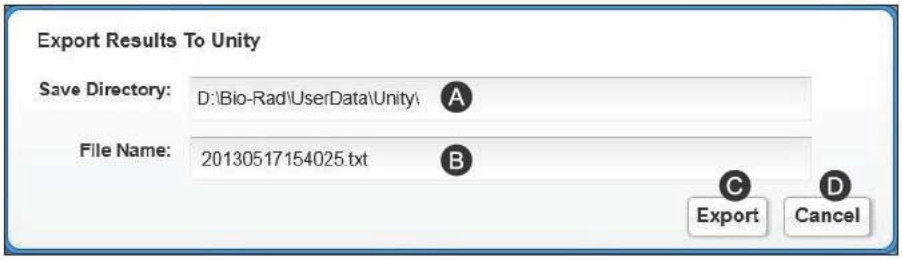
A	**Exclude patient demographics**: Select this checkbox to exclude demographic information from the results; if not selected, patient demographics will be included in the results.
B	Save to: The default location for exported files is the D:\Bio-Rad\UserData\Exports folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
C	File Name: The default file name is "Export_yyyymmddhhmmss.csv". Touch the field to change the file name.
D	**Export** button: Touch this button to export the selected results. A pop-up message confirms that the export was completed successfully. Touch **OK**.
E	**Cancel** button: Closes the dialog box without exporting results.

4.3.3.2 Manually Exporting QC Results to Unity™

QC results can be exported automatically to Unity (see Section 4.4.5). Alternatively, to manually export QC results, follow these instructions.

1. From the **Results** screen, select the corresponding checkbox(es) for the desired QC sample(s). Selected results are highlighted blue.
2. Touch  .
3. From the Results Menu, touch **Export to Unity**.

Figure 4-51: *Export Results to Unity Dialog Box*



A	Save Directory: The default location for manually exported QC Unity files is the D:\Bio-Rad\UserData\Unity folder. The location is set in the **QC/Unity** screen (see Section 4.4.5).
B	File Name: The default file name is simply the export date and time: "yyyymmddhhmmss.txt". A prefix can be added to the file name in the **QC/Unity** screen (see Section 4.4.5).
C	**Export** button: Touch this button to export the selected results. A pop-up message confirms that the export was completed successfully. Touch **OK**.
D	**Cancel** button: Closes the dialog box without exporting results.

4.3.3.3 Adding or Deleting Columns in the Results Table

The results table can be customized to meet your laboratory's needs.

1. From the **Results** screen, touch  .
2. From the Results Menu, touch **Add or Delete Columns**.

Figure 4-52: *Add or Delete Columns Dialog Box*

A	Secondary Identifier: In the default table configuration, the Accession Number appears on the first line of each result as the primary identifier and the Position (i.e., rack ID/tube position) appears on the second line. The **Patient Name, Patient ID,** or **Date of Birth** can be displayed as a secondary identifier below the Accession Number by selecting the desired option; when a secondary identifier is displayed, the Position appears in a separate column in the table.
B	Results: A column for **Total Area** can be added to the table by selecting this option.
C	Peak Results: Columns can be added to the table for up to 3 additional peaks by selecting peak(s) in the list.
NOTE:	*The maximum number of columns that can be added to the table is 3.*
D	**Save** button: Touch this button to save the changes to the table.
E	**Cancel** button: Closes the dialog box without saving any changes to the table.

4.3.3.4 View Archive

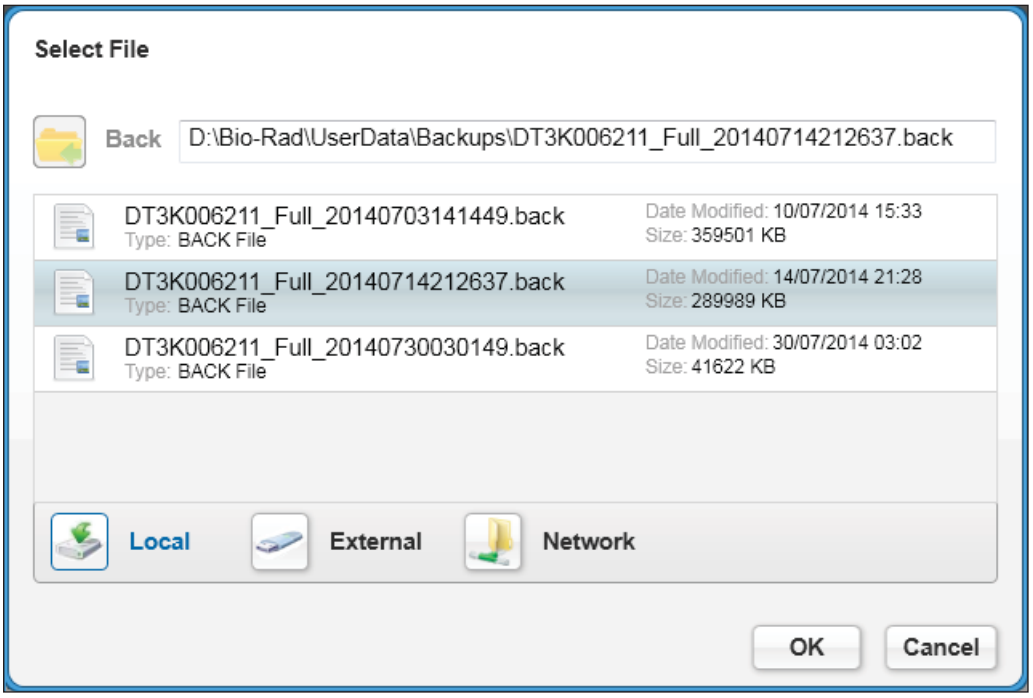
Sample results from a backed-up database can be viewed on the system.

1. From the **Results** screen, touch .
2. From the Results Menu, touch **View Archive**.
3. The View Archive dialog box appears. To locate the database backup file, touch the browse button ...

Figure 4-53: *View Archive Dialog Box*

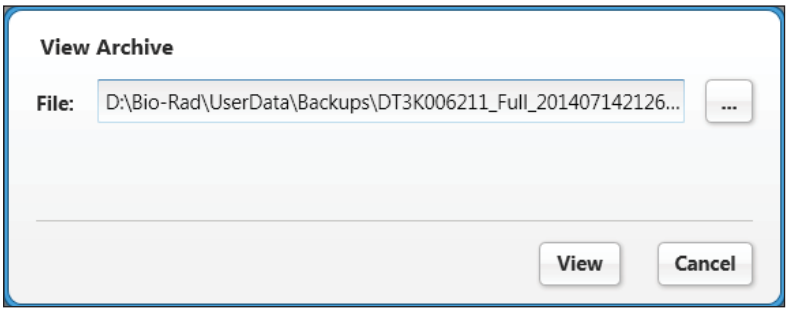
4. In the Select File dialog box, select the file to view from the applicable drive/folder and touch **OK**.

Figure 4-54: *Select File Dialog Box*



5. The file path appears in the File field of the View Archive dialog box. Touch **View** to open the file.

   Figure 4-55: *View Archive Dialog Box after file selected*



   NOTE: *The time to open the file depends on the size of the database and the speed of the drive.*

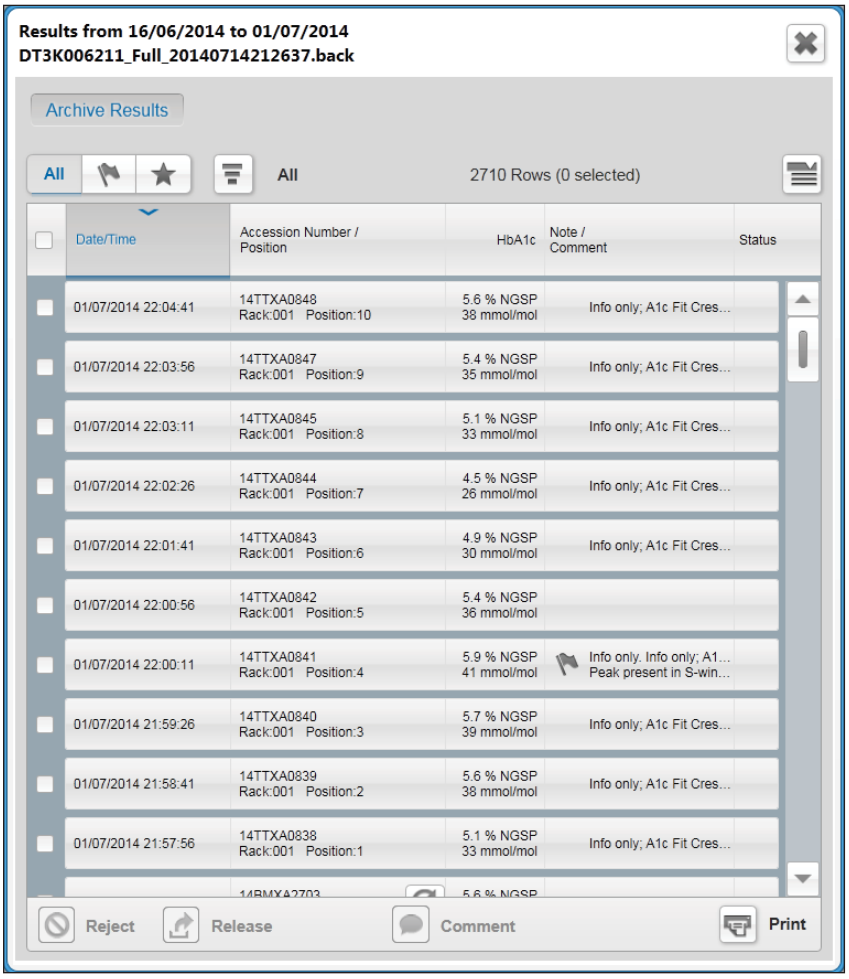
6. The Archive Results window appears. See Figure 4-56. The window header provides the date range of the results and the backup file name. Results are displayed in table format identical to the **Results** screen.

   The Archive Results window has the same functionality as the **Results** screen, with the exception of changing results:

   - Results can be filtered.
   - Results can be exported.
   - Columns can be added or deleted.
   - Result Details can be viewed.

     NOTE: *After viewing the Result Details for an archived sample result, you can return to the results table by touching the* Archive Results *button.*
   - Results can be printed.
   - Comments cannot be changed or entered.
   - Results cannot be Rejected or Released.

Figure 4-56: *Archive Results Window*

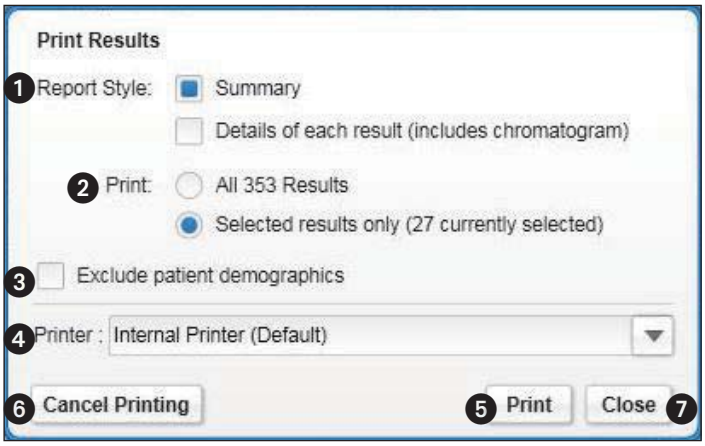


7. After you are finished viewing the archived results, touch  to close the file. A pop-up message asks if you are sure you want to close the archive. Touch **OK**.

4.3.4 Printing Results

From the **Results** screen, touch **Print**.

Figure 4-57: *Results Screen, Print Results Dialog Box*



1	Report Style: Select one or both option(s). • **Summary**: The selected results are printed in table format just like they appear in the **Results** screen; also included is a separate table listing the lot numbers and serial numbers of the consumables used for the analysis. • **Details of each result (includes chromatogram)**: A detailed report is printed for each selected sample, including the chromatogram and peak table.
2	Print: Select option. • **All Results**: Results will be printed for all samples in the results table. • **Selected results only**: Results will be printed for only the selected samples in the results table.
3	**Exclude patient demographics**: Select this checkbox to exclude demographic information from the printed report; if not selected, patient demographics will be included in the report.
4	Printer: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). **NOTE:** *The default printer is set in the **Settings/Reports** screen. See Section 4.7.2.* The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Summary Report is "Summary-System Name-yyyy-mm-dd_hh-mm-ss.pdf". The default file name for the detailed report (i.e., Patient Report) is "Accession Number-yyyy-mm-dd_hh-mm-ss.pdf".
5	**Print** button: After selecting options, touch this button to print results.
6	**NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Results** screen, then touch **Cancel Printing** in the dialog box.*
7	**Close** button: Closes the dialog box without printing.

4.4 QC Tab

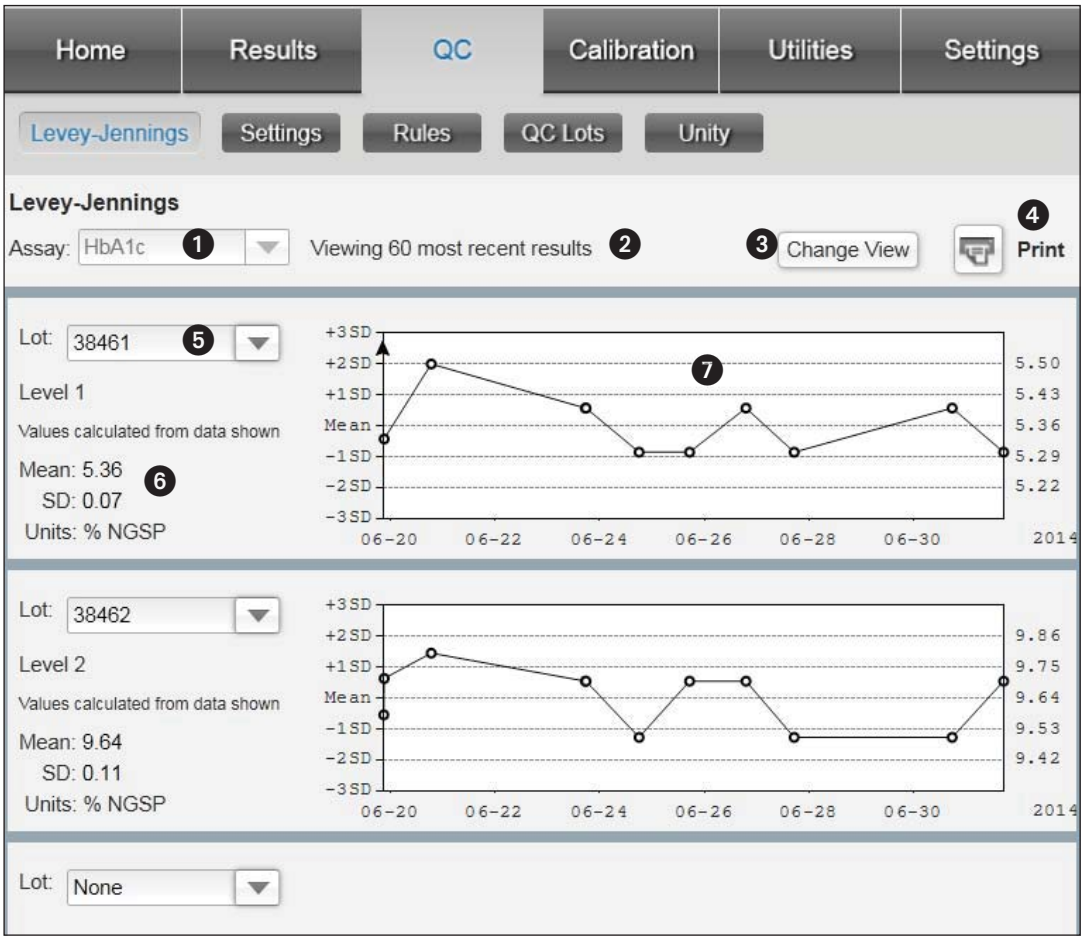
The **QC** tab includes the following secondary navigation buttons:

- **Levey-Jennings** (See Section 4.4.1)
- **Settings** (See Section 4.4.2)
- **Rules** (See Section 4.4.3)
- **QC Lots** (See Section 4.4.4)
- **Unity** (See Section 4.4.5)

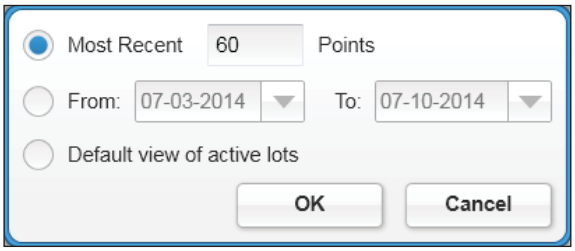
4.4.1 QC/Levey-Jennings Screen

QC results are presented in the form of a Levey-Jennings chart. A separate chart is displayed for each control level (up to 3 levels).

Figure 4-58: *QC/Levey-Jennings Screen*



1	Assay: The assay name (HbA1c).
2	Indicates the results included in the Levey-Jennings chart.

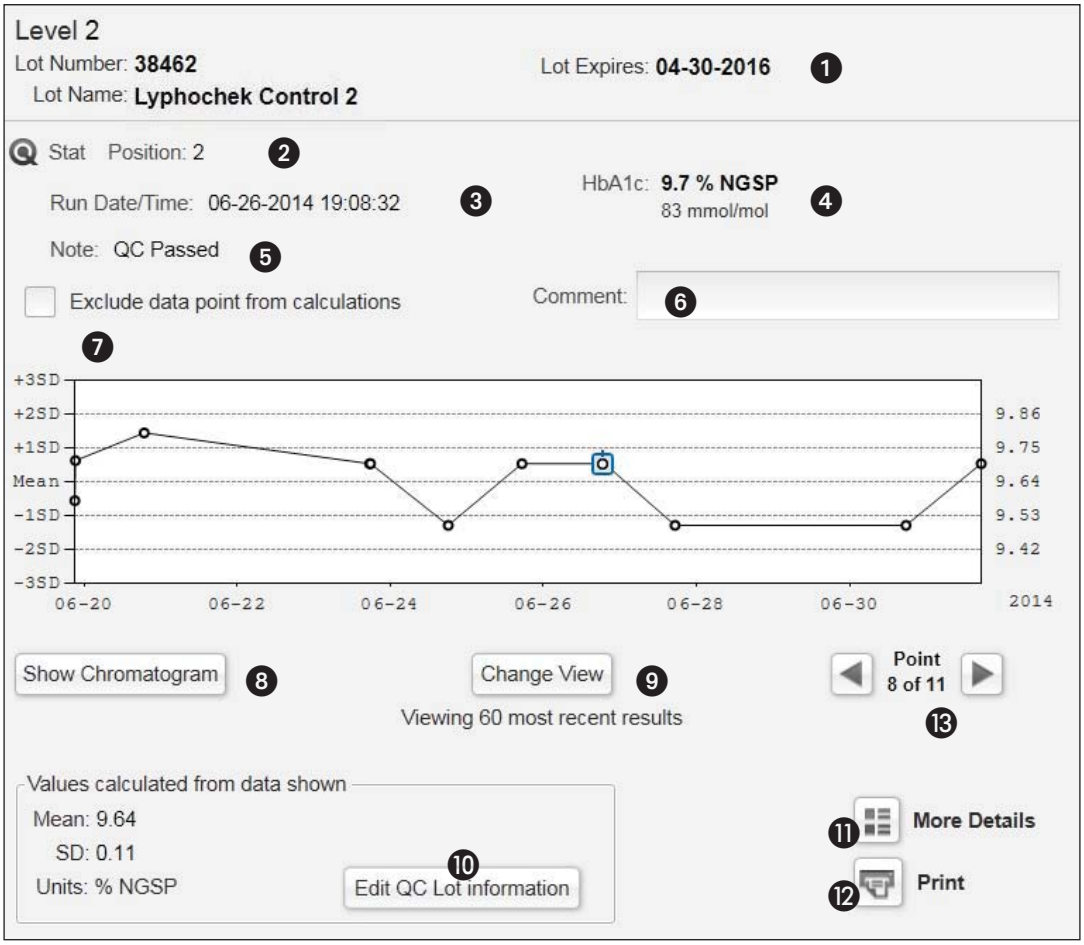
❸	**Change View** button: Opens a dialog box, allowing you to select the control data to be viewed. ***Figure 4-59:*** *Change View Dialog Box*  Select from the following options: • **Most recent**: Enter a number of data points. • **From/To**: Enter a "From" date and a "To" date. • **Default view of active lots**: The most recent 60 points from the control lots in use are included. Touch **OK** to save the entry and close the dialog box.
❹	**Print** button: Touch to print the QC Summary Report. The report includes the Levey-Jennings charts plus the individual QC results in table format for all control levels. ***Figure 4-60:*** *Print QC Summary Report Dialog Box*  • **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the QC Summary Report is "QC-System Name-yyyy-mm-dd_hh-mm-ss.pdf". • **Print** button: After selecting options, touch this button to print the report. **NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **QC/Levey-Jennings** screen, then touch **Cancel Printing** in the dialog box.* • **Close** button: Closes the dialog box without printing.
❺	Lot: The lot number of the control results being displayed is indicated. A different lot can be selected from the drop-down list.
❻	The mean, standard deviation (SD), and primary reporting unit (% NGSP or mmol/mol IFCC) are indicated.
❼	The Levey-Jennings chart plots the dates of analyses along the X-axis and control values on the Y-axis. The mean and standard deviation (SD) limits are also marked on the Y-axis. **NOTE:** *An arrow pointing above or below the chart indicates there is a data point outside ±3 standard deviations (SD) from the mean.*

NOTE: *To view the Levey-Jennings results in detail, touch the Levey-Jennings chart. See Section 4.4.1.1.*

4.4.1.1 Viewing Levey-Jennings Details

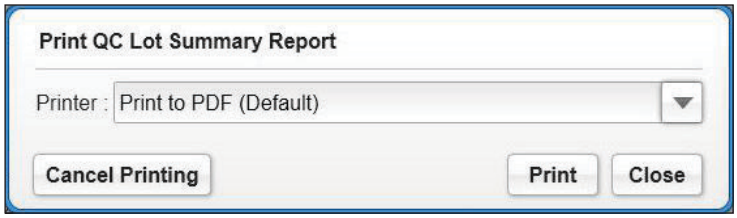
From the **QC/Levey-Jennings** screen, touch the chart. The Levey-Jennings Details screen appears. This screen displays the individual QC results for the specific control lot.

Figure 4-61: *Levey-Jennings Details Screen*



In addition to the information already provided in the **QC/Levey-Jennings** screen (described in Section 4.4.1), the Levey-Jennings Details screen displays the following:

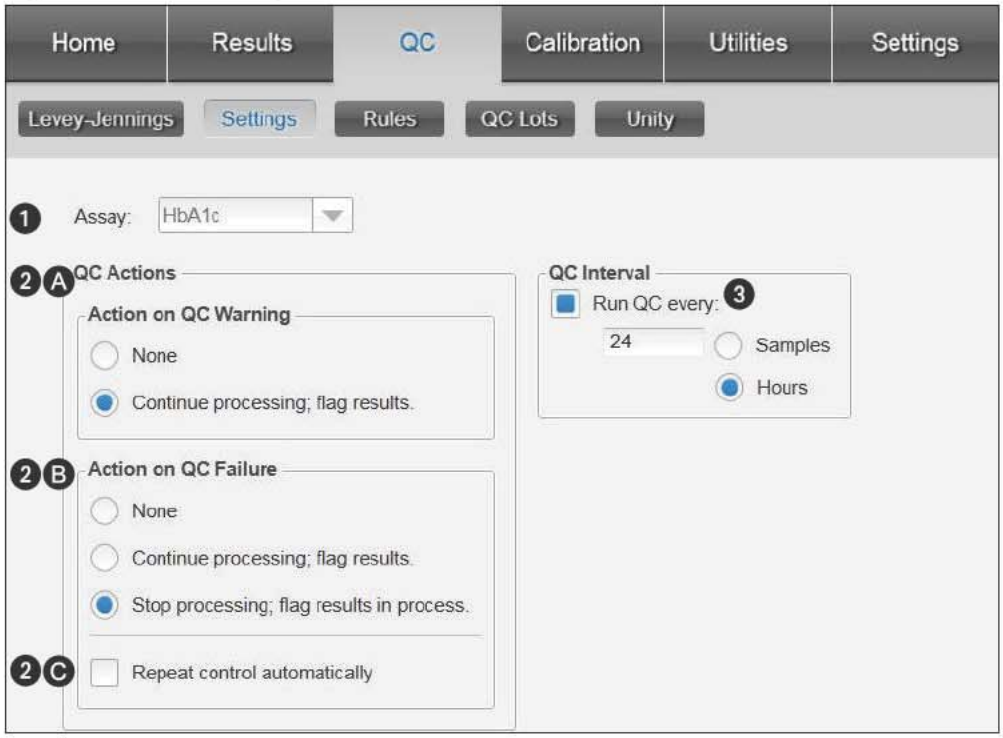
1	Lot Expires: The QC lot expiration date.
2	The Sysmex rack ID and tube position, or the position in the Stat rack.
3	Run Date/Time: The date and time the control result was generated.
4	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s). The primary reporting unit appears first and is bolded.
5	Note: Any notes associated with QC or Advisor rules (e.g., QC Passed) appear in this field. • indicates the QC result has been flagged for QC failure or violating a rule.

❻	Comment: Any comments entered regarding the control appear in this field. • Comments can be predefined for Advisor rules or can be entered by the user. To enter a comment or edit an existing comment, touch this field (no limit to number of characters).
❼	**Exclude data point from calculations**: Select this checkbox to exclude the result from the Levey-Jennings calculations. A pop-up message confirms that the QC result will be excluded from the calculations. Touch **OK**. **NOTE:** *Failed QC results are automatically excluded from the calculations.*
❽	**Show Chromatogram** button: Touch this button to view the chromatogram for the control result. Touch ✖ to close the chromatogram view.
❾	**Change View** button: Opens a dialog box, allowing you to select the control data to be viewed. See Figure 4-59.
❿	**Edit QC Lot information** button: Opens a dialog box, allowing you to edit the control lot information. See Figure 4-68.
⓫	**More Details** button: To view additional details regarding the control analysis, touch this button. See Figure 4-46.
⓬	**Print** button: Touch to print the QC Lot Summary report. The report includes the Levey-Jennings chart plus the individual QC results in table format for the control lot. ***Figure 4-62:*** *Print QC Lot Summary Report Dialog Box*  • **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the QC Summary Report is "QC-System Name-yyyy-mm-dd_hh-mm-ss.pdf". • **Print** button: After selecting options, touch this button to print the report. **NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Levey-Jennings Details screen, then touch **Cancel Printing** in the dialog box.* • **Close** button: Closes the dialog box without printing.
⓭	Touch ▶ to display the next control result or ◀ to display the previous control result. **NOTE:** *To exit the Levey-Jennings Details screen, touch any navigation tab (e.g., touch **Levey-Jennings** to return to the **Levey-Jennings** screen).*

4.4.2 QC/Settings Screen

The settings for QC actions are defined in the **QC/Settings** screen. These settings cannot be changed when the instrument is in Running state.

Figure 4-63: *QC/Settings Screen*



1	Assay: HbA1c.
2	There are 3 actions to define the system behavior when QC rules are violated or QC fails. A Action on QC Warning – Select from the following 2 options to define the system behavior when there is a QC Warning for violating a QC rule (see Section 4.4.3 for QC rules): • **None**: the run will continue and the subsequent patient samples will not be flagged. • **Continue processing; flag results**: the run will continue, but the subsequent patient samples will be flagged, with the Note "QC Warning". B Action on QC Failure – Select from the following 3 options to define the system behavior when there is a QC Failure (i.e., QC result is outside of the fixed control range or fails a QC rule): • **None**: the run will continue and the subsequent patient samples will not be flagged. • **Continue processing; flag results**: the run will continue, but the subsequent patient samples will be flagged, with the Note "QC Failed". • **Stop processing; flag results in process**: the run will stop and any subsequent patient samples that were in process will be flagged, with the Note "QC Failed". C **Repeat control automatically**: To automatically repeat the QC when it fails, select this checkbox; the QC will be repeated one time. **NOTE:** *By default, when a QC warning occurs, the message panel displays the yellow message "QC Warning"; when QC fails, the message panel displays the red message "QC Failed".*

❸	QC Interval: To have the system alert you to run QC at a specific interval, select the **Run QC every** checkbox, select a unit (**Samples** or **Hours**), and enter the desired number. The system indicates when the QC is “Due Next” in the **Home** screen QC Status area. See Section 4.2.4.

4.4.3 QC/Rules Screen

The rules for QC results are defined in the **QC/Rules** screen.

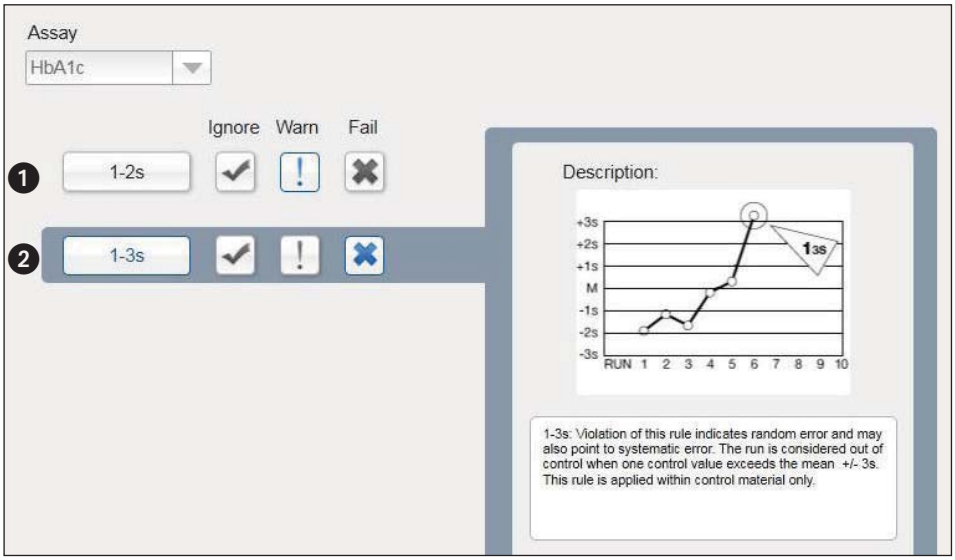
NOTE:

- *These rules apply only when the "**Use QC rules with**" validation method is selected in the Add/Edit Control Lot dialog box (see Section 4.4.4.1, No. 8B).*
- *The actions taken by the system when these rules are violated are defined in the **QC/Settings** screen (see Section 4.4.2).*

Figure 4-64: *QC/Rules Screen, 1-2s Rule*

1	**1-2s** button: Touch to view or edit the 1-2s rule. See Figure 4-64. This rule is violated when a single control result is outside ±2 SD from the mean. Select an option for handling this rule violation. (The selected option icon appears in blue.) • **Ignore** ✓ : The QC result will not be flagged. • **Warn** ! : The QC result will be flagged, with the Note "QC Warning". • **Fail** ✖ : The QC result will be flagged, with the Note "QC Failure".

❷ ***Figure 4-65:*** *QC/Rules Screen, 1-3s Rule*



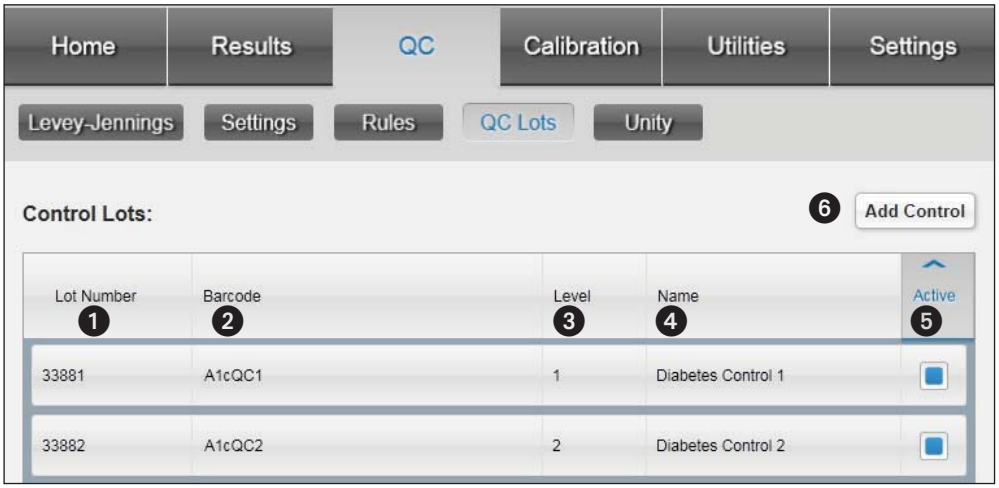
1-3s button: Touch to view or edit the 1-3s rule. See Figure 4-65. This rule is violated when a single control result is outside ±3 SD from the mean. Select an option for handling this rule violation. (The selected option icon appears in blue.)

- **Ignore** : The QC result will not be flagged.
- **Warn** : The QC result will be flagged, with the Note “QC Warning”.
- **Fail** : The QC result will be flagged, with the Note “QC Failure”.

4.4.4 QC/QC Lots Screen

The **QC/QC Lots** screen provides control lot information in table format. QC lots cannot be added or edited when the instrument is in Running state.

Figure 4-66: *QC/QC Lots Screen*



1	Lot Number: control lot number.
2	Barcode: microvial adapter barcode label name.
3	Level: control level (i.e., 1, 2, or 3).
4	Name: control name.
5	Active: The checkbox is selected (blue) if the control lot is currently in use. Clear the checkbox when the lot is no longer in use. The QC Status of the active controls is displayed in the **Home** screen (see Section 4.2.4).

NOTE:

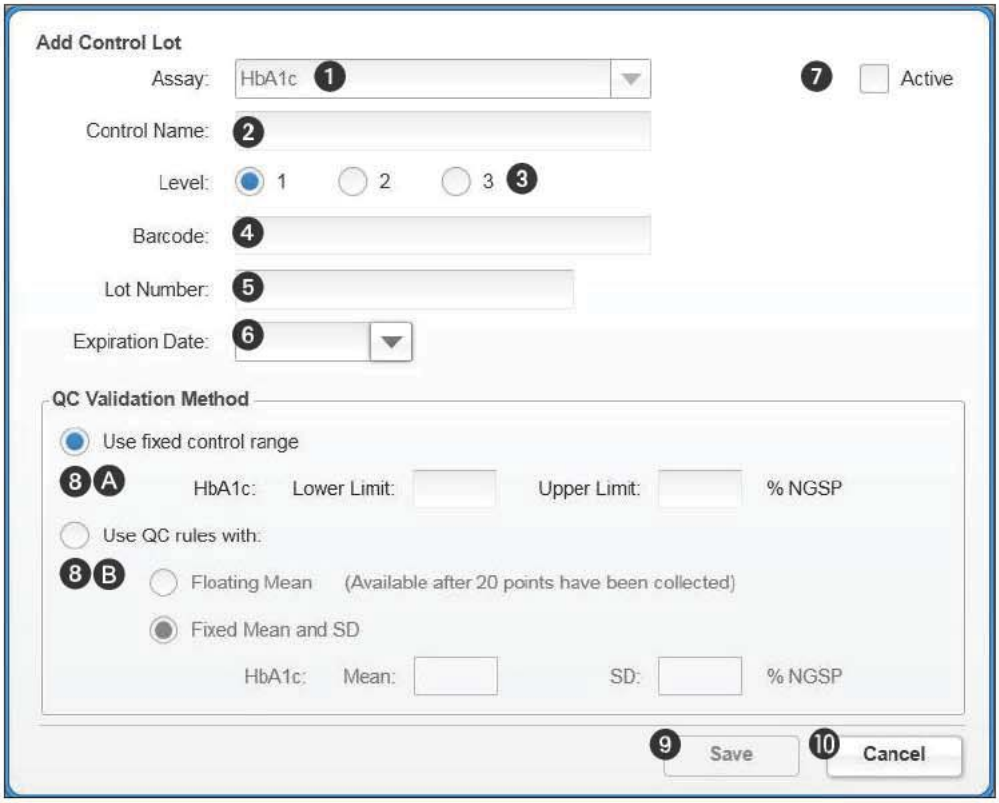
- *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.*
- *To view or edit the control lot information, touch the lot row. See Section 4.4.4.2.*

6	**Add Control** button: To add a new control lot to the system, touch this button. See Section 4.4.4.1.

4.4.4.1 Adding a New QC Lot

From the **QC/QC Lots** screen, touch **Add Control**. The Add Control Lot dialog box appears.

Figure 4-67: *Add Control Lot Dialog Box*



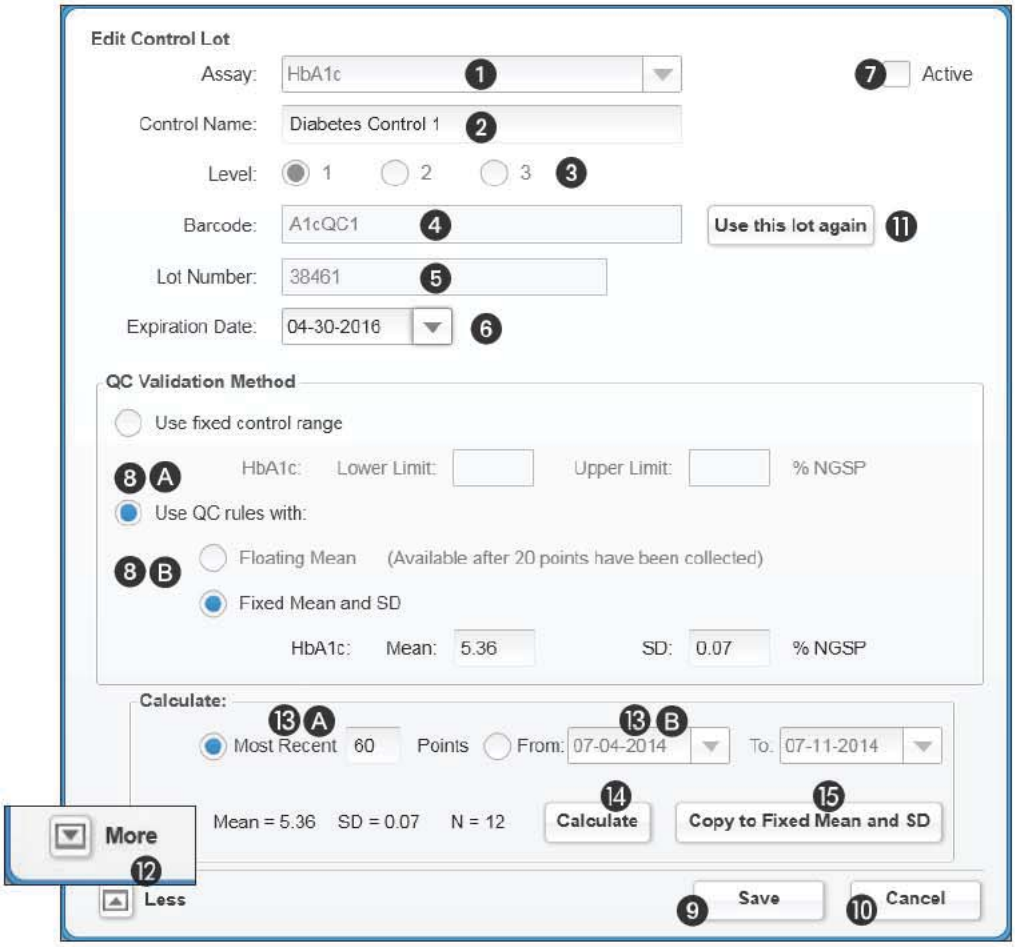
1	Assay: HbA1c.
2	Control Name: Enter the name of the control (maximum: 20 characters).
3	Level: Select control level (1, 2, or 3).
4	Barcode: Enter the microvial adapter barcode label name (maximum: 22 characters; no spaces).
5	Lot Number: Enter the control lot number (maximum: 15 characters).
6	Expiration Date: Enter the control lot expiration date.
7	Active: Select this checkbox to indicate that this is the QC lot currently in use on the system. You will clear the checkbox when the lot is no longer in use.

❽	Select a QC validation method: Ⓐ **Use fixed control range**: Enter the HbA1c **Lower Limit** and **Upper Limit** provided in the QC insert. Ⓑ **Use QC rules with**: This method uses either a **Floating Mean** (which is automatically determined after 20 data points have been collected) or a **Fixed Mean and SD**. If you select the latter option, enter a **Mean** and **SD**. **NOTE:** • *The Floating Mean option cannot be selected until 20 data points have been collected.* • *Ensure all values entered are appropriate for the primary reporting unit shown (i.e., % NGSP or mmol/mol IFCC).*
❾	**Save** button: Touch this button to save the control lot information. A pop-up message confirms that the new lot will now be used for QC rules evaluation. Touch **OK**.
❿	**Cancel** button: Closes the dialog box without saving any information.

4.4.4.2 Viewing or Editing QC Lot Information and Calculation

From the **QC/QC Lots** screen, touch the desired lot row in the table. The Edit Control Lot dialog box appears.

Figure 4-68: *Edit Control Lot Dialog Box (Extended)*



See Section 4.4.4.1 for a description of No. ❶ through ❿ in the dialog box.

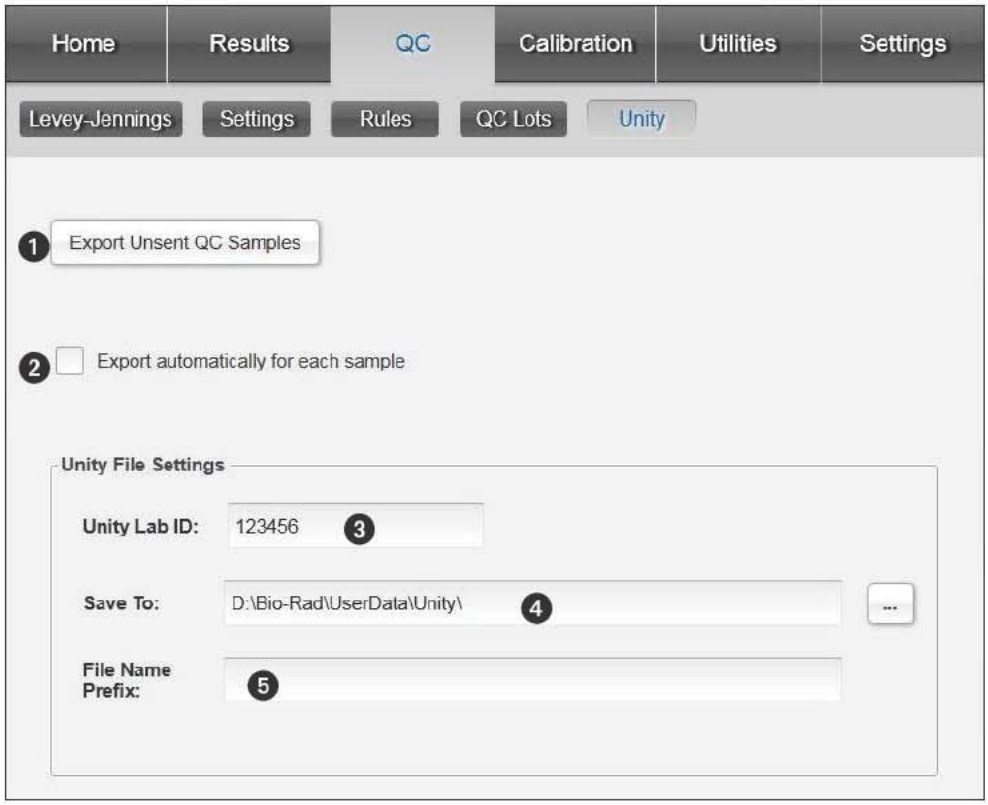
❺	The lot number cannot be edited (appears dimmed) if there is at least one result in the database for that lot.
⓫	**Use this lot again** button: If you want to make a previously used control lot "Active" again, you must touch this button. The same microvial adapter barcode labels are used for all control lots, so this step is necessary to ensure the barcodes are associated with the control lot you are currently using.
⓬	Touch the **More** button to extend the dialog box to view/edit the QC calculation information; touch the **Less** button to condense the dialog box.
⓭	Select from 2 Levey-Jennings calculation options: Ⓐ **Most recent**: Enter the number of most recent data points you want to include in the calculations. Ⓑ **From/To**: Enter a "From" date and a "To" date.

⓮	**Calculate** button: After selecting a calculation option, touch this button to display the calculated Mean, SD, and N.
⓯	**Copy to Fixed mean and SD** button: If you have selected **Use QC rules with Fixed Mean and SD** as your QC validation method (see No. ❽ Ⓑ), touch this button to automatically copy the values to the respective fields.

4.4.5 QC/Unity Screen

The settings for the Bio-Rad Unity Real Time® program are defined in the **QC/Unity** screen.

Figure 4-69: *QC/Unity Screen*



❶	**Export Unsent QC Samples** button: If QC results are not automatically exported to Unity, touch this button to export results.
❷	**Export automatically for each sample**: To automatically export each QC result to Unity immediately after analysis, select this checkbox.
❸	Unity Lab ID: Enter your 6-digit Unity lab ID.
❹	Save To: The default location for exported Unity files is the D:\Bio-Rad\UserData\Unity folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
❺	File Name Prefix: The default file name is "yyyymmddhhmmss.txt". Touch this field to add a prefix to the file name.

4.5 Calibration Screen

The **Calibration** screen provides access to all calibrator results in a table format. This screen can be accessed at any time with the instrument in any state.

Figure 4-70: *Calibration Screen*

1	Date/Time: The date and time the calibration results were generated.
2	Calibrator Lot #: The Calibrator Pack lot number.
3	Cartridge: The lot number and serial number of the cartridge installed for this calibration.
4	Result: The calibration slope, intercept, and reporting unit.
5	Status: The calibration status (i.e., Pass or Fail).
NOTE:	*To view the calibrator results in detail, touch the calibration row. See Section 4.5.1.*

6 **Calibrate Now** button: Touching this button opens the following dialog box.

NOTE: ***Calibrate Now*** *is disabled when the instrument is in Running state.*

Figure 4-71: *Calibration Dialog Box*

- **A** Assay: HbA1c.
- **B** Lot Number: The Calibrator Pack lot number.
- **C** Expiration Date: The Calibrator Pack expiration date.
- **D** Level 1: Calibrator Level 1 assigned value in the primary reporting unit.
- **E** Level 2: Calibrator Level 2 assigned value in the primary reporting unit.

F **Calibrate without reconstituting (calibrate with prediluted material)**: If the Calibrator Pack was previously run/reconstituted on the instrument, this checkbox must be selected.

G **Open Stat Area/Load Stat Area** button: This toggle button is used to eject the Stat rack from the Stat Area so that the Calibrator Pack and controls can be inserted, and then load the Stat rack into the Stat Area.

NOTE: *The calibrator information* **B** *through* **E** *is automatically entered from the barcode label when the Calibrator Pack is loaded into the Stat Area.*

H **Calibrate Now** button: Touch to start the calibration run.

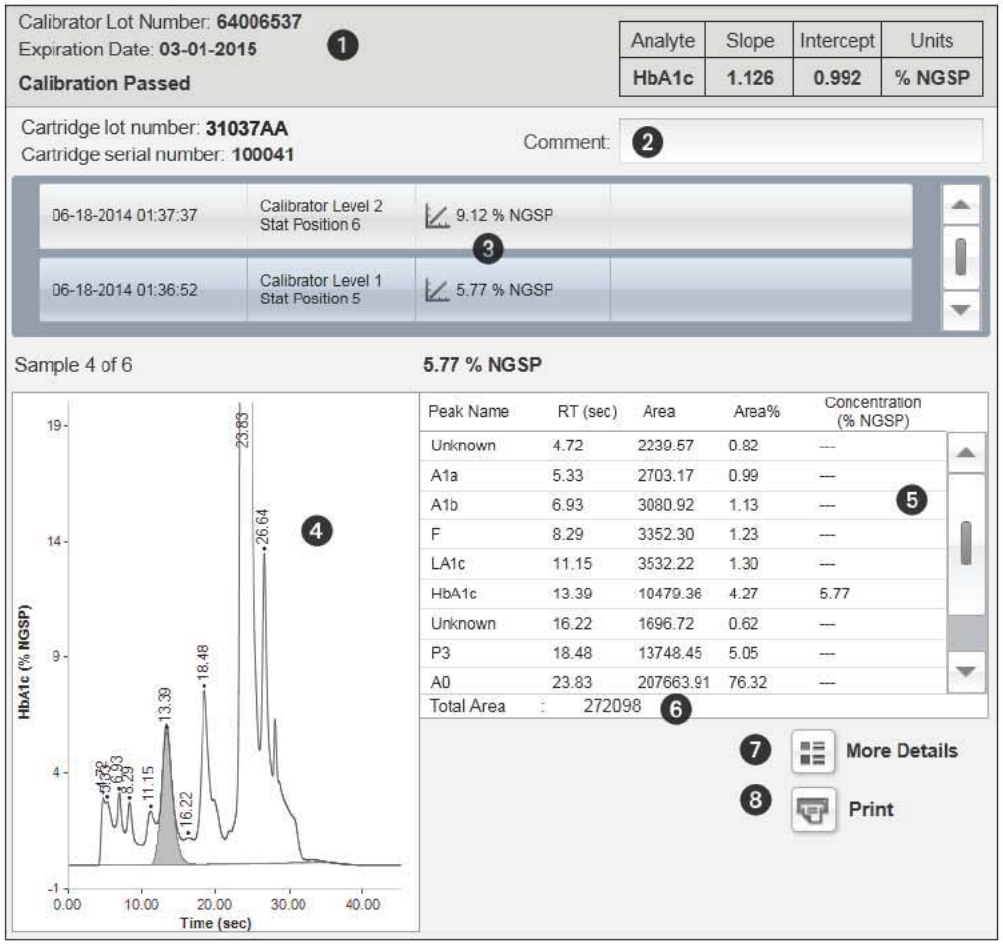
I **Cancel** button: Closes the dialog box without running the Calibrator Pack.

See Section 5.3 for more information regarding running the Calibrator Pack. See Section 5.9 for information regarding running third-party calibrators.

4.5.1 Viewing Calibration Details

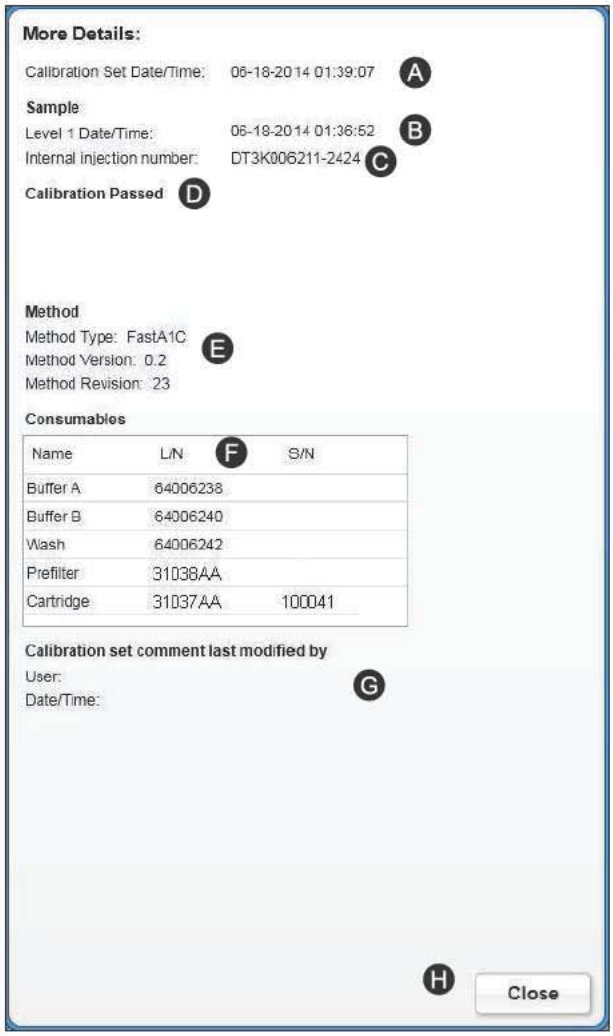
From the **Calibration** screen, touch the desired calibration row in the table. The Calibration Details screen appears.

Figure 4-72: *Calibration Details Screen*



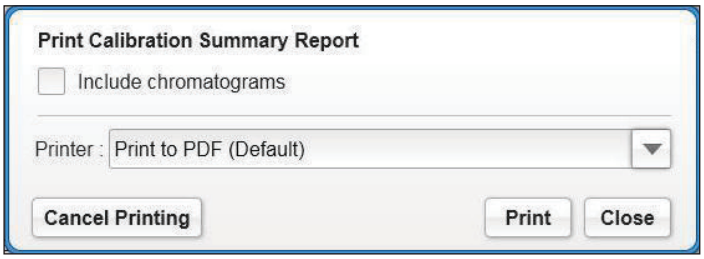
In addition to the calibrator information already provided in the **Calibration** screen (described in Section 4.5), the Calibration Details screen displays the following:

1	Expiration Date: The Calibrator Pack expiration date.
2	Comment: To enter a comment or edit an existing comment for the calibration, touch this field (no limit to number of characters).
3	The results for Calibrator Level 1 and Calibrator Level 2 are displayed in table format, similar to the **Results** screen. For each calibration, there are 3 results for Calibrator Level 1 and 3 results for Calibrator Level 2. The result details displayed are for the result highlighted blue. Touch another result in this table to display the details for that result. **NOTE:** *Use the scroll bar to move up/down the table.*
4	Chromatogram: graph of detector output vs time. For an enlarged view of the chromatogram and peak table, touch the chromatogram (e.g., see Figure 4-45). Touch ✖ to close the enlarged view.

5	Peak Table: This table includes the list of detected peaks, retention time (RT) in seconds, area, area % of non-calibrated peaks, and concentration of HbA1c peak. The HbA_{1c} result appears in the primary reporting unit.
6	Total Area: The sum of all detected peak areas.
7	***Figure 4-73:*** *More Details Pop-Up Window*  **More Details** button: to view additional details regarding the calibrator analysis, touch this button. A pop-up window displays the following: A Calibration Set Date/Time B Sample Date/Time C Internal injection number D Status (i.e., Calibration Passed or Calibration Failed) E Method name, version, and revision F Consumables lot numbers and serial numbers G User name, date, and time of specific user actions H Touch **Close** to close the window.

❽ **Print** button: Touch to print the Calibration Summary Report.

Figure 4-74: *Print Calibration Summary Report Dialog Box*



- **Include chromatograms**: Select this checkbox to include the calibrator chromatograms and peak tables in the printed report.
- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).
  The default location for reports printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Calibration Summary Report is "Calib-System Name-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print the report.

  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Calibration Details screen, then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

4.6 Utilities Tab

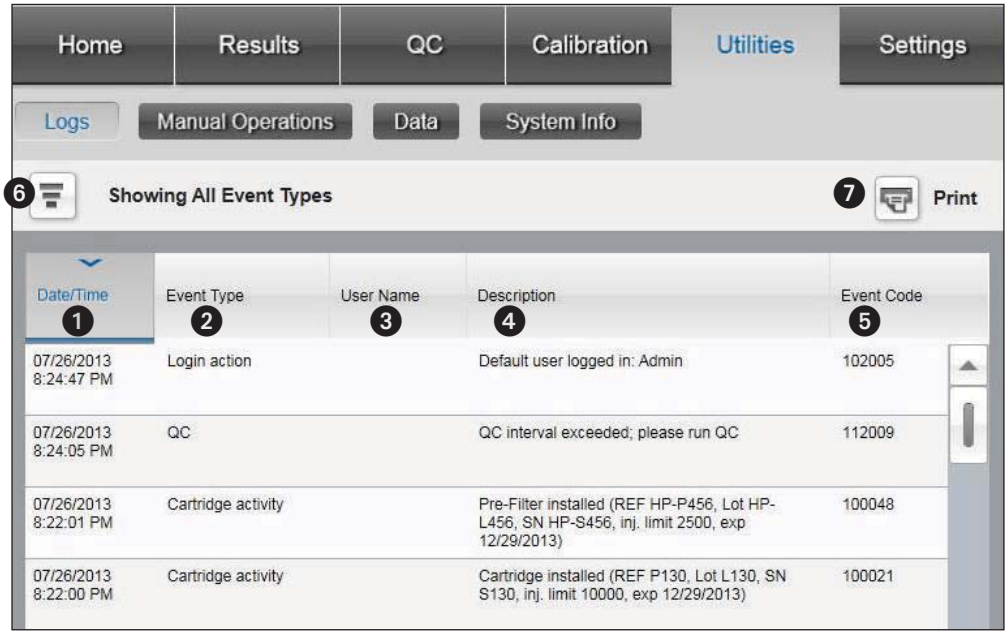
The **Utilities** tab includes the following secondary navigation buttons:

- **Logs** (See Section 4.6.1)
- **Manual Operations** (See Section 4.6.2)
- **Data** (See Section 4.6.3)
- **System Info** (See Section 4.6.4)

4.6.1 Utilities/Logs Screen

Every event that occurs on the system is logged in table format in the **Utilities/Logs** screen.

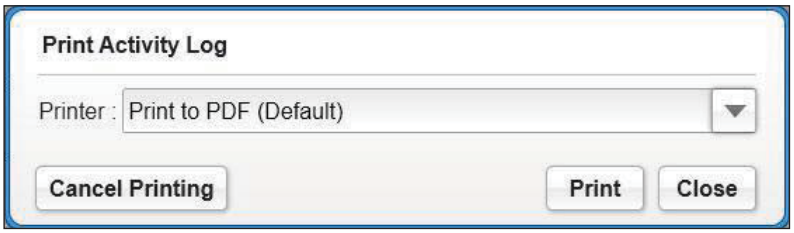
Figure 4-75: *Utilities/Logs Screen*



1	Date/Time: The date and time of the event.
2	Event Type: The general type of event (e.g., Reagent activity, Cartridge activity, Hardware state change, User action, Fault).
3	User Name: The user logged in to the system at the time of the event.
4	Description: The specific description of the event.
5	Event Code: The numerical code for the event.
6	button: Opens the Activity Log Filter dialog box. See Section 4.6.1.1. The filter currently in use is indicated to the right of the button.

❼ **Print** button: Touch to print the Activity Log Report (entire table of filtered log entries). If the log report is lengthy, a pop-up message indicates the number of lines in the log and asks if you want to continue. Touch **Yes** to proceed.

Figure 4-76: *Print Activity Log Dialog Box*



- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).

  The default location for logs printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Activity Log is "Activity Log-System Name-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print the report.

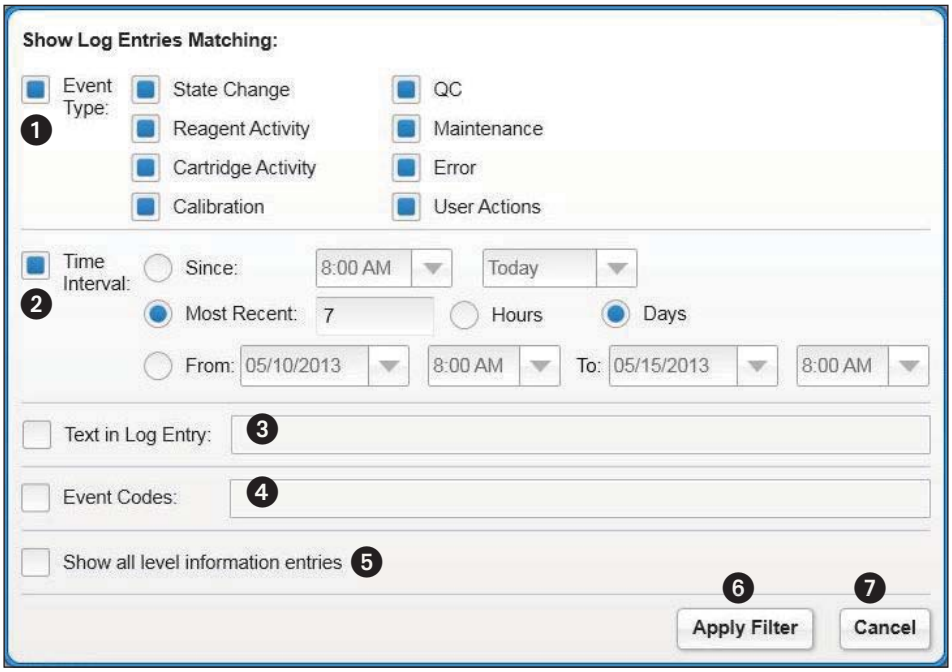
  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Utilities/Logs** screen, touch **Yes** in the pop-up message, and then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

NOTE: *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.*

4.6.1.1 Filtering Activity Log Entries

From the **Utilities/Logs** screen, touch  . The Activity Log Filter dialog box appears. The default filter ("Showing All Event Types") criteria are shown in Figure 4-77.

Figure 4-77: *Activity Log Filter Dialog Box, Default Filter*



To create a custom filter for your lab, select or enter the applicable criteria as follows:

1	To filter by the type of event, select **Event Type** and then select one or more of the following options: • **State Change** (i.e., instrument state changes) • **Reagent Activity** (i.e., buffer, wash, and waste events) • **Cartridge Activity** (i.e., cartridge, prefilter, and low-pressure filter events) • **Calibration** (i.e., calibrator events) • **QC** (e.g., QC warning, QC failure, QC lot expired, etc.) • **Maintenance** (e.g., cleaning, change probe, etc.) • **Error** (e.g., SW fault, HW error, etc.) • **User Actions** (e.g., user logged in/out, user requested Run, QC settings changed, etc.)
2	To filter by an interval of time, select **Time interval** and then select one of the following options: • **Since**: enter a time and select a day (**Today** or **Yesterday**) from the drop-down list. • **Most recent**: enter a number and select **Hours** or **Days**. • **From/To**: enter a "From" date and time plus a "To" date and time.
3	To filter by the text in the Description field, select **Text in Log Entry** and then enter the applicable text.
4	To filter by the Event Code, select **Event Codes** and then enter the applicable code.

❺	**Show all level information entries**: Select this checkbox to include all log entries, regardless of level; if not selected, low-level (insignificant) messages will not be shown.
❻	**Apply Filter** button: To apply the filter, touch this button. The Activity Log table will display the filtered results (under the name "Custom Filter") until you log out of the system or change the filter.
❼	**Cancel** button: Closes the dialog box without saving filter selections.

4.6.2 Utilities/Manual Operations Screen

From the **Utilities/Manual Operations** screen, the following functions can be performed:

- **General: Sleep** (See Section 4.6.2.1), **Clean Screen** (See Section 7.4 for instructions), and **Calibrate Touch Screen** (See Section 7.5 for instructions)
- **Clean System** (See Section 7.8 for instructions)
- **Change Probe** (See Section 7.9 for instructions)
- **Prime/Flush** (See Section 7.6 for instructions)
- **Update Method** (See Section 4.6.2.2)

4.6.2.1 Transitioning to Sleeping State

Some functions cannot be performed unless the instrument is in Sleeping state.

Figure 4-78: *Utilities/Manual Operations Screen, General and Sleep Buttons*

1. Go to the **Utilities/Manual Operations** screen.
2. Touch **General**.
3. Touch **Sleep**.

4.6.2.2 Updating Method

Test parameters are automatically updated when the analytical cartridge RFID is read. However, there may be a case where a new method file must be downloaded from a USB flash drive.

Figure 4-79: *Utilities/Manual Operations Screen, Update Method Buttons*

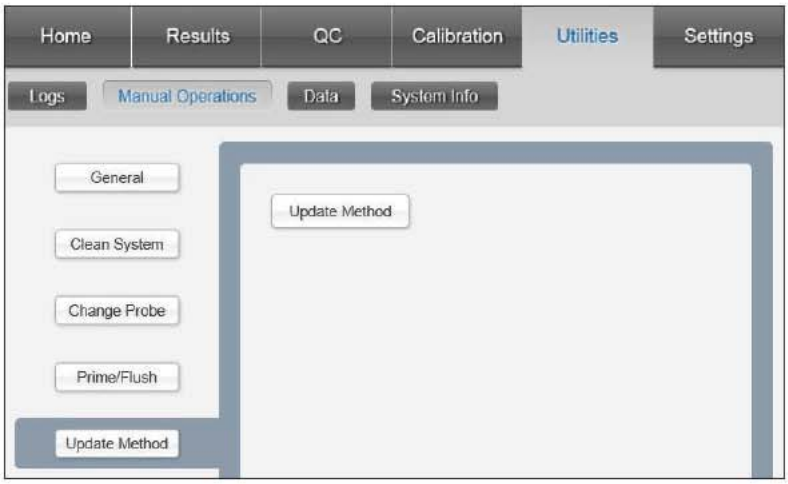
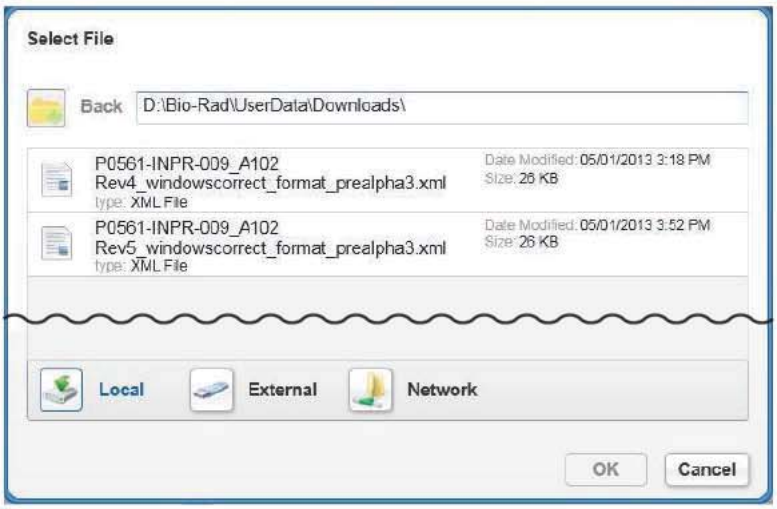


Figure 4-80: *Select File Dialog Box*

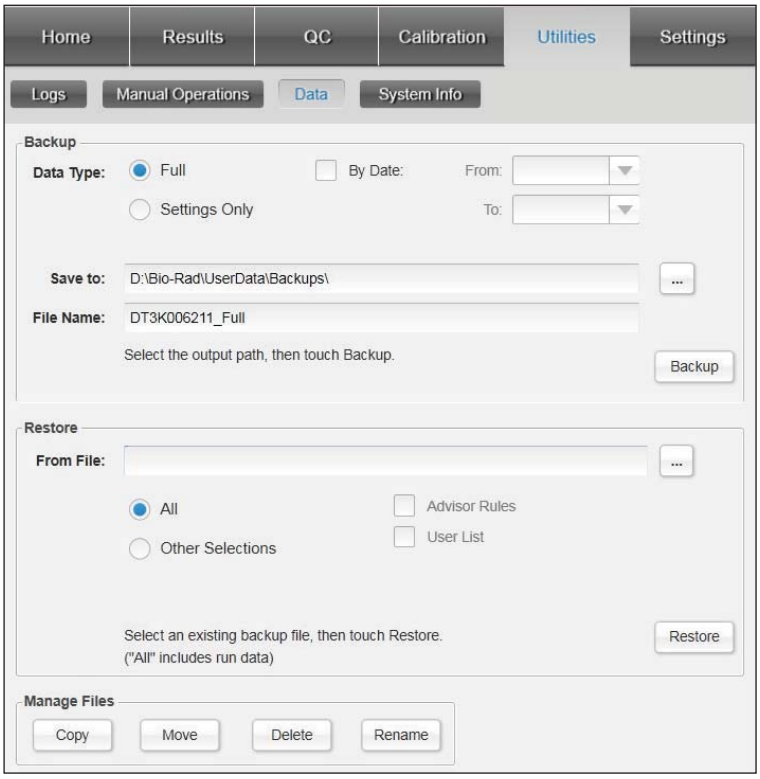


1. The instrument must be in Sleeping or Standby state.
2. Insert the flash drive containing the method file into one of the D-100 USB ports.
3. Go to the **Utilities/Manual Operations** screen.
4. Touch **Update Method**.
5. Touch the second **Update Method** button.
6. In the Select File dialog box, select the corresponding drive and the file name.
7. Touch **OK**.
8. A pop-up message confirms that the new method was imported. Touch **OK**.

4.6.3 Utilities/Data Screen

From the **Utilities/Data** screen, the following functions can be performed:

Figure 4-81: *Utilities/Data Screen*



- Backing up the database (See Section 4.6.3.1)
- Restoring a database (See Section 4.6.3.2)
- Managing files (See Section 4.6.3.3)

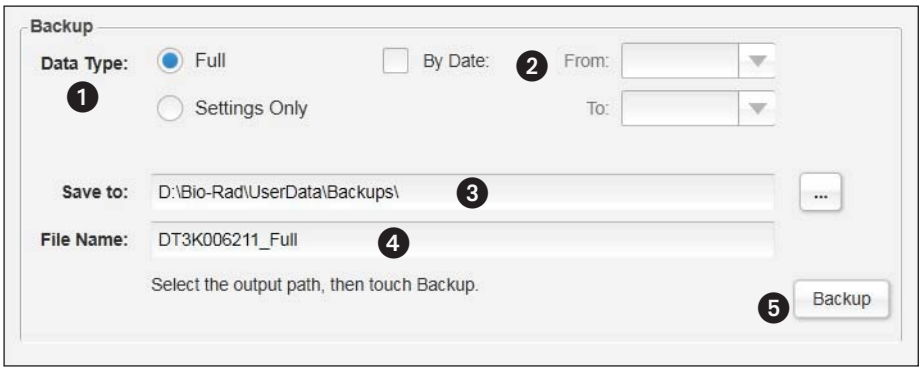
4.6.3.1 Backing Up the Database

IMPORTANT! ***It is highly recommended to back up data on a regular basis.***

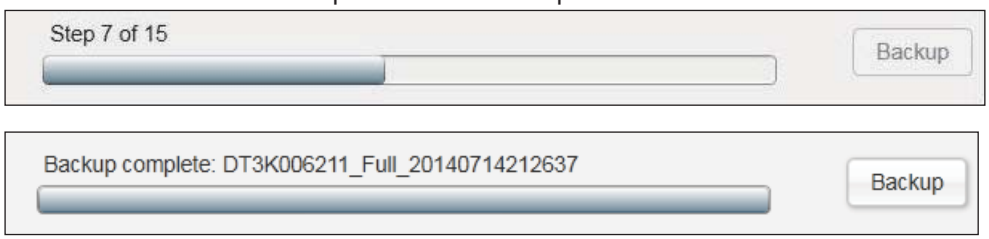
- All data is maintained in a database by the D-100 software.
- Each D-100 database is limited to approximately 100,000 results. When the database reaches its limit, results will be managed on a FIFO (first in, first out) basis as new results are added to the database. There is no warning when the database reaches its limit.
- During the backup process, the **Utilities/Data** screen cannot be exited to perform other system functions. Complete any urgent activities before backing up data.
- To view or print previous data in a backed-up database, use the **View Archive** function (see Section 4.3.3.4). It is not necessary to restore the database.

The instrument must be in Sleeping or Standby state. Go to the **Utilities/Data** screen.

Figure 4-82: *Utilities/Data Screen, Backup Dialog Box*



1	Data Type: Select **Full** to back up the entire database (i.e., configuration, test properties, sample data, and event logs) or **Settings Only** to back up the configuration and user settings only.
2	To back up data from a specific date interval, select **By Date** and then enter a "From" date plus a "To" date.
3	Save to: The default location for database backups is the D:\Bio-Rad\UserData\Backups folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
4	File Name: The default file name is "Instrument Serial Number_Full/Set_yyyymmddhhmmss.back". The File Name field is autopopulated with the instrument serial number and "Full" or "Set", depending on the data type selected. To enter a different prefix for the file name, touch the field and edit the prefix.

❺	**Backup** button: Touch this button to back up the database. A progress bar to the left of the button indicates the status and completion of the backup.  **NOTE:** *The time to back up a database depends on the size of the database and the speed of the drive. For a complete database of 100,000 results, backup may require approximately 45 minutes. More frequent backups of a smaller database using a* ***By Date*** *range will reduce the backup time.*

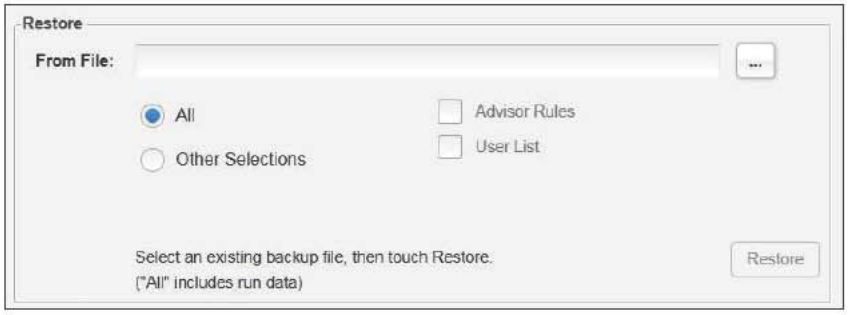
4.6.3.2 Restoring a Database

- The Restore function lets you restore a backed-up database for use on a different D-100 instrument or for troubleshooting purposes.
- During the restore process, the **Utilities/Data** screen cannot be exited to perform other system functions. Complete any urgent activities before restoring data.

NOTE: *When you restore a database, the current database is replaced by the restored database; the current database cannot be restored once it is replaced. To simply view or print previous data, use the* ***View Archive*** *function (see Section 4.3.3.4).*

1. The instrument must be in Sleeping state.
2. Go to the **Utilities/Data** screen.

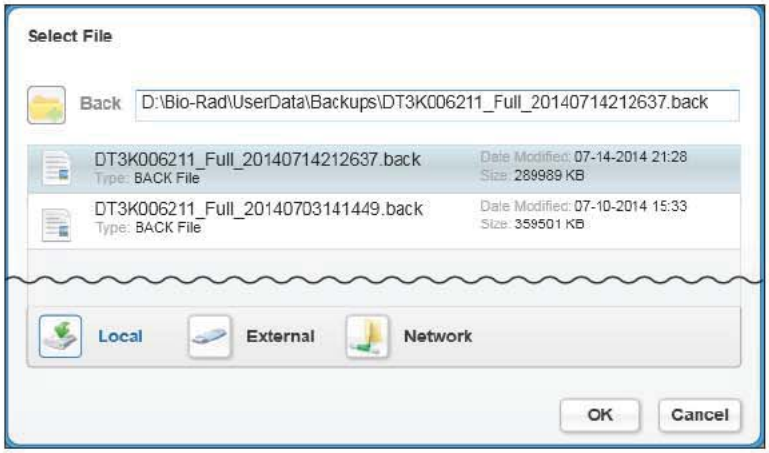
Figure 4-83: *Utilities/Data Screen, Restore Dialog Box*



3. In the Restore dialog box, touch the From File field or the browse button ... to locate the database backup file.
4. In the Select File dialog box, select the file to restore from the applicable drive/folder and touch **OK**.

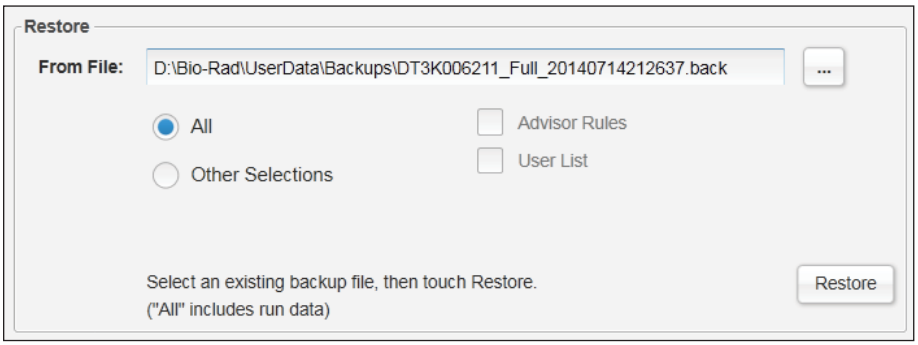
   The default location for database backup files is the D:\Bio-Rad\UserData\Backups folder. Only files with the extension .back are displayed.

Figure 4-84: *Select File Dialog Box*

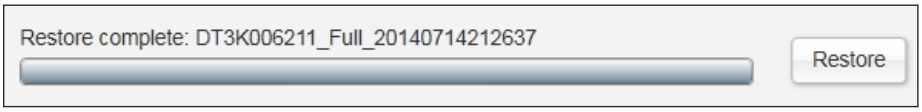


5. The file path appears in the From File field of the Restore dialog box.

Figure 4-85: *Restore Dialog Box after file selected*



6. Select one of the following options for the type of data you want to restore:
   - **All**: The entire database (i.e., configuration, test properties, sample data, and event logs) will be restored.
   - **Other Selections**: Select this option to restore only specific information from the database, and select one or both of the following checkboxes:
     - **Advisor Rules**
     - **User List**
7. Touch **Restore** to restore the file.
8. A pop-up message indicates that your current database will be replaced by the restored database and asks if you want to continue. Touch **Yes** to proceed.
9. Another pop-up message indicates that the system is restoring the database, and a progress bar to the left of the Restore button indicates the status and completion of the restore process.



NOTE: *The time to restore a database depends on the size of the database and the speed of the drive. For a database of 100,000 results, the restore process may require approximately 40 minutes.*

4.6.3.3 Managing Files

The D-100 software lets you copy, move, delete, or rename files.

Figure 4-86: *Utilities/Data Screen, Manage Files Buttons*

4.6.3.3.1 Copying Files

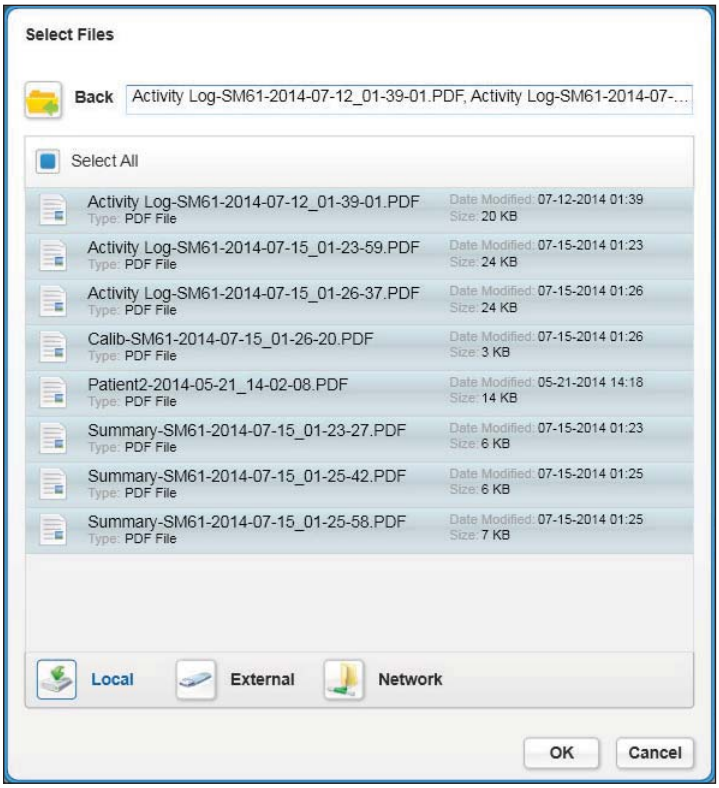
1. Go to the **Utilities/Data** screen.
2. Touch **Copy**.

Figure 4-87: *Utilities/Data Screen, Copy Files Dialog Box*

3. In the Copy Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to copy.
4. In the Select Files dialog box, select the file(s) to copy from the applicable drive/folder and touch **OK**.

   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*

Figure 4-88: *Select Files Dialog Box*



5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Copy Files dialog box.

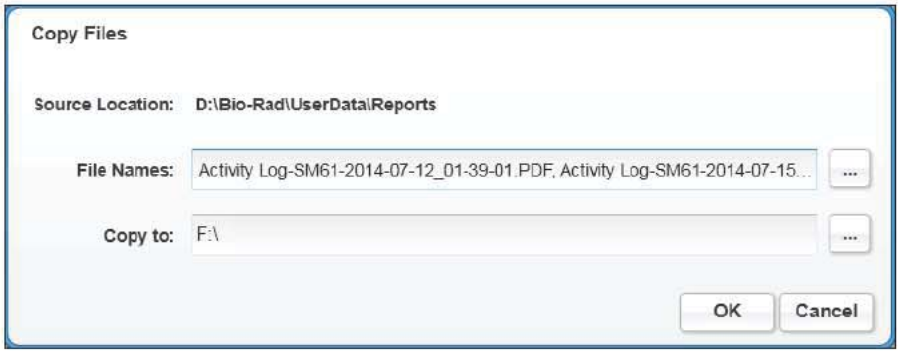
Figure 4-89: *Copy Files Dialog Box after files selected*

6. Touch the Copy to field or the browse button ... to select the destination location.
7. In the Select Folder dialog box, select the destination for the copied files and touch **OK**.

Figure 4-90: *Select Folder Dialog Box*

8. The selected destination location appears in the Copy to field of the Copy Files dialog box.

Figure 4-91: *Copy Files Dialog Box after destination selected*



9. Touch **OK** to copy the file(s).

4.6.3.3.2 Moving Files

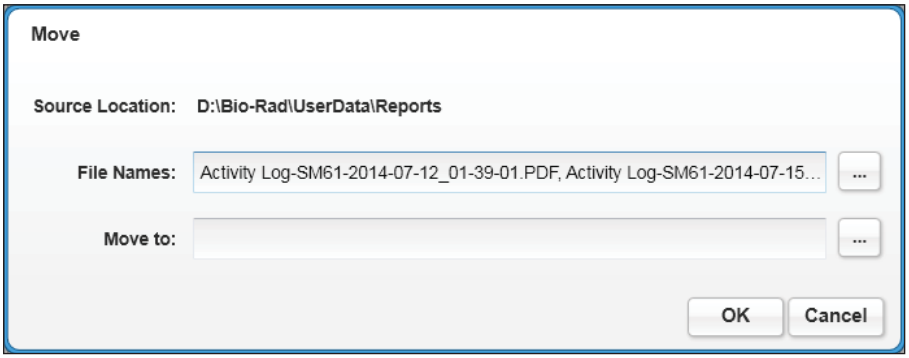
1. Go to the **Utilities/Data** screen.
2. Touch **Move**.

Figure 4-92: *Utilities/Data Screen, Move Files Dialog Box*

3. In the Move Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to move.
4. In the Select Files dialog box (see Figure 4-88), select the file(s) to move from the applicable drive/ folder and touch **OK**.

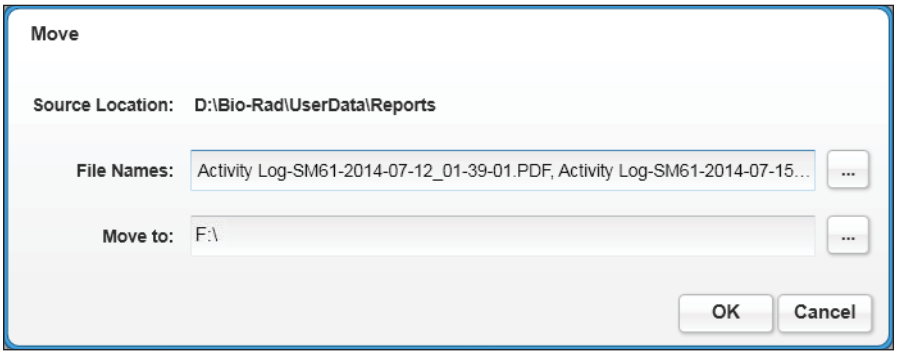
   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*
5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Move Files dialog box.

Figure 4-93: *Move Files Dialog Box after files selected*



6. Touch the Move to field or the browse button ... to select the destination location.
7. In the Select Folder dialog box (see Figure 4-90), select the destination for the moved files and touch **OK**.
8. The selected destination location appears in the Move to field of the Move Files dialog box.

Figure 4-94: *Move Files Dialog Box after destination selected*



9. Touch **OK** to move the file(s).

4.6.3.3.3 Deleting Files

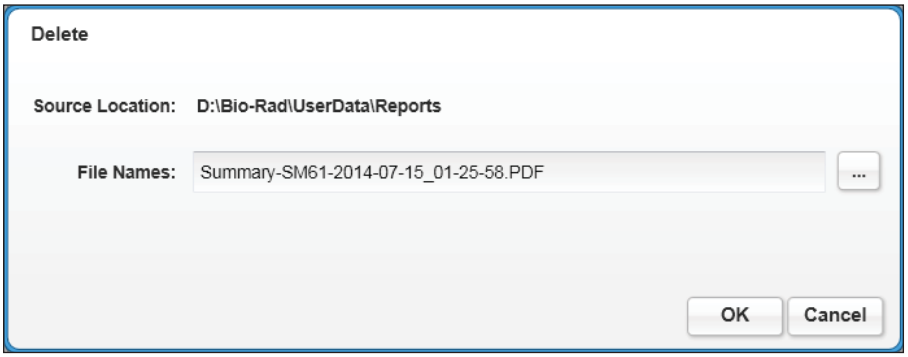
1. Go to the **Utilities/Data** screen.
2. Touch **Delete**.

Figure 4-95: *Utilities/Data Screen, Delete Files Dialog Box*

3. In the Delete Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to delete.
4. In the Select Files dialog box (see Figure 4-88), select the file(s) to delete from the applicable drive/folder and touch **OK**.

   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*
5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Delete Files dialog box.

Figure 4-96: *Delete Files Dialog Box after files selected*



6. Touch **OK** to delete the file(s).
7. A pop-up message asks if you are sure you want to delete the files. Touch **Yes** to delete.

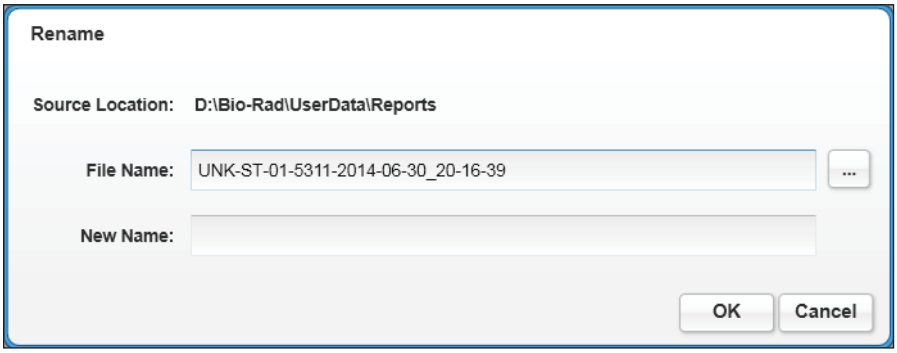
4.6.3.3.4 Renaming Files

1. Go to the **Utilities/Data** screen.
2. Touch **Rename**.

Figure 4-97: *Utilities/Data Screen, Rename File Dialog Box*

3. In the Rename File dialog box, touch the File Name field or the browse button ... to locate the file to rename.
4. In the Select File dialog box (see Figure 4-88), select the file to rename from the applicable drive/ folder and touch **OK**.
5. The selected file path appears in the Source Location field and the selected file appears in the File Name field of the Rename File dialog box.

Figure 4-98: *Rename File Dialog Box after file selected*



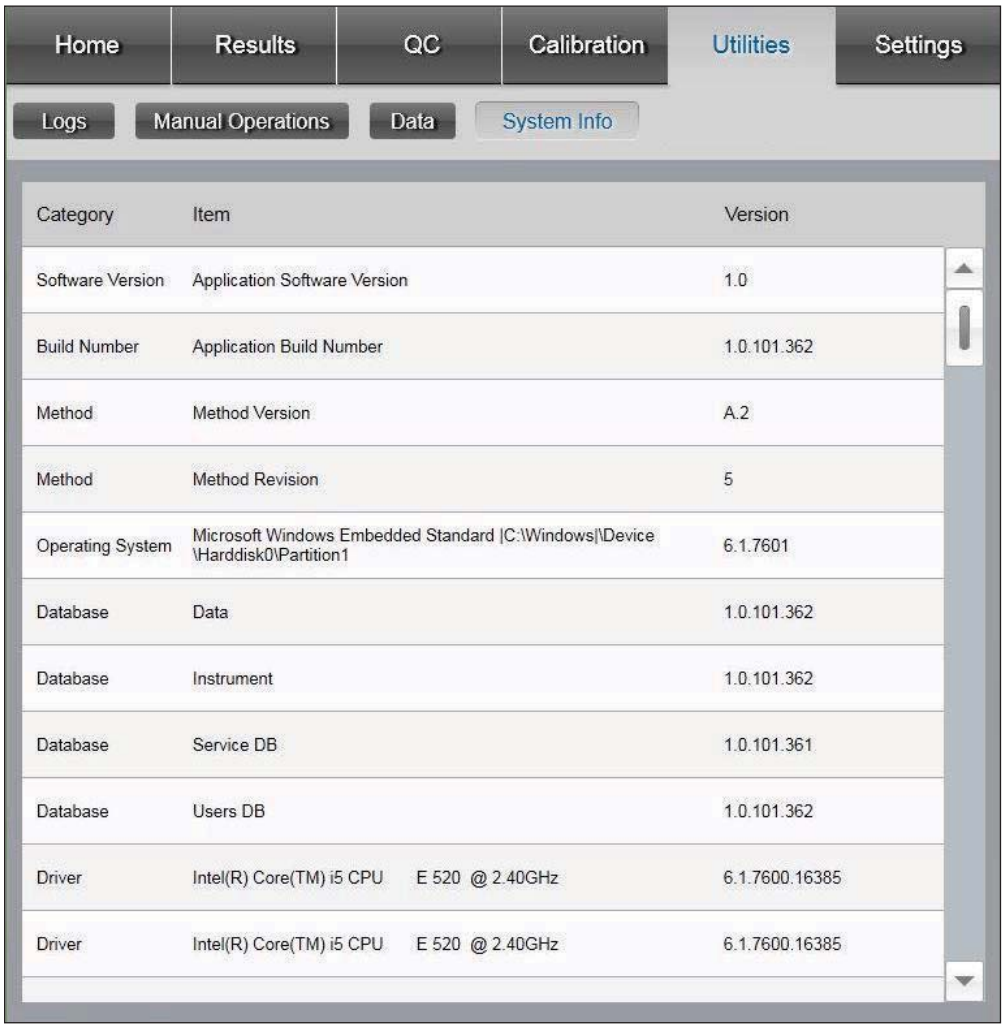
6. Touch the New Name field to enter the new file name.
7. Touch **OK**.

4.6.4 Utilities/System Info Screen

The **Utilities/System Info** screen displays the version numbers for all D-100 software, method, database, and hardware/firmware items currently installed on the instrument.

Use the scroll bar to move up/down the table.

Figure 4-99: *Utilities/System Info Screen*



4.7 Settings Tab

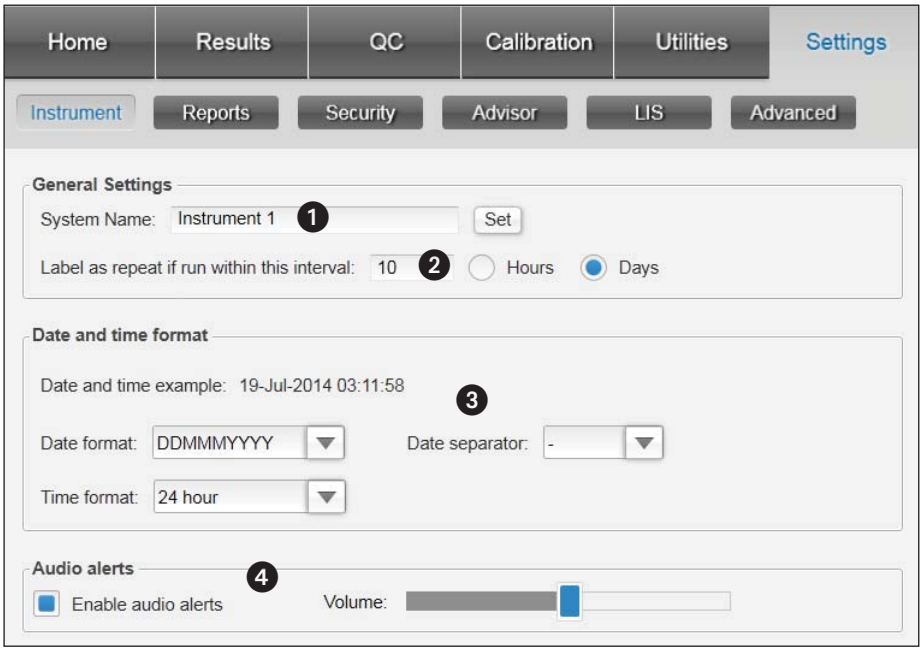
The **Settings** tab includes the following secondary navigation buttons:

- **Instrument** (See Section 4.7.1)
- **Reports** (See Section 4.7.2)
- **Security** (See Section 4.7.3)
- **Advisor** (See Section 4.7.4)
- **LIS** (See Section 4.7.5)
- **Advanced** (See Section 4.7.6)

NOTE: *All settings in the **Settings** tab require Level3 access to modify, except changing one's own password in the **Security** screen.*

4.7.1 Settings/Instrument Screen

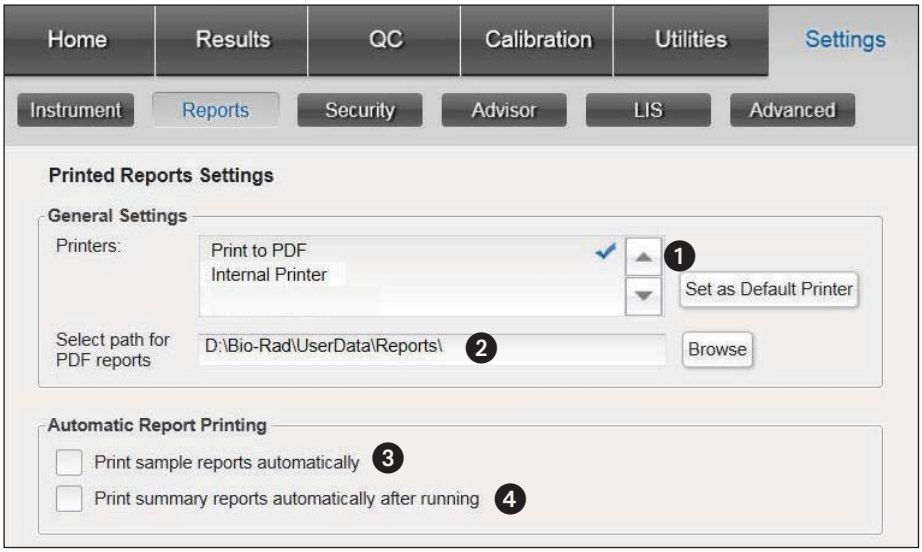
Figure 4-100: *Settings/Instrument Screen*



1	System Name: enter a name for the instrument (maximum: 12 characters). Touch **Set** to save the name.
2	A sample result will be labeled as a repeat (i.e., ⟳) if the sample is processed multiple times within a defined interval. Enter a number and select **Hours** or **Days**.
3	Select the preferred date format, date separator, and time format from the corresponding drop-down lists.
4	**Enable audio alerts**: If this checkbox is selected, audible alarms will accompany important instrument messages or conditions. Select the preferred volume for the alarms; moving the slider to the right increases the volume.

4.7.2 Settings/Reports Screen

Figure 4-101: *Settings/Reports Screen*

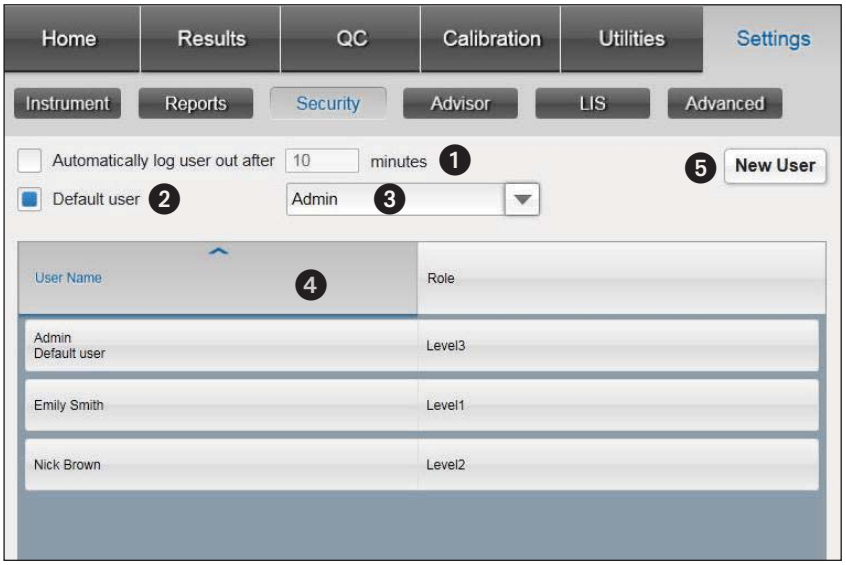


1	Printers: All printers configured for your instrument are listed. Define the default printer by selecting a printer from the drop-down list and then touch **Set as Default Printer**. The default printer is indicated by a blue checkmark and moves to the top of the list.
2	Select path for PDF reports: The default location for all reports (e.g., results, Activity Logs, rules sets, etc.) printed to PDF is the D:\Bio-Rad\UserData\Reports folder. To select a different location, touch **Browse**. In the Select Folder dialog box, select the drive/folder and touch **OK**.
3	**Print sample reports automatically**: Select this checkbox to automatically print the sample report after each sample is processed.
4	**Print summary reports automatically after running**: Select this checkbox to automatically print a summary report after the run is finished.

4.7.3 Settings/Security Screen

The system provides user access through user name and password combinations. This access information is displayed and managed in the **Settings/Security** screen. This screen can be accessed at any time with the instrument in any state.

Figure 4-102: *Settings/Security Screen*

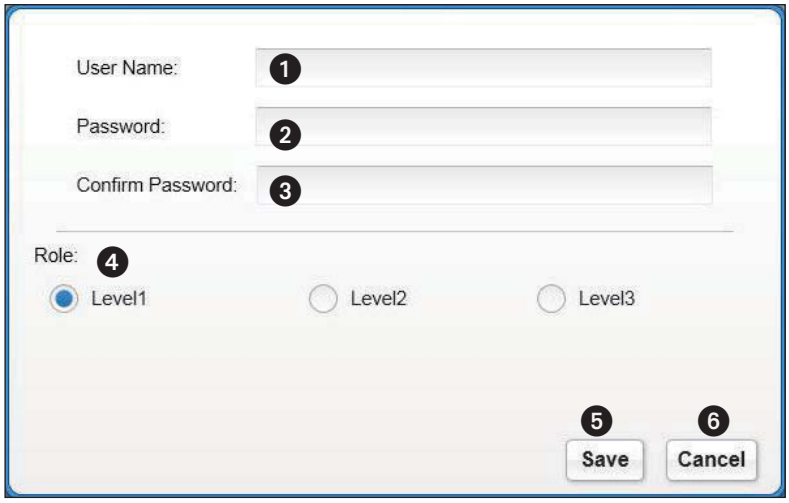


1	**Automatically log user out:** To have the system automatically log out the user after a period of time, select this checkbox and enter the number of minutes. **NOTE:** *If automatic log out occurs during a critical process (i.e., Backup or Restore), the system will delay the log out until the process is completed.*
2	**Default user:** To define a default user profile for the system, select this checkbox and select a user name from the drop-down list. This feature allows system use without manual log-in/password entry. For example, setting a Level 1 user as the default user allows any user to perform daily operation functions without logging in; access to higher level features would require log-in. The default user is automatically logged in when the system is powered on or if a logged-in user logs out.
3	The system is configured with a predefined user profile: Admin. The password is provided to authorized users by Bio-Rad Technical Service. This administrator profile provides full access to all customer-permissible features. This user profile cannot be edited or deleted.
4	Each user name and role (i.e., access level) is displayed in this table. **NOTE:** • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To edit or delete a user profile, touch the row. See Section 4.7.3.2.*
5	**New User** button: To add a new user to the system, touch this button. See Section 4.7.3.1.

4.7.3.1 Adding a New User Profile

From the **Settings/Security** screen, touch **New User**. The User dialog box appears. Only authorized users can add a user profile.

Figure 4-103: *User Dialog Box, New User*



1	User Name: enter the new user name (maximum: 32 characters).
2	Password: enter a password (maximum: 16 characters). **NOTE:** *When logging into the system, user names are not case-sensitive; passwords are case-sensitive.*
3	Confirm Password: re-enter the password to confirm.

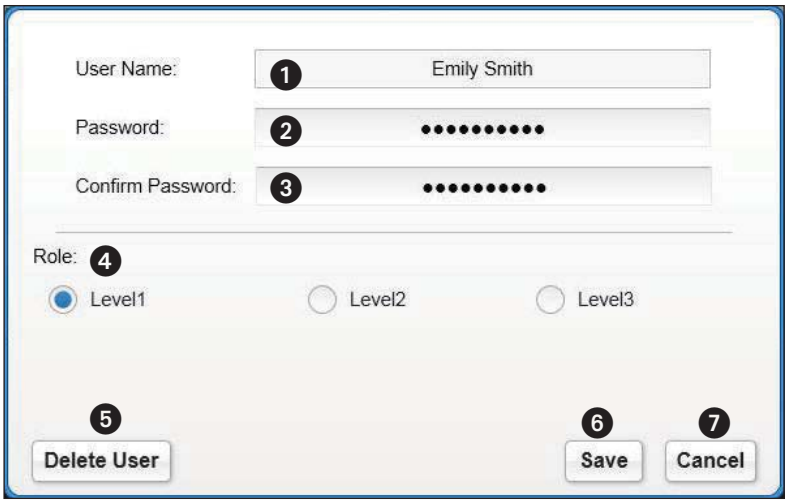
❹	Role: select an access level for the user.	
	Level3 (Supervisor)	Permission to access all customer-permissible features, except cannot restore a database (accessible to Admin user only). Level3 users can release flagged results, modify all instrument settings including Advisor and QC rules and settings, and run with expired consumables.
	Level2 (Lead Technician)	Same permissions as Level3, except the following: • Cannot modify any settings in the **Settings** tab (except own user password) • Cannot modify any settings in the **QC/Settings** or **QC/Rules** screens • Cannot exclude QC data points from calculations • Cannot release or reject flagged results • Cannot run with expired calibrators • Cannot manage files • Cannot calibrate the touchscreen Level2 users can release non-flagged results, enter new QC lot information, back up a database, and export data.
	Level1 (Secondary Technician)	Same permissions as Level2, except the following: • Cannot release or reject any results • Cannot add or edit QC lots • Cannot back up a database • Cannot export data Level1 users can replace consumables, calibrate the D-100, perform a run, and view, print, and add comments to results.
❺	**Save** button: Touch this button to save the user profile.	
❻	**Cancel** button: Closes the dialog box without saving any information.	

4.7.3.2 Editing or Deleting a User Profile

From the **Settings/Security** screen, touch the desired user row in the table. The User dialog box appears. Only authorized users can change or delete a user profile.

NOTE: *Users cannot change their own role or delete themselves; another user with appropriate permission must change the role or delete the user profile.*

Figure 4-104: *User Dialog Box, Existing User*



1	User Name: The user name cannot be edited.
2	Password: To change the password, touch the field and enter a new password (maximum: 16 characters).
3	Confirm Password: If applicable, re-enter the new password to confirm.
4	Role: To change the user's permissions, select the applicable access level.
5	**Delete User** button: To delete the user profile from the system, touch this button.
6	**Save** button: Touch this button to save the changes.
7	**Cancel** button: Closes the dialog box without saving any changes.

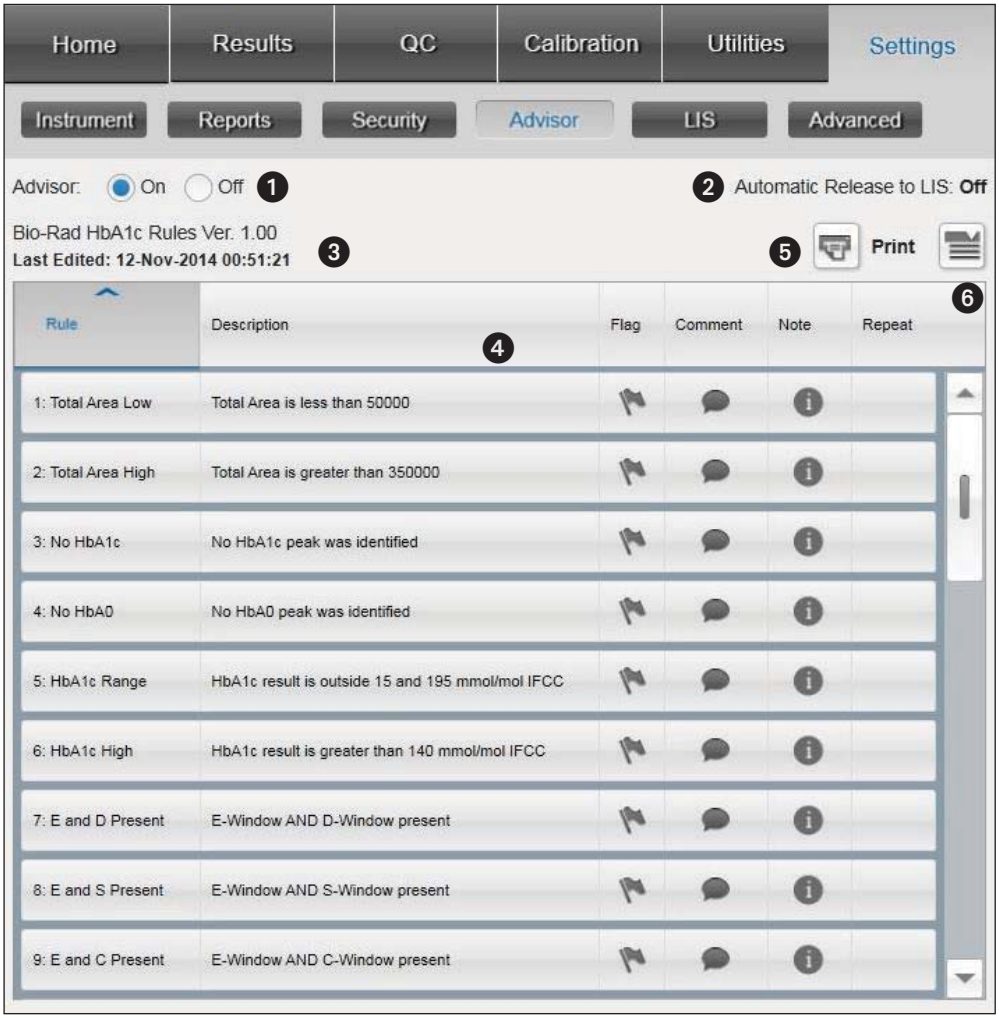
4.7.4 Settings/Advisor Screen

The D-100 System is configured with a set of predefined rules (i.e., "Advisor") that facilitates results reporting by identifying sample results that require further review before they are released to the LIS.

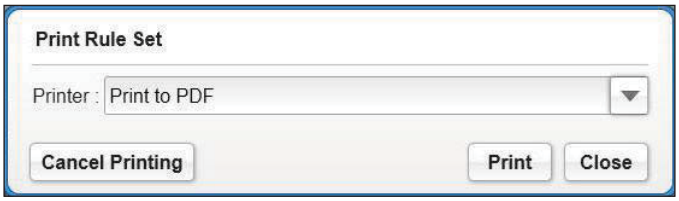
Bio-Rad provides a default rules set based on performance claims in the assay Instructions For Use and additional parameters. See Appendix C for rule details.

The **Settings/Advisor** screen provides access to all rules in table format.

Figure 4-105: *Settings/Advisor Screen*



1	Advisor: You have the option to enable (**On**) or disable (**Off**) rules processing.
2	Indicates whether the Automatic Release to LIS function for patient results is selected (On) or not selected (Off); this function is set in the **Settings/LIS** screen. See Section 4.7.5.
3	Indicates the name of the rules set currently in use and the date/time it was last edited. **NOTE:** *Once a named rule set has been modified, the saved name is no longer displayed. It becomes a modified rule set identified only by the date/time of the last saved edit.*

❹	Each Advisor rule is displayed as a row in this table. The rule name and description are provided. Icons in the last 4 columns indicate the action(s) that will occur when a result triggers the rule: • Flag : The result will be flagged. • Comment : The result will include a comment. • Note : The result will include a note. • Repeat : The sample will automatically be repeated. **NOTE:** • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To view or edit the rule details, touch the row. See Section 4.7.4.1.*
❺	**Print** button: Touch to print the rules set. ***Figure 4-106:*** *Print Rule Set Dialog Box*  • **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for rules sets printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the rules set (i.e., Advisor Report) is "Advisor-System Name-yyyy-mm-dd_hh-mm-ss.pdf". • **Print** button: Touch this button to print the rules set. **NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Settings/Advisor** screen, then touch **Cancel Printing** in the dialog box.* • **Close** button: Closes the dialog box without printing.
❻	Advisor Menu button: See Section 4.7.4.2.

4.7.4.1 Viewing/Editing Rule Details

From the **Settings/Advisor** screen, touch the desired rule row in the table. The Rule Details screen appears.

NOTE:

- *Rules can be edited, if necessary, by authorized users only.*
- *Rules cannot be edited while the instrument is in Running state.*
- *Any use of a rule, whether modified or provided as a default rule, is undertaken at the Customer's sole risk. The content and use of each rule depends on the Customer's parameters for processing patient test results. Because the Customer's parameters cannot possibly be known by Bio-Rad Laboratories, Inc., the Customer undertakes sole responsibility for the contents, and resulting actions or uses, of any rule.*

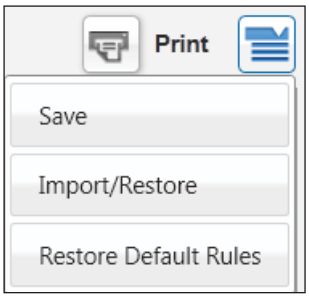
Figure 4-107: *Rule Details Screen, HbA1c Range*

1	Rule Set: The name of the rules set currently in use. **NOTE:** *Once a named rule set has been modified, the saved name is no longer displayed. It becomes a modified rule set identified only by the date/time of the last saved edit.*
2	Rule: The name of the rule.
3	**Enable Rule**: The rule will be included in the rules processing if this checkbox is selected; clear the checkbox to disable the rule.
4	Conditions: The rule parameters are displayed. Parameters vary by rule.

❺	Actions: The rule actions are displayed. The available actions vary by rule. • **Flag Sample**: If this checkbox is selected, the result will be flagged when the rule is triggered. • **Comment**: If this checkbox is selected, the result will include the comment shown when the rule is triggered. Comments can be transmitted to the LIS. • **Note to user**: If this checkbox is selected, the result will include the note shown when the rule is triggered. Notes are not transmitted to the LIS. • **Repeat Sample**: If this checkbox is selected, the sample will automatically be repeated one time (if it is available for processing) when the rule is triggered.
❻	**Save** button: Touch this button to save the changes to the rule. This button is enabled only if the rule has been edited.
❼	**Cancel** button: Closes the Rule Details screen without saving any changes.

4.7.4.2 Advisor Menu

Figure 4-108: *Advisor Menu*



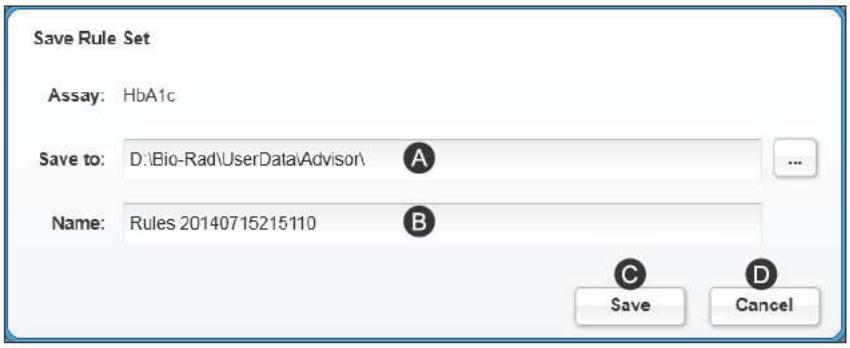
From the **Settings/Advisor** screen, touch . The Advisor Menu appears. There are 3 actions available:

- **Save** (See Section 4.7.4.2.1)
- **Import/Restore** (See Section 4.7.4.2.2)
- **Restore Default Rules** (See Section 4.7.4.2.3)

4.7.4.2.1 Saving a Rules Set

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch .
3. From the Advisor Menu, touch **Save**.

Figure 4-109: *Save Rule Set Dialog Box*



A	Save to: The default location for saving rules sets is the D:\Bio-Rad\UserData\Advisor folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
B	Name: The default file name is "Rules yyyymmddhhmmss.brs". The date/time is when the rule set was last edited. Touch the field to change the file name.
C	**Save** button: Touch this button to save the rules set.
D	**Cancel** button: Closes the dialog box without saving the rules set.

4.7.4.2.2 Importing/Restoring a Rules Set

The **Import/Restore** function lets you import a saved rules set for use on a different D-100 instrument or import default rules set updates from Bio-Rad.

NOTE: *When you import a rules set, the current rules set is replaced by the imported rules set; the current rules set cannot be restored unless it is saved.*

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch .
3. From the Advisor Menu, touch **Import/Restore**.

Figure 4-110: *Import Rule Set Dialog Box*

A	Rules to Import: Touch this field or the browse button ... to locate the rules set to import. In the Select File dialog box, select the file to restore from the applicable drive/folder and touch **OK**. The default location for rules set files is the D:\Bio-Rad\UserData\Advisor folder. Only files with the extension .brs are displayed.
B	The rules set name and date/time last edited appear in the Import Rule Set dialog box. ***Figure 4-111:*** *Import Rule Set Dialog Box after file selected* 
C	**Import** button: Touch this button to import the rules set. A pop-up message indicates that your current rules will be replaced by the imported rules and asks if you want to continue. Touch **Yes** to proceed.
D	**Cancel** button: Closes the dialog box without saving the rules set.

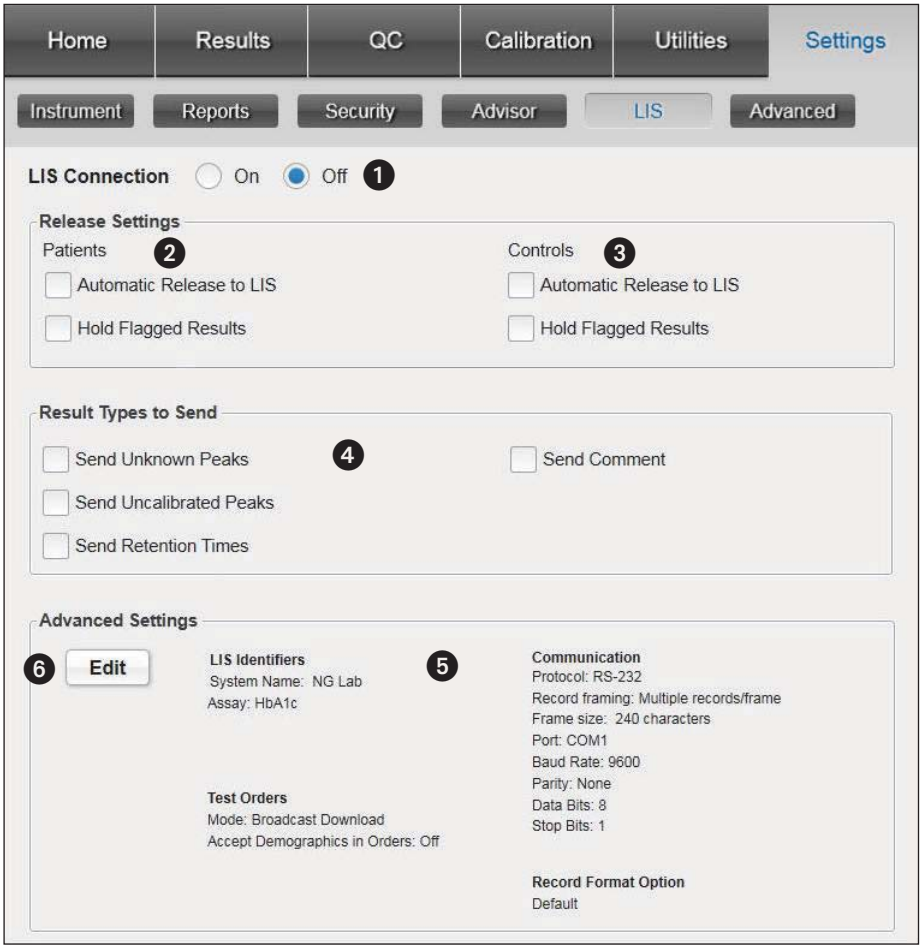
4.7.4.2.3 Restoring the Default Rules Set

The Bio-Rad Default Rules Set can be restored as follows:

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch  .
3. From the Advisor Menu, touch **Restore Default Rules**.
4. A pop-up message indicates that your current rules will be replaced by the default rules and asks if you want to continue. Touch **Yes** to proceed.

4.7.5 Settings/LIS Screen

Figure 4-112: *Settings/LIS Screen*

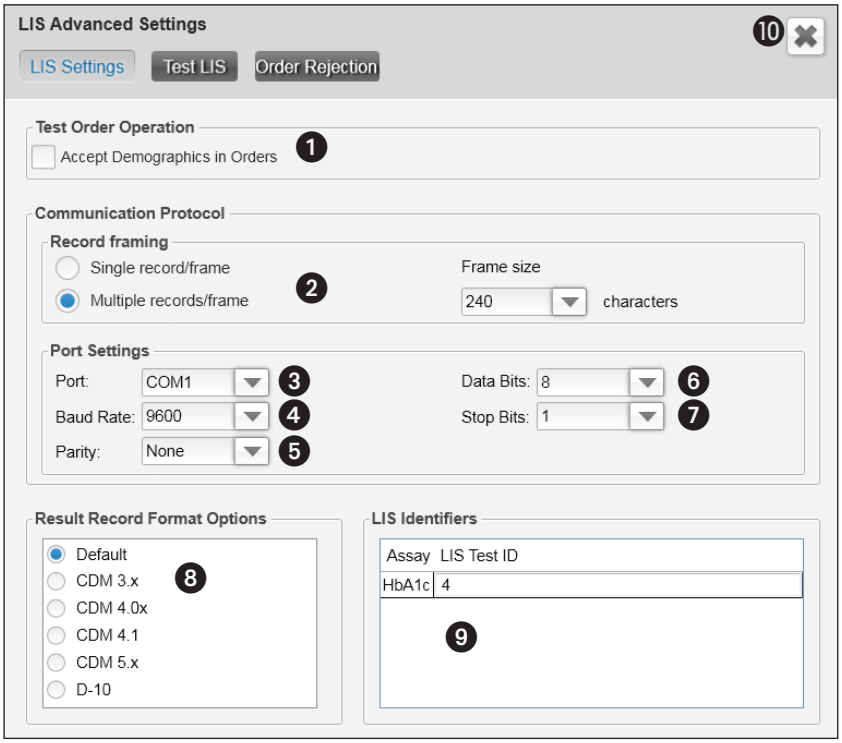


1	LIS Connection: You have the option to enable (**On**) or disable (**Off**) the LIS connection.
2	Release Settings for Patients: • To have the system automatically release patient results to the LIS, select the **Automatic Release to LIS** checkbox. • To have the system hold flagged patient results for manual review, select the **Hold Flagged Results** checkbox.

❸	Release Settings for Controls: • To have the system automatically release QC results to the LIS, select the **Automatic Release to LIS** checkbox. • To have the system hold flagged QC results, select the **Hold Flagged Results** checkbox.
❹	Result Types to Send: You have the option to send specific types of data. • **Send Unknown Peaks**: Peaks that are detected but not identified are labeled "Unknown". If transmission of unknown peaks is required, select this checkbox. **NOTE:** ***Send Uncalibrated Peaks*** *must also be selected for unknown peaks to be transmitted to the LIS.* • **Send Uncalibrated Peaks**: For the HbA1c assay, only the HbA1c peak is calibrated. If transmission of uncalibrated peaks is required, select this checkbox. • **Send Retention Times**: If transmission of peak retention times is required, select this checkbox. • **Send Comment**: If transmission of result comments is required, select this checkbox.
❺	Advanced Settings: Other LIS settings are displayed.
❻	**Edit** button: To edit any of the LIS Advanced Settings, touch this button. The LIS Advanced Settings are organized under 3 tabs: • **LIS Settings** (See Section 4.7.5.1) • **Test LIS** (See Section 4.7.5.2) • **Order Rejection** (See Section 4.7.5.3)

4.7.5.1 LIS Settings Tab

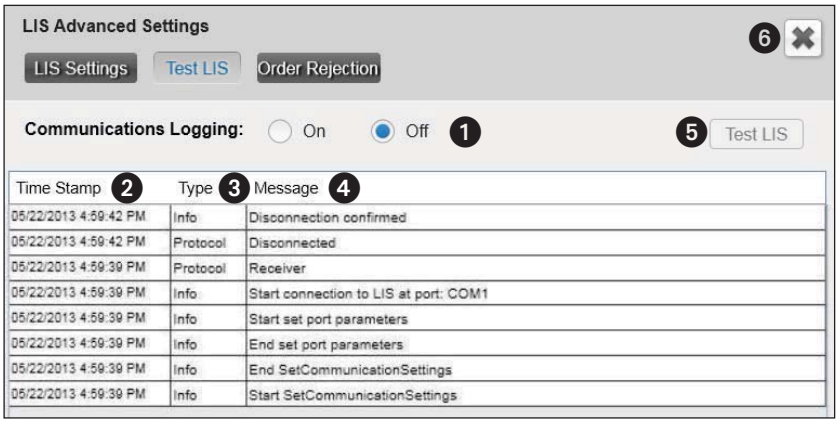
Figure 4-113: *LIS Advanced Settings/LIS Settings Tab*



❶	**Accept Demographics in Orders**: To allow patient demographic data to be included with the test order, this checkbox must be selected.
❷	Record framing: Select one option. • **Single record/frame** • **Multiple records/frame** (default); also select the frame size (**240** or **63993** characters) from the drop-down list. Default is 240.
❸	Port: Select the communication port (**COM1**, **COM2**, etc.) from the drop-down list.
❹	Baud Rate: Select the baud rate from the drop-down list. Default is 9600.
❺	Parity: Select a parity option (**None, Odd, Even, Mark**, or **Space**) from the drop-down list. Default is None.
❻	Data Bits: Select the data bits (**4, 5, 6, 7**, or **8**) from the drop-down list. Default is 8.
❼	Stop Bits: Select the stop bits (**None, 1, 2**, or **1.5**) from the drop-down list. Default is 1.
❽	Result Record Format Options: You can select from a list of existing Bio-Rad result record formats to match the format your lab is currently using.
❾	LIS Test ID: Enter the Test ID for the assay. Default is 4 for the HbA1c assay.
❿	Touch ✖ to close the tab.

4.7.5.2 Test LIS Tab

Figure 4-114: *LIS Advanced Settings/Test LIS Tab*

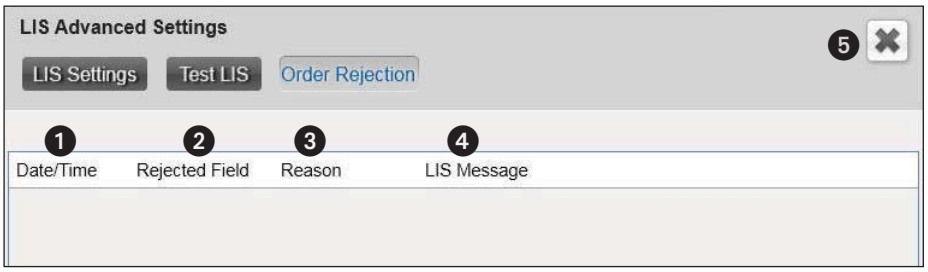


❶	**Communications Logging**: You have the option to enable (**On**) or disable (**Off**) the logging of Low Level transmissions between the instrument and the LIS (both directions). **NOTE:** *Disable (turn **Off**) Communications Logging when you are finished checking LIS communications.*
❷	Time Stamp: The date and time of the transmission.
❸	Type: The type of transmission (e.g., Info, Protocol, Send, Receive).
❹	Message: The full contents of the transmission.
❺	**Test LIS** button: Touch this button to reset the LIS ports and send a test message to the LIS to verify the connection is working and the LIS is responding.
❻	Touch ✖ to close the tab.

4.7.5.3 Order Rejection Tab

Every test order sent by the LIS that is rejected by the D-100 System is logged in table format in the **Order Rejection** tab.

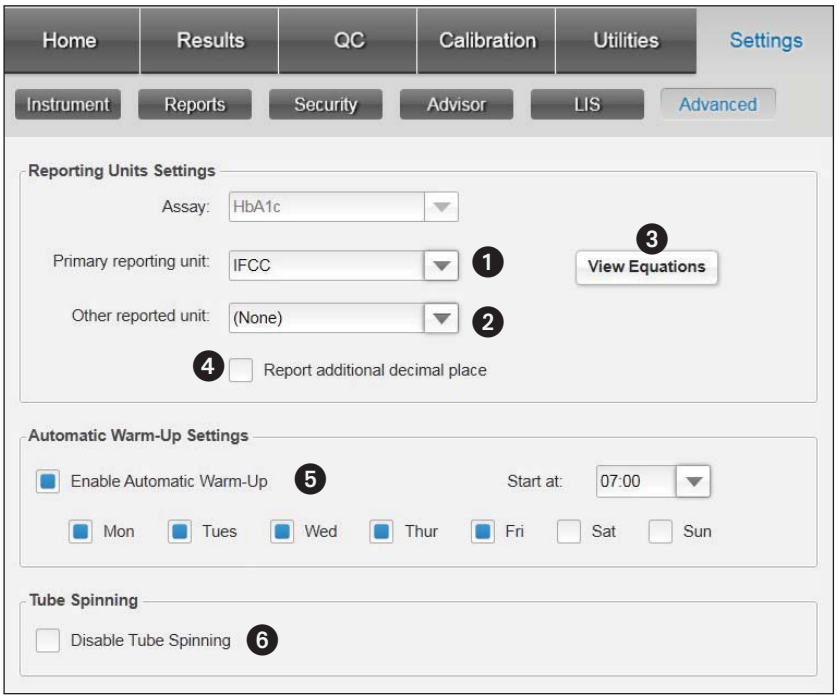
Figure 4-115: *LIS Advanced Settings/Rejection Log Tab*

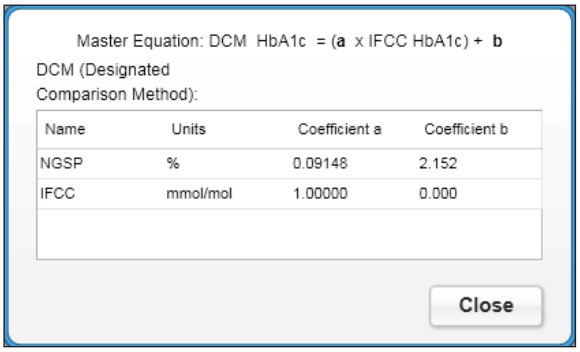


1	Date/Time: The date and time the order was rejected.
2	Rejected Field: “LIS field rejected” or “LIS message rejected”.
3	Reason: A description of why the field or message was invalid.
4	LIS Message: The LIS message that was rejected or that contains the field that was rejected.
5	Touch ✖ to close the tab.

4.7.6 Settings/Advanced Screen

Figure 4-116: *Settings/Advanced Screen*



1	Primary reporting unit: Select your preferred HbA1c primary reporting unit [**IFCC** (mmol/mol) or **NGSP** (%)] from the drop-down list.	
2	Other reported unit: If you want to report using both units, select the secondary reporting unit from this drop-down list.	
3	***Figure 4-117:*** *Master Equation* 	**View Equations**: Touch this button to view the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) standardized HbA1c master equation and DCMs (Designated Comparison Methods) supported by D-100.
4	**Report additional decimal place:** To display and report results with an additional decimal place, select this checkbox. Patient and QC results are reported at the same decimal precision.	
	Default decimal precision • IFCC = xxx (e.g., 43 mmol/mol) • NGSP = xx.x (e.g., 6.1%)	*Additional decimal precision* • IFCC = xxx.x (e.g., 42.7 mmol/mol) • NGSP = xx.xx (e.g., 6.06%)
5	**Enable Automatic Warm-Up:** To have the system automatically perform startup operations, select this checkbox. Also select the checkbox(es) for the applicable day(s) of the week and select the start time.	

❻	**Disable Tube Spinning:** You have the option to disable the tube spinning if you use sample racks from another system that do not allow tube spinning (i.e., tube positions do not have rotary bottoms).

1 Run Operations

5.1 Overview of Sample Analysis

5.1.1 Processing Primary Sample Tubes

a. Barcode information for the sample tube is read by the barcode reader.
b. The sample probe pierces the tube cap, then withdraws sample from the tube.
c. The sample is diluted in the dilution well.
d. The sample is injected into the buffer stream (analytical flow path).
e. The sample probe and line are flushed to prevent cross-contamination between samples.
f. The sample and buffer mixture flows through the cartridge, where the sample is separated into its constituents.
g. The sample constituents and buffer flow through the detector, where the absorbance of each sample constituent is measured.
h. The detector output is plotted as a chromatogram in a result report.
i. A system flush removes any residual sample components.

5.1.2 Processing Prediluted Samples

a. A sensor detects the presence of a microvial adapter in the rack.
b. If present, barcode information on the microvial or adapter is read by the barcode reader.
c. The sample probe pierces the microvial cap, then withdraws sample from the microvial.
d. The sample is injected into the buffer stream (analytical flow path).
e. The sample probe and line are flushed to prevent cross-contamination between samples.
f. The sample and buffer mixture flows through the cartridge, where the sample is separated into its constituents.
g. The sample constituents and buffer flow through the detector, where the absorbance of each sample constituent is measured.
h. The detector output is plotted as a chromatogram in a result report.
i. A system flush removes any residual sample components.

5.2 System Startup

NOTE: *It is not necessary to perform System Startup on a daily basis. During normal operation, it is recommended to keep the D-100 powered on.*

1. Turn on the D-100 power switch (on the rear of the instrument). If the instrument power switch is already turned on, press the soft power button on the front of the instrument to activate the instrument from standby power mode to full operating power. See Figure 2-1, No. 3.
2. The Bio-Rad software startup screen will display while the software is loading.
3. To log into the system, see Section 4.1.1, No. 5.
4. After the software is loaded, touch **Run** in the banner to initiate the system warm-up. The instrument will transition from Sleeping to Warming Up state.

   NOTE: *To enable Automatic Warm-Up, see Section 4.7.6.*
5. After the warm-up is complete, the instrument transitions to Standby state. It is now ready to run.

5.3 Running the Calibrator Pack

See the assay Instructions For Use for information regarding storage of the Calibrator Pack and calibration frequency.

1. The instrument must be in Sleeping or Standby state.
2. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
3. Insert the Calibrator Pack in the dedicated position of the Stat rack with the barcodes facing you.
4. Insert QC samples in positions 1–3 of the Stat rack as instructed in Section 5.4.

   NOTE:

   - *All barcodes are read before processing the first sample in the Stat rack. The Calibrator Pack is processed first, followed by the other Stat rack samples in sequential order.*
   - *Verify that the control lots and barcodes in the **QC/QC Lots** screen match the QC samples being run (see Section 4.4.4).*
5. Touch ⏏ **Load**. The Stat rack is loaded into the Stat Area. The calibrator information is automatically entered from the barcode.
6. The Calibration dialog box appears (see Figure 4-71). Touch **Calibrate Now**.

   NOTE:

   - *If the Calibrator Pack was previously run/reconstituted on the instrument, the **Calibrate without reconstituting** checkbox must be selected.*
   - *Calibration takes approximately 30 minutes. Once the calibration run is started, it cannot be stopped.*
7. After the Stat rack samples are finished being processed, touch ⏏ **Open**.
8. Remove the Calibrator Pack and QC samples from the Stat rack.

5.4 Running Controls (QC)

See the assay Instructions For Use for information regarding preparation of the controls and recommended QC frequency.

1. Check the message panel for red or yellow messages and address as needed.
2. Before running a new lot of QC, go to the **QC/QC Lots** screen (while in Sleeping or Standby state). Touch **Add Control**. Enter the necessary information in the Add Control Lot dialog box (see Section 4.4.4.1).
3. Insert the prediluted QC microvials into the appropriately barcode-labeled microvial adapters.
4. The prediluted QC samples can be run in the Stat Area or in a Sysmex rack.
   a. To run in the Stat Area, touch ⏏ **Open** in the **Home** screen to retrieve the Stat rack. Insert the microvial adapters in positions 1–3 of the Stat rack with the barcodes facing you so they are visibly displayed through the rack slots. Touch ⏏ **Load**. If not in Running state, touch **Run**. After the QC samples are finished being processed, touch ⏏ **Open**. Remove the QC microvials and adapters from the Stat rack.
   b. To run in a Sysmex rack, insert the microvial adapters with the magnet facing the back of the rack. Place the rack in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you. If not in Running state, touch **Run**. Remove the processed Sysmex rack from the output area.

   NOTE: *To avoid processing empty sample positions, ensure empty barcoded microvial adapters are removed from racks before the next run.*

5.5 Running Patient Samples

1. Check the message panel for red or yellow messages and address as needed.
2. Insert the primary sample tubes in the Sysmex racks.

   NOTE:
   - *If the sample is in an abnormal size/type tube, or if the height of the sample in the tube appears to be ≤1 cm, then the sample must be prediluted and placed in a microvial adapter. See the assay Instructions For Use for Specimen Preparation instructions. Insert the microvial adapter with the magnet facing the back of the rack.*
   - *All prediluted sample microvials and primary sample tubes should be capped before loading them onto the system.*
3. Place the racks in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you.
4. If not in Running state, touch **Run**.

5. Remove the processed Sysmex racks from the output area.

NOTE:

- *A maximum of 9 racks can be positioned in the rack input area at one time; a 10th rack can be inserted after the run starts and the 1st rack is in the sampling position.*
- *A maximum of 10 racks are permitted in the rack output area at one time. You may continue adding racks to the input area as you remove completed racks from the output area.*

5.6 Running Urgent (Stat) Samples

Priority samples can be analyzed during a run in progress.

1. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
2. Insert the sample(s) (i.e., primary sample tubes or prediluted samples in microvial adapters) in positions 1–3 of the Stat rack, with the barcode(s) facing you.
3. Touch ⏏ **Load**. The Stat rack is loaded into the Stat Area.
4. The Stat sample(s) will automatically be processed after completion of the last aspirated sample. After the Stat sample(s) are finished being processed, the instrument resumes processing samples in Sysmex racks.
5. After the Stat samples are finished being processed, touch ⏏ **Open**.
6. Remove the samples from the Stat rack.

5.7 Handling Unread Barcodes

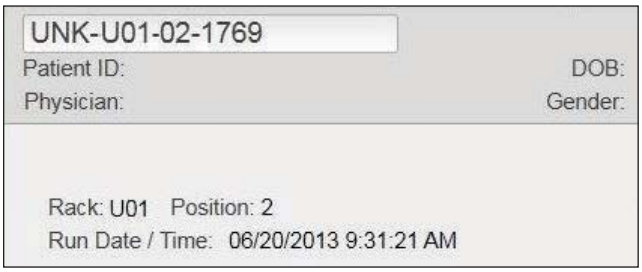
5.7.1 Unread Sample Barcodes

When a patient sample has no barcode or has a damaged barcode that cannot be read, the system automatically generates a “UNK” (Unknown) Sample ID based on the Rack ID (i.e., Sysmex rack number or ST for Stat rack), tube position, and unique injection number (e.g., UNK-001-01-injection number).

The Sample ID can be edited after the sample analysis is complete.

1. From the **Results** screen, touch the desired sample result row in the table. The Result Details screen appears.

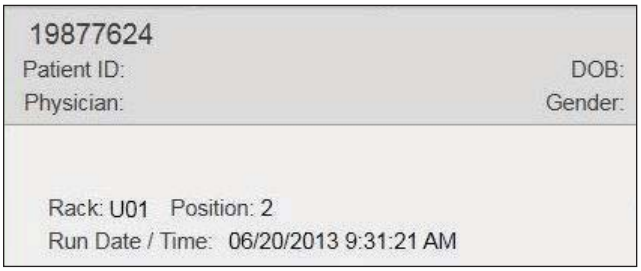
Figure 5-1: *Result Details Screen, Editable Sample ID Field*



2. Touch the Accession Number/Sample ID field and enter the correct sample ID.
3. The system automatically looks for a corresponding order from the LIS and enters the Patient ID, Physician, DOB, and/or Gender if present.
4. The Confirm New Barcode dialog box appears. Touch **Confirm** to save the corrected Sample ID; once the Sample ID is saved, it cannot be edited.

Figure 5-2: *Confirm New Barcode Dialog Box*

Figure 5-3: *Result Details Screen, Corrected Sample ID*



5.7.2 Unread Rack Barcodes

When a Sysmex rack barcode is missing or cannot be read, the system automatically generates a 3-character Rack ID (e.g., U01, U02, up to U99). The “U” indicates unknown; the number is incremented for each auto-generated ID; the number is reset to 01 on system startup and whenever it reaches 99.

5.8 Repeating Samples

Under certain circumstances, samples will be automatically repeated (if available):

- If a sample's total area exceeds a limit defined in the method, the results for subsequent samples potentially impacted (due to risk of carryover) are not reported. The system is automatically flushed and the subsequent samples are repeated.
- If a sample's HbA_{1c} level exceeds a certain limit defined in the method, the result for the subsequent sample is not reported (due to risk of carryover); the subsequent sample is automatically repeated.
- The system can be configured to automatically repeat a QC sample after a QC rules failure. See Section 4.4.2.
- The system can be configured to automatically repeat a sample when a specific Advisor rule is triggered. See Section 4.7.4.1. The repeat sample will be processed as the next test.

Otherwise, to manually repeat a sample, run it in a Sysmex rack or run it as a Stat sample (see Section 5.6).

5.9 Running Third-Party Calibrators

If there is an occasion when your lab is required to use a third-party liquid calibrator (e.g., IFCC calibrators) to calibrate the D-100, please follow these instructions.

1. Run the D-100 Calibrator Pack to ensure the cartridge is properly conditioned (see Section 5.3).
2. Predilute each third-party liquid calibrator 1:300:
   a. Mix the calibrator by inverting the vial several times.
   b. Pipet 1.5 mL of D-100 Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the calibrator.
   c. Cap the microvial and mix thoroughly.
3. The instrument must be in Sleeping or Standby state.
4. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
5. Insert a microvial containing 1.5 mL of Sample Diluent into position 1 to substitute the conditioner.
6. Insert the prediluted calibrator 1 (low level) and calibrator 2 (high level) microvials into unlabeled microvial adapters in positions 2 and 3 of the Stat rack, respectively. Touch ⏏ **Load** or wait until step 8 to load the rack into the Stat Area.
7. Go to the **Calibration** screen and touch **Calibrate Now**.
8. In the Calibration dialog box (see Figure 4-71), touch **Load Stat Area**. The Stat rack is loaded into the Stat Area.
9. Enter the required calibrator information (i.e., assigned values) and select the **Calibrate without reconstituting** checkbox.
10. Touch **Calibrate Now**.
11. After the Stat Area samples are finished being processed, touch ⏏ **Open**.

12. Remove the samples from the Stat rack.

5.10 Stopping a Run

Touch **Stop** in the banner. The instrument state changes from Running to Stopping. The system finishes processing the last aspirated sample and ejects the racks from the shuttle. The instrument performs end-of-run operations and transitions to Standby state.

5.11 Shutting Down and Restarting the D-100 System

NOTE: *It is not necessary to shut down the D-100 on a daily basis. During normal operation, it is recommended to keep the D-100 powered on.*

1. To shut down the system, touch **Log Out**.
2. In the Log Out dialog box, touch **Shut Down**.
3. To restart the system, press the soft power button on the front of the instrument. See Figure 2-1, No. 3.

5.12 Long-Term System Shutdown

The D-100 should remain powered on when idle to keep the reagents pressurized. However, if the instrument is to be shut down for more than 7 days, perform the following procedure to ensure the system remains in optimal operating condition.

1. The instrument must be in Sleeping state. To transition the instrument, go to the **Utilities/Manual Operations** screen and touch **General**, then **Sleep**.
2. Touch **Log Out** in the banner.
3. Touch **Shut Down** in the dialog box.

   NOTE: *If the Stat rack is not loaded in the Stat Area, a pop-up message asks if you are sure you want to exit. It is recommended that you touch **No** and load the Stat rack to ensure it is not removed while the instrument is shut down.*
4. Remove all bottles from the reagent compartment.
5. Remove the analytical cartridge and prefilter from their holders. Cap the ends of the analytical cartridge and prefilter and store at 2–8 °C for reuse.
6. Install the gray utility cartridge and utility prefilter.
7. After the touchscreen has turned off, turn off the power switch on the rear of the instrument.

Restarting the System after a Long-Term Shutdown

1. Turn on the D-100 power switch (on the rear of the instrument).
2. Press the soft power button on the front of the instrument to activate the instrument from standby power mode to full operating power. See Figure 2-1, No. 3.
3. The Bio-Rad software startup screen will display while the software is loading.
4. After the software is loaded, install the reagent bottles.

5. Remove the gray utility cartridge and utility prefilter from their holders.
6. Install the analytical cartridge and prefilter.
7. Touch **Run** in the banner to initiate the system warm-up. The instrument will transition from Sleeping to Warming Up state.
8. After the warm-up is complete, the instrument transitions to Standby state.
9. It is recommended that the system be recalibrated by running the Calibrator Pack and QC samples.

1 Results and Data Management

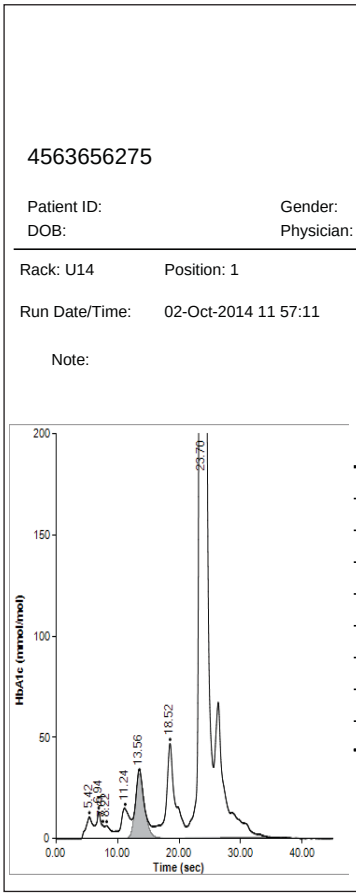
6.1 Reviewing Results

Results are reviewed in the **Results** screen. See Section 4.3 for information.

NOTE: *Bio-Rad recommends that all sample chromatograms be reviewed before releasing results from the D-100.*

Figure 6-1 indicates the items that should be checked on each sample. All items are automatically checked by the D-100 Advisor rules. See Appendix C for rule details.

Figure 6-1: *D-100 HbA1c Example Result Report*



	Item
1	Total Area
2	HbA1c peak
3	A0 peak
4	F peak area
5	P3 peak area
6	Area of Unknown peak
7	HbA1c result

See the assay Instructions For Use and Quick Guide for specific criteria used to interpret the result report.

6.1.1 Viewing Flagged Results

1. In the **Home** screen, touch Flagged to go to the **Results** screen, where results have been filtered to display only flagged samples (i.e., results that meet the **Flagged** filter criteria).
2. In the results table, see the reason why the sample was flagged in the Note/Comment column.
3. To view the sample result in detail, touch the result row.

6.2 Releasing or Rejecting Results

Results can be released or rejected in the results table or in the Result Details screen.

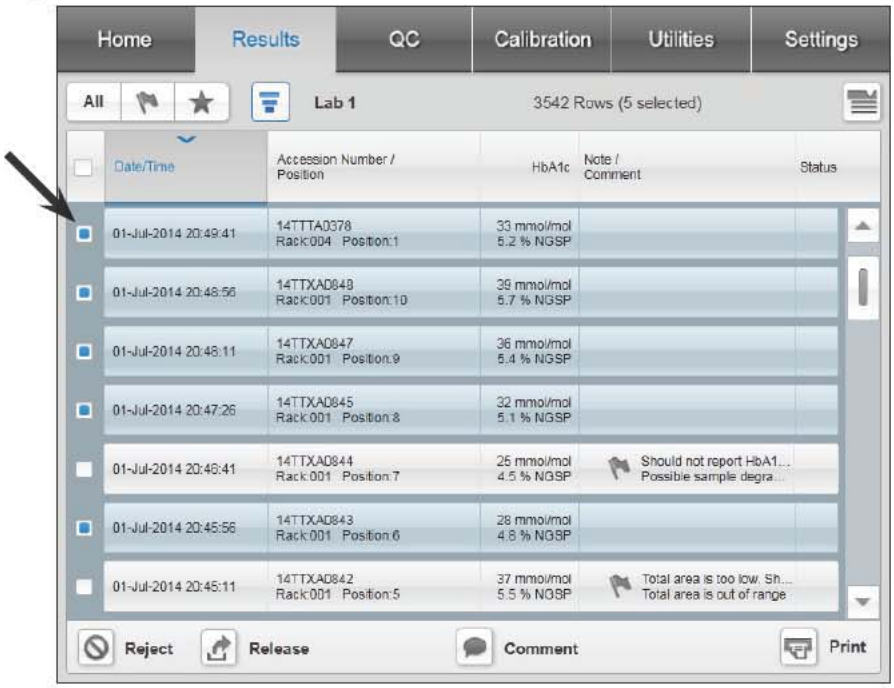
NOTE:

- *If the LIS Connection is **On** (see Section 4.7.5), releasing a result sends it to the LIS. If a questionable result is accidentally released, the result must be manually rejected at the LIS.*
- *Rejected results are not sent to the LIS. If a good result is accidentally rejected, the action can be undone by releasing the result.*

6.2.1 Releasing or Rejecting Results in the Results Table

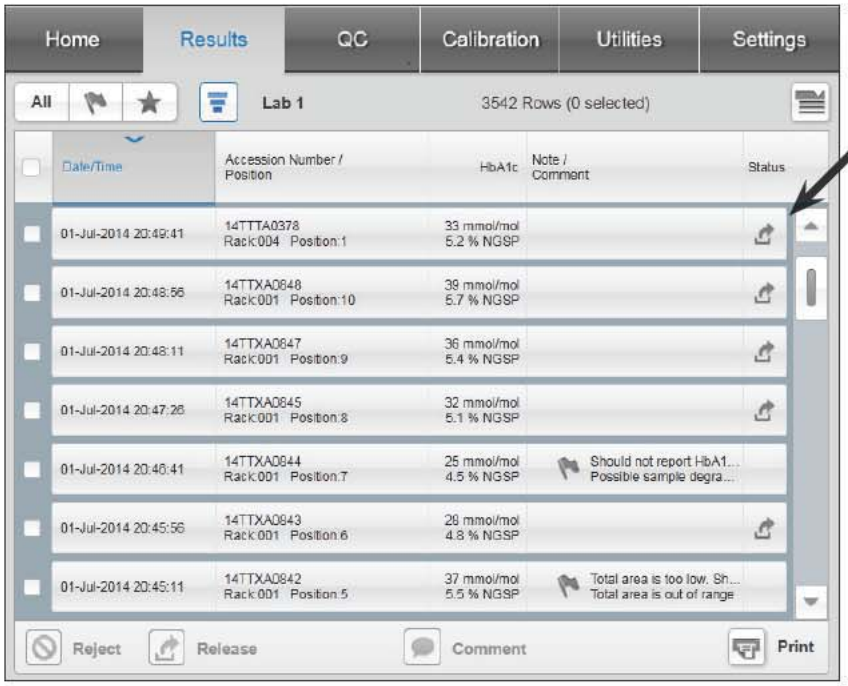
1. In the results table, select the corresponding checkbox for each sample result you want to release.

***Figure 6-2:** Results Table, 5 Results Selected to be Released*



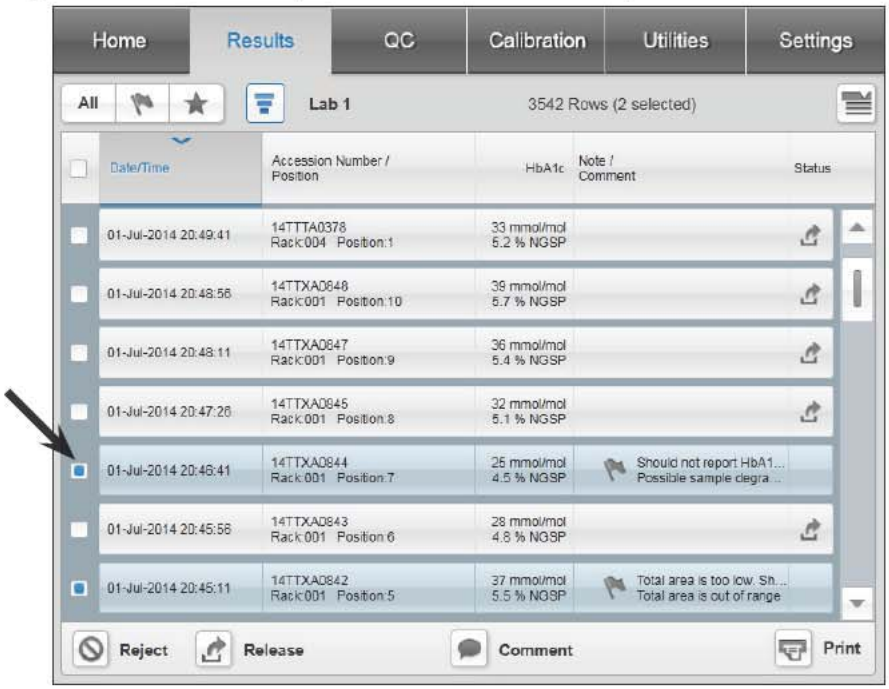
2. Touch **Release** at the bottom of the screen. The result(s) status now indicates .

Figure 6-3: *Results Table, 5 Results Released*



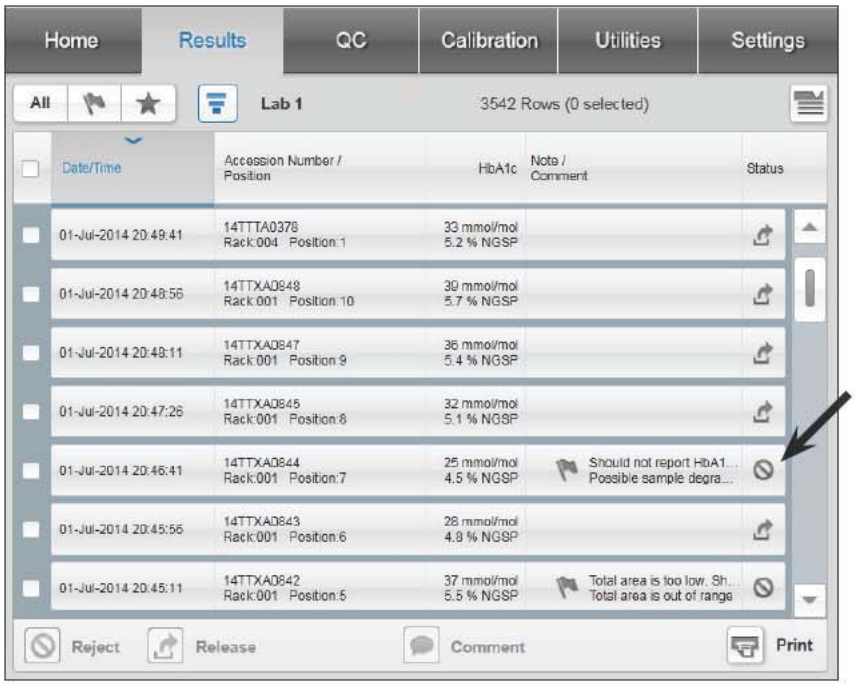
3. Select the corresponding checkbox for each sample result you want to reject.

Figure 6-4: *Results Table, 2 Results Selected to be Rejected*



4. Touch **Reject** at the bottom of the screen. The result(s) status now indicates .

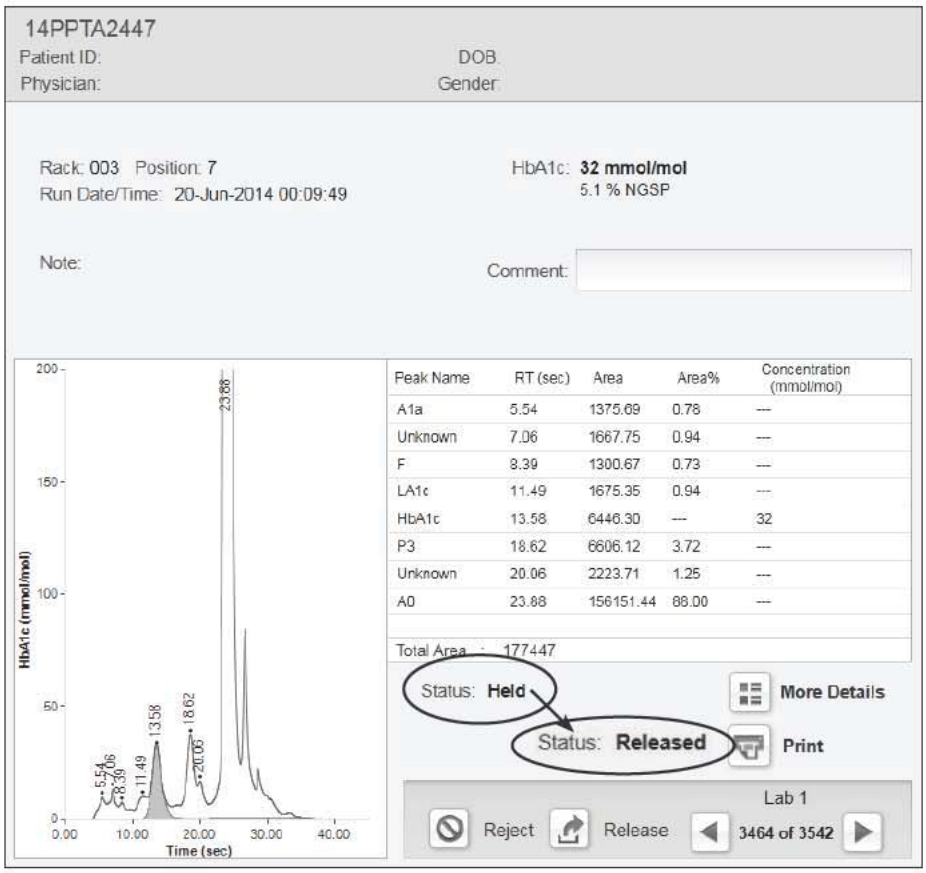
Figure 6-5: *Results Table, 2 Results Rejected*



6.2.2 Releasing or Rejecting Results in the Result Details Screen

1. In the results table, touch the sample result row to open the Result Details screen. The Status indicates "Held".

Figure 6-6: *Result Details Screen, Status*



2. After viewing the details, touch **Release** to release the result or touch **Reject** to reject the result.
3. The system updates the result status ("Released" or "Rejected") accordingly and displays the next sample result.

6.3 Transmitting Results to the LIS

The D-100 can receive orders and send results to a Laboratory Information System (LIS).

When LIS is enabled (i.e., the LIS Connection setting is **On**), results are sent to the LIS according to the settings defined in the **Settings/LIS** screen. See Section 4.7.5 for information.

- Results can be sent to the LIS manually by Releasing the results in the **Results** screen (see Section 6.2).
- Results can be sent to the LIS automatically by selecting the **Automatic Release to LIS** checkbox in the **Settings/LIS** screen.

6.4 Exporting Results

Results can be exported from the **Results** screen. See Section 4.3.3.1 for information.

6.5 Quality Control (QC)

QC results are presented in the **QC** tab. See Section 4.4 for information.

QC results can be exported to the Bio-Rad Unity Real Time® program according to the settings defined in the **QC/Unity** screen. See Section 4.4.5.

- QC results can be sent to Unity manually by selecting **Export Unsent QC Samples** in the **QC/Unity** screen.
- QC results can also be sent to Unity manually by selecting **Export to Unity** from the Results Menu in the **Results** screen. See Section 4.3.3.2.
- QC results can be sent to Unity automatically by selecting the **Export automatically for each sample** checkbox in the **QC/Unity** screen.

6.6 Backing Up Data

To back up the D-100 database, go to the **Utilities/Data** screen. See Section 4.6.3.1 for information.

6.7 Viewing Archived Data

To view or print previous data from a backed-up database, select **View Archive** from the Results Menu in the **Results** screen. See Section 4.3.3.4 for information.

6.8 Restoring Data

To restore a backed-up database for use on a different D-100 instrument or for troubleshooting purposes, go to the **Utilities/Data** screen. See Section 4.6.3.2 for information.

6.9 Managing Files

To copy, move, delete, or rename files, go to the **Utilities/Data** screen. See Section 4.6.3.3 for information.

7 Maintenance

Routine user maintenance for the D-100 is performed at the following intervals:

As Needed:

- Emptying the Waste Bottles (Section 7.1)
- Cleaning Up Spills and Decontaminating Surface Area (Section 7.2)
- Cleaning the Stat Rack and Sysmex Racks (Section 7.3)
- Cleaning the Touchscreen (Section 7.4)
- Calibrating the Touchscreen (Section 7.5)
- Priming/Flushing (Section 7.6)
- Manually Priming the Pumps (Section 7.7)

Every 3 cartridges or 30,000 tests:

- Cleaning the System (Section 7.8)

Maintenance is necessary to maintain optimum system performance.

WARNING: *All maintenance procedures described in this manual can be safely performed by qualified personnel. Maintenance not covered in this manual should be performed only by a Bio-Rad representative.*

BIOHAZARD: *Performing maintenance procedures may expose you to biohazardous conditions. Wear appropriate personal protective equipment.*

NOTE: *The user has responsibility for appropriate decontamination in case of spillage of hazardous material on or inside the equipment.*

Before the use of any decontamination or cleaning methods other than those recommended, users should check with Bio-Rad that the proposed method will not damage the equipment.

7.1 Emptying the Waste Bottles

The Waste indicator becomes red in the consumables panel when a waste bottle is full. A red warning message appears in the message panel when both waste bottles are full.

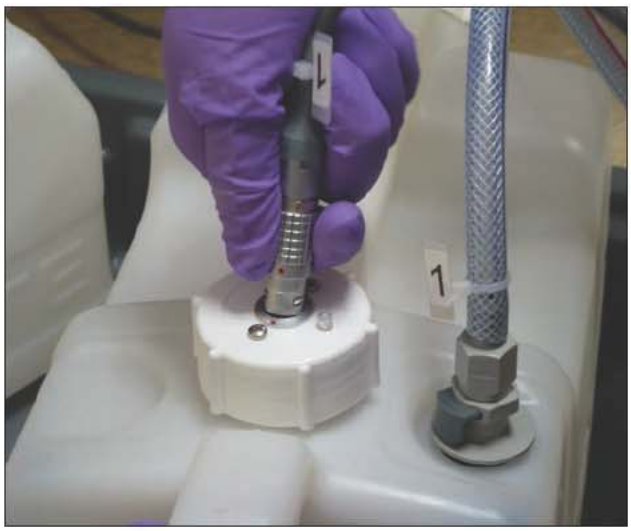
A waste bottle can be emptied at any time, even when the instrument is in Running state, as long as a second waste bottle is installed and not full. If the bottle being disconnected is active, the D-100 automatically switches to the second bottle.

NOTE: *The waste tubings and level sensor cables are labeled as 1 or 2 to correspond with the Waste indicators in the consumables panel.*

WARNING: *Some reagents used with the D-100 contain sodium azide as a preservative (see labels). Azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of reagents containing sodium azide, always flush with large volumes of water to prevent metal azide buildup. For further information, consult the manual Safety Management, No. CDC-22, "Decontamination of Laboratory Sink Drains to Remove Azide Salts" (Centers for Disease Control and Prevention, Atlanta, GA, April 30, 1976).*

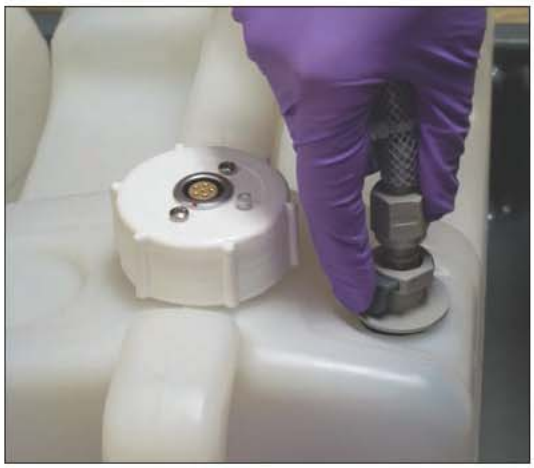
1. Disconnect the gray electrical cable from the level sensor cap by pulling the connector.

Figure 7-1: *Disconnecting Level Sensor Cable from Waste Bottle*



2. Disconnect the braided waste tubing from the bottle by pressing the thumb latch on the quick-disconnect valve. Place the tubing on an absorbent towel.

Figure 7-2: *Disconnecting Waste Tubing from Waste Bottle*



3. Transfer the waste bottle to the appropriate disposal area.

4. Unscrew the waste cap on the rear of the bottle. Place the cap on an absorbent towel.

   Figure 7-3: *Unscrewing Waste Cap*

5. Dispose of the waste properly, as directed by laboratory safety procedures.

   NOTE: *Liquid waste should be decontaminated on site if possible. It is recommended to mix liquid waste with household bleach (5% sodium hypochlorite) at a ratio of 1 part bleach to 10 parts waste. For example, add 500 mL of household bleach to a full 5 L waste bottle. Let the mixture stand for at least 30 minutes before emptying.*

6. Reinstall and secure the waste cap.
7. Reconnect the braided waste tubing to the valve. Ensure that the waste tubing is not looped or crimped and is sloped downward.
8. Reconnect the gray electrical cable to the level sensor cap. The red dots on the level sensor cap and the connector must be aligned to reconnect (see Figure 7-1).

7.2 Cleaning Up Spills and Decontaminating Surface Area

Clean up any spills when they occur. Sample spills are potentially biohazardous; treat appropriately. If any sample spills occur in the rack handler area, decontaminate the area using 70% isopropyl alcohol.

1. Ensure the instrument is not in Running state.
2. Remove all racks from the rack handler.
3. Prepare a 70% isopropyl alcohol solution. Do not use corrosive liquids (e.g., bleach).
4. Dampen a disposable towel with the decontamination solution.
5. Wipe the rack handler area and conveyor belts with the damp towel.
6. After decontamination, let the belts air-dry before use.

7.3 Cleaning the Stat Rack and Sysmex Racks

Clean the racks as needed.

1. Remove all samples from the racks.
2. Remove the Stat rack from the instrument: press and hold the button on the front of the rack while lifting up the rack.

Figure 7-4: *Removing Stat Rack*

3. Clean the Stat rack and Sysmex racks using 70% isopropyl alcohol and a disposable towel.

   If a rack is contaminated with dried blood, first wet the area with a detergent disinfectant. Carefully remove with a disposable towel to prevent scattering potentially infectious material. After removal, disinfect the cleaned surface with 70% isopropyl alcohol.
4. Inspect the sample racks to ensure they are in good working condition.
5. Reinstall the Stat rack: while pressing and holding the button on the front of the rack, set the rack in its loading position on the instrument. Release the button to secure the rack.

   NOTE: *The 3 slots beneath the rack align with the 3 posts on the instrument to ensure proper installation.*

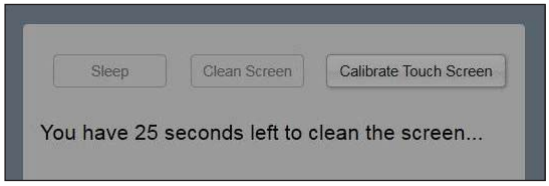
7.4 Cleaning the Touchscreen

Clean the touchscreen as needed.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Clean Screen**.

Figure 7-5: *Utilities/Manual Operations Screen, Clean Screen Button*

4. The touchscreen is disabled for a 30-second period to allow cleaning. The countdown is displayed on the screen.
   During this period, clean the touchscreen using 70% isopropyl alcohol and a soft towel.



7.5 Calibrating the Touchscreen

If the touchscreen is not responding accurately (i.e., the touch targets are misaligned), it may require recalibration. Calibrate the touchscreen as needed.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Calibrate Touch Screen**.

Figure 7-6: *Utilities/Manual Operations Screen, Calibrate Touch Screen Button*

4. A series of 4 red calibration points are displayed one at a time on each corner of the screen. Touch each calibration point within the 15-second countdown period indicated, until the point turns blue and the countdown timer changes to “OK!”.
5. After acknowledging the 4th calibration point, a pop-up message indicates the parameters are being read. The touchscreen calibration is now completed.

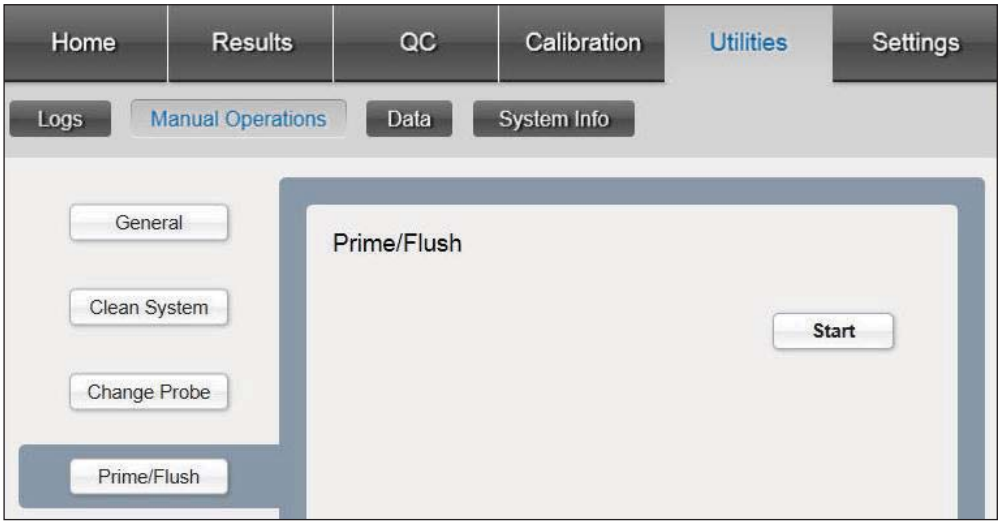
7.6 Priming/Flushing

The **Prime/Flush** function flushes the fluidics with buffer.

Pressure variations >±5% may indicate the presence of air in the buffer lines. Perform the **Prime/Flush** to remove air bubbles.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Prime/Flush**.

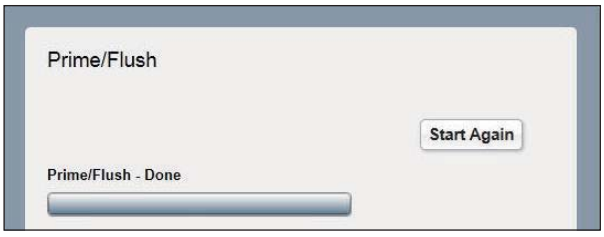
Figure 7-7: *Utilities/Manual Operations Screen, Prime/Flush Button*



4. Touch **Start**. The screen indicates that the Prime/Flush is in progress and when it is complete. The process takes approximately 8 minutes.

5. To repeat the Prime/Flush, touch **Start Again**.

NOTE: *If the pressure variations continue, perform the manual pump prime procedure. See Section 7.7.*



7.7 Manually Priming the Pumps

This manual pump prime procedure can be performed as a supplement or alternative to the automated **Prime/Flush** (Section 7.6). Perform this procedure as needed to remove air bubbles from the buffer lines.

1. The instrument must be in Sleeping state with the reagents pressurized.
2. Open the cartridge/prefilter compartment door to access the high-pressure pumps.
3. Insert a 10–20 mL syringe into the pump A purge valve to collect the leaking buffer. Open the pump A purge valve approximately ½-turn counterclockwise or until buffer begins flowing.

Figure 7-8: *Syringe in Pump B Purge Valve*

4. Leave the valve open until there is a uniform buffer flow without any bubbles, then close the valve by turning it clockwise until secured.
5. Remove the syringe and dispose of the liquid. Use a disposable towel to wipe any buffer from the outside of the purge valve.
6. Repeat steps 3–5 with the pump B purge valve.
7. Close the cartridge/prefilter compartment door.

7.8 Cleaning the System

Clean the system every 3 cartridges or 30,000 tests. Depending on usage conditions, a message may appear prompting you to clean the system sooner. If that occurs, clean the system as soon as possible in addition to the typical cleaning frequency.

NOTE: *Failure to clean the system every 30,000 tests may result in carryover.*

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Clean System**. The software provides step-by-step instructions for cleaning the system.

Figure 7-9: *Utilities/Manual Operations Screen, Clean System Button*

4. Touch **Open** to retrieve the Stat rack from the Stat Area.

5. Insert the Cleaning Tube in a microvial adapter in the Stat rack. Touch **Clean Now**.

6. The screen indicates that the cleaning is in progress and when it is complete. The process takes approximately 2 minutes.

7. The Stat Area opens automatically when the cleaning is complete. Remove the Cleaning Tube from the Stat rack and discard. Touch **Load**.

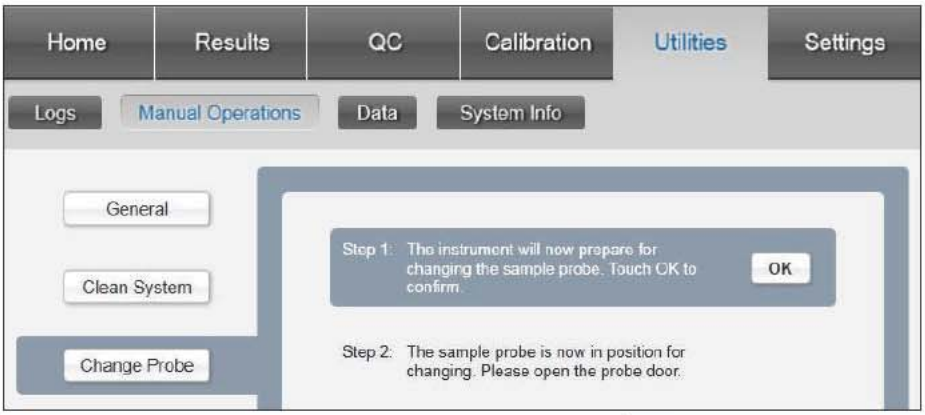
7.9 Replacing the Sample Probe

WARNING: *The sample probe is very sharp. Use caution when handling to avoid injury. Do not attempt to replace a broken probe that has detached from its base. Contact your local Bio-Rad office for technical assistance.*

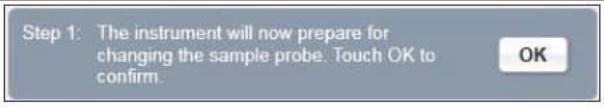
The sample probe will be replaced by Bio-Rad Service during scheduled maintenance; however, you may need to replace the probe if instructed by Technical Service. As an extra biohazard precaution, Bio-Rad recommends cleaning the system (see Section 7.8) before replacing the sample probe. If the probe is damaged, and it is not possible to perform that procedure, carefully wipe down the probe using a towel moistened with 5% sodium hypochlorite solution prior to removing.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Change Probe**. The software provides step-by-step instructions for changing the sample probe.

Figure 7-10: *Utilities/Manual Operations Screen, Change Probe Button*



4. Touch **OK** to move the sample probe to the change position.



5. Open the probe door.

   NOTE:

   - *The probe door is interlocked. When open, the interlock switch shuts off power to the probe assembly.*
   - *The Change Probe screen indicates when the probe door is open.*

6. Unscrew the tube fitting from the top of the sample probe. See Figure 7-11.
7. Grasp the probe and slide it up and out through the side of the probe carrier. See Figure 7-12.

Figure 7-11: *Unscrewing the Tube Fitting from the Sample Probe**

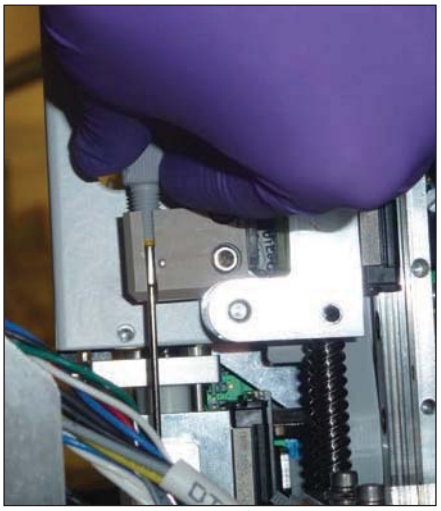
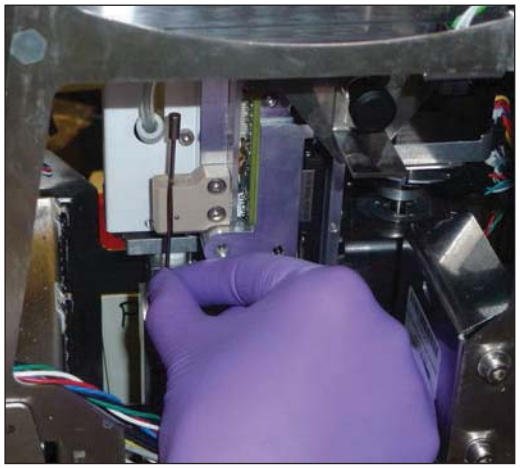


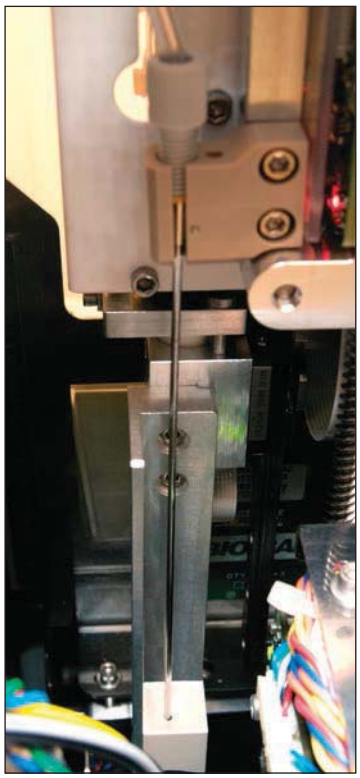
Figure 7-12: *Removing the Sample Probe from the Probe Carrier**



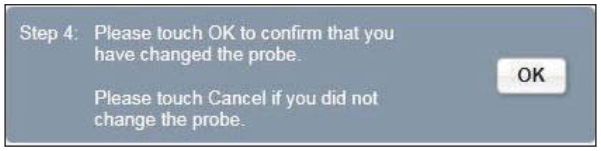
8. Discard the old probe according to your laboratory's standard operating procedures for sharps.
9. Remove a new sample probe from its shipping tube; remove the tip cover.
10. Insert the new probe into the probe carrier, being careful to lower the probe tip into the center of the tube holder, which has a conical base (see Figure 7-13). There is a flat surface on the probe base to ensure a proper fit; rotate the probe until it drops into place.
11. Reconnect the tube fitting.
12. Close the probe door.

** The instrument cover was removed for visibility in these photos.*

Figure 7-13: *Probe Tip in Center of Tube Holder Base**



13. Touch **OK** to confirm that you changed the sample probe. (If you did not change the probe, touch **Cancel**.)



8 Troubleshooting

8.1 Important Troubleshooting Information

Troubleshooting advice for problems you may encounter while using the D-100 is organized as follows:

- General Error Messages (Section 8.2)
- Hardware Error Messages (Section 8.3)
- Chromatography Problems (Section 8.4)
- Other Problems (Section 8.5)

The recommended solutions include abbreviated procedures; see appropriate sections for detailed procedures.

If the problem persists after completing the recommended solution(s), or if the problem is not addressed in this operation manual, contact Bio-Rad Technical Service.

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

Outside the USA: Contact your regional Bio-Rad office. Go to www.bio-rad.com for contact information.

NOTE: *When performing the recommended solution to a problem requires turning the main power switch off, always attempt to shut down the instrument first by touching* ***Log Out*** *in the banner and touching* ***Shut Down*** *in the dialog box. Wait for the touchscreen to turn off, then turn off the power switch on the rear of the instrument.*

After the troubleshooting is completed, turn the power switch back on. Press the soft power button on the front of the instrument to activate the power-on sequence.

8.2 General Error Messages

The following error messages appear in the message panel when the errors occur. Check the **Utilities/Logs** screen for the description and event code.

Error Message	Probable Cause	Event Code	Recommended Solution
X exceeded recommended test limit	The stated cartridge or prefilter is now past the recommended number of tests.	113033, 113042	Replace the cartridge or prefilter at your earliest convenience.
X is empty	Both bottles of the stated reagent are empty.	113006, 113009, 113012	You must replace the reagent before you can start a run.
X level is low	The stated reagent level is low.	113005, 113008, 113011, 113017	Replace the reagent at your earliest convenience to prevent the run from stopping.

Error Message	Probable Cause	Event Code	Recommended Solution
X lot expired	The stated consumable has reached its expiration date.	113031, 113034, 113036, 113038, 113040, 113043, 113078, 113081, 113084	Replace the consumable at your earliest convenience.
X onboard stability expired	The open/installed stability of the stated consumable has expired.	113032, 113035, 113037, 113039, 113041, 113044, 113079, 113082, 113085	Replace the consumable at your earliest convenience.
X unavailable	There are no bottles of the stated reagent installed.	113062, 113064, 113066, 113072	Install reagent.
Calibration failed for X	Calibrator failed for the stated assay.	123003, 123005	See Section 8.4 for “Calibrator Pack failure” solutions.
Calibration required – new cartridge installed	A new cartridge was installed on the instrument.	113057	Perform calibration.
Calibrator skipped; please load in Stat rack	Calibrator pack inserted in Sysmex rack	123009	Remove calibrator pack from Sysmex rack and insert in Stat rack.
Cannot print PDF file	Selected folder for PDF reports is full.	NA	Move or delete files from the applicable drive to provide space (Section 4.6.3.3).
Cartridge reloaded – calibration recommended	A previously installed cartridge was reinstalled on the instrument.	113058	Recalibration is recommended, but not required.
Cartridge reloaded – calibration required	There is no passed calibration for the cartridge.	113013	Perform calibration.
Cartridge unavailable	No cartridge installed	113060, 113070	Install cartridge.
Cartridge unusable – Data tag error	RFID tag missing	100040, 100044	Discard and replace the cartridge. Contact Bio-Rad Technical Service for a replacement.
	RFID tag failed		1. Remove and reinstall the cartridge. 2. If problem persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
	Electromagnetic interference		Identify and remove the interfering device.
	Failed RFID reader		Contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
Could not close stat area; reopening	Obstruction to movement of Stat Area door	101009	Check for and remove any obstacles from the Stat Area.
Error (see activity log)	Varies	100006, 100014, 100015, 100016, 100017, 100018	Go to the **Utilities/Logs** screen for a description of the error. Contact Bio-Rad Technical Service.
Error in Stat Area	Obstruction to movement of Stat Area door	101013, 101014	Check for and remove any obstacles from the Stat Area.
Export failed	Connectivity failure	100026	• Verify the "Save to" location is correct. • If the "Save to" location is an "External" USB drive, ensure that it is properly connected to a USB port on the D-100. • If the "Save to" location is on the "Network": 1. Ensure the D-100 is connected to the network (i.e., the cable is connected to the correct network port and the cable is connected to the LAN port on the rear of the D-100). 2. Verify the network location is active (i.e., navigate to that folder from another connected instrument to confirm it is online and available). 3. Verify the network location is not full.
Fluidics error; Clean system or contact service	System cleaning required	101051	You must clean the system (Section 7.8) before you can start a run.
Fluidics warning; please clean system	System cleaning recommended	101050	Warning will be cleared after cleaning the system (Section 7.8) or if the subsequent system check passes.
Incompatible bottle in X position	The batch number of reagent installed in the stated position is not compatible.	113022, 113024, 113026, 113028, 113030	Replace the reagent with one that has a compatible batch number.
Invalid calibrator pack loaded	Incompatible lot of calibrator pack installed	123010	Remove and reinstall the calibrator pack.

Error Message	Probable Cause	Event Code	Recommended Solution
LIS is not responding	Incorrect LIS settings	107001	Check LIS settings (Section 4.7.5) and correct as needed.
	LIS is not connected to D-100		1. Ensure LIS is connected to the D-100 serial port via RS-232 cable. 2. Touch the **Test** button in the LIS Advanced Settings/**Test LIS** tab to verify the connection.
One or more active control lots has expired	The QC currently in use has expired.	112001	Use a new lot of QC.
Paused – Insufficient level resource	The system has transitioned from Running to Paused state because it is out of reagents or waste space.	101045	1. Replenish the resource. 2. Touch **Resume** to continue the run.
Prefilter unavailable	No prefilter installed	113068	Install prefilter.
Printer error	External printer is not available	100024	Verify the external printer is connected, powered on, and has no errors indicated.
	Check the internal printer error LED. A red light indicates a problem: 1 flash = Printer memory is full and cannot receive additional data		Turn the D-100 main power switch off and then on again as instructed in the NOTE in Section 8.1. If error persists, contact Bio-Rad Technical Service.
	2 flashes = Printer head temperature too high		1. Remove the thermal paper from the printer and leave the paper door open until the printer head has cooled down. 2. Reinstall the paper (Section 3.11).
	Permanent blink = No paper or paper door is open		• If needed, install new roll of paper (Section 3.11). • Close the printer paper door.
	1 long flash and 1 short flash = Printer head failure		Contact Bio-Rad Technical Service.
Printer error: internal sw error	NA	NA	Shut down and restart the D-100 (Section 5.11).
Printer: PDF Error: folder is not available	Selected folder for PDF reports is not available	100057	Verify the selected path for PDF reports (Section 4.7.2) in the **Settings/Reports** screen.

Error Message	Probable Cause	Event Code	Recommended Solution
Probe door is not locked	Probe compartment door is open	101020	Close the door.
QC Failed	QC result is outside of the fixed control range or failed a QC rule	100030, 100031	Rerun QC.
	The QC values entered do not correspond to the primary reporting unit (i.e., % NGSP or mmol/mol IFCC).		Enter the correct values for the primary reporting unit in the Edit Control Lot dialog box (Section 4.4.4.2) in the **QC/QC Lots** screen.
QC interval exceeded; please run QC	QC is now due to be run	112009	Run QC.
QC Warning	QC has violated the 1-2s or 1-3s QC rule	100032	Rerun QC.
Rack handler error	Obstruction to movement of rack handler	101015, 101016	Check for and remove any obstacles from the rack input, output, and sampling areas.
Rack loaded backwards; please reload rack	A Sysmex rack was positioned incorrectly in the rack input area.	101006	To prevent the run from stopping, immediately remove and reposition the rack so that the rack barcode faces the instrument and the Sysmex logo faces you.
Remove racks: Output full.	The rack output area contains the maximum number of racks.	101047	Remove the completed Sysmex racks from the rack output area.
Run stopped due to full disk	D drive is full	110001	Move or delete files from the D drive to provide space (Section 4.6.3.3).
Sample path maintenance due; contact service	Low-pressure filter is approaching its test limit	114001	Contact Bio-Rad Technical Service to replace the low-pressure filter at your earliest convenience. **NOTE:** *You may continue to run the system until Service arrives.*
Sample path maintenance warning; contact service	Low-pressure filter is now past its test limit	113045	Contact Bio-Rad Technical Service to replace the low-pressure filter as soon as possible.

Error Message	Probable Cause	Event Code	Recommended Solution
Stat area is jammed; please clear	Stat rack is incorrectly installed	101004	Remove and reinstall the Stat rack (Section 7.3).
	Sample or Calibrator Pack is incorrectly inserted in the Stat rack		Remove and reinsert the sample or Calibrator Pack (Section 3.6).
	There is an obstacle inside the Stat Area.		Contact Bio-Rad Technical Service.
Stopped due to backwards rack	The run has stopped and the system has entered Standby state because the next Sysmex rack to be processed is positioned incorrectly in the rack input area.	101007	1. Remove and reposition the rack so that the rack barcode faces the instrument and the Sysmex logo faces you. 2. Restart the run.
Stopped due to full output area	The Sysmex rack just finished being processed cannot be ejected from the shuttle because the rack output area contains the maximum number of racks. The system has transitioned from Running to Paused state.	101001	1. Remove the completed Sysmex racks from the rack output area. 2. Touch **Resume** to continue the run.
Units changed – Calibration required.	The primary reporting unit (i.e., NGSP or IFCC) has been changed.	104003	Perform calibration.
Unity folder error (see activity log)	Network folder is unavailable	100037, 100055	• Ensure the D-100 is connected to the network. • Ensure the computer that hosts the network folder is connected and running.
Waste level is almost full	The external waste level is almost full.	113049	Empty the waste bottles at your earliest convenience to prevent the run from stopping.
Waste level is full	Both external waste bottles are full.	113050	You must empty the waste bottles before you can start a run.

8.3 Hardware Error Messages

The following hardware error messages appear in the message panel when the errors occur. Check the **Utilities/Logs** screen for the description and event code.

Error Message	Probable Cause	Event Code	Recommended Solution
Buffer error; check bottles, touch Run or Reset	A reagent bottle is not correctly installed.	131206	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Touch **Run** or **Reset**.
	A faulty reagent bottle is leaking air.	133209	1. Replace the applicable buffer bottle. 2. Touch **Run** or **Reset**.
Cannot read tag; please replace consumable	Checksum verification failed.	130504	1. Remove and reinstall the consumable. 2. If error persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
Cartridge error; check cartridge, touch Reset/ Run	The Cartridge holder was opened while in use.	131704	1. Ensure the cartridge is still installed. 2. Close the cartridge holder door and the cartridge/prefilter compartment door. 3. Touch **Reset** or **Run**.
	The pumps were running with no cartridge installed.	133074	1. Wipe up any leaks. Leaks from the cartridge holder are potentially biohazardous; handle and treat appropriately. 2. Install a cartridge. 3. Close the cartridge holder door and the cartridge/prefilter compartment door. 4. Touch **Reset** or **Run**.
Close all doors to continue	Failed to lock a door because it is not closed.	132203	Close the open instrument door(s).
Data tag error; replace bottle A/B/Wash position 1/2	Failed to write data to the RFID tag for the stated reagent bottle.	132766-132771	• Replace the stated reagent. • If error persists, contact Bio-Rad Technical Service.
Data tag error; replace Cartridge	Writing on the RFID tag of the cartridge failed.	131228	• Replace the cartridge. • If error persists, contact Bio-Rad Technical Service.
	Failed to write data to the RFID tag for the Cartridge.	132772	

Error Message	Probable Cause	Event Code	Recommended Solution
Data tag error; replace consumable bottle	Writing on the RFID tag of a consumable bottle failed.	131230	• Replace the applicable bottle. • If error persists, contact Bio-Rad Technical Service.
Data tag error; replace Low Pressure Filter	Failed to write data to the RFID tag for the Low Pressure Filter.	132773	Contact Bio-Rad Technical Service.
Data tag error; replace Prefilter	Writing on the RFID tag of the Prefilter failed.	131229	• Replace the prefilter. • If error persists, contact Bio-Rad Technical Service.
	Failed to write data to the RFID tag for the Prefilter.	132774	
Detector warning; contact Technical Service	LED could be at the end of its life.	132507, 132513, 132514	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Door error; check doors and touch Reset or Run	Door open (and not allowed) or interlock error.	133012	1. Close the open instrument door(s). 2. Touch **Reset** or **Run**.
Door open; close doors and touch Reset or Run	The operation failed because a door is open.	131203	1. Close the open instrument door(s). 2. Touch **Reset** or **Run**.
Error; check output area and touch Reset or Run	Moving the rack failed (Pushers motors error).	133058	1. Check for and remove any obstacles from the rack output area. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Eject sequence failed (Output motor error).	133060	
In-use waste bottle removed; check for spills	A waste bottle was removed while being filled.	132699	1. Wipe up any spills. Waste spills are biohazardous; handle and treat appropriately. 2. Ensure at least one empty waste bottle is available and correctly installed (Section 7.1).
Low Buffer A/B; replace bottle, touch Run or Reset	The process failed because there is not enough of the stated buffer to complete.	131208, 131209	1. Replace the applicable buffer bottle(s). 2. Touch **Run** or **Reset**.
Low Pressure filter error; please check	The Low Pressure Filter holder was opened while in use.	131705	Contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
No cap; check tube or contact Technical Service	No cap was detected while moving into the sample tube.	131231	1. Locate and correct sample tube with no cap. 2. Touch **Run** or **Reset**. 3. If error persists, contact Bio-Rad Technical Service.
Please replace cartridge; cannot read tag	The calculated checksum of the Cartridge extended data does not match the stored value.	131406	1. Remove and reinstall the cartridge. 2. If error persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
	The Cartridge extended data could not be read.	131407	
Please replace consumable; cannot read tag	Decoding of the Data Tag failed (i.e., the RFID tag could not be read).	130500	1. Remove and reinstall the consumable. 2. If error persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
	Reading of a data block failed.	130501	
	Reading of a (signed) value block failed.	130502	
	Reading of a (unsigned) value block failed.	130503	
Power supply warning; contact Technical Service	The power supply is beginning to fail.	132698	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Prefilter error; check prefilter, touch Reset/ Run	The Prefilter holder was opened while in use.	131706	1. Ensure the prefilter is still installed. 2. Close the prefilter holder door and the cartridge/prefilter compartment door. 3. Touch **Reset** or **Run**.
	The pumps were running with no prefilter installed.	133076	1. Wipe up any leaks. Leaks from the prefilter holder are potentially biohazardous; handle and treat appropriately. 2. Install a prefilter. 3. Close the prefilter holder door and the cartridge/prefilter compartment door. 4. Touch **Reset** or **Run**.

Error Message	Probable Cause	Event Code	Recommended Solution
Pressure fault; Replace Prefilter	Pressure higher than upper limit.	133068, 133208	1. Replace prefilter. 2. If error persists, replace cartridge. Contact Bio Rad Technical Service for a replacement.
Pressure warning: Replace Prefilter	Pressure higher than warning limit.	132546	1. Replace prefilter. 2. If error persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
Rack jam; clear shuttle and touch Reset or Run	Unexpected motors error while testing the shuttle.	133061	1. Check for and remove any obstacles from the shuttle. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Unexpected motors error while clearing the shuttle.	133062	
Rack jam; clear shuttle to avoid stopping the run	A Sysmex rack is jammed in the shuttle.	132710	Remove Sysmex rack(s) from the shuttle and correctly position racks in the rack input area to prevent the run from stopping.

Error Message	Probable Cause	Event Code	Recommended Solution
System error; contact Technical Service	Dilution module pressure low. Possible clog in sample path.	133047	Contact Bio-Rad Technical Service.
	Dilution module pressure high. Possible clog in sample path.	133048	Contact Bio-Rad Technical Service.
	Leak has been detected. Leak in the reagent compartment.	133085	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Wipe up any reagent in the leak tray. 3. If error persists, contact Bio-Rad Technical Service.
	The pressure is too low when running gradients (Separation).	133207	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Prime/flush system (Section 7.6). 3. If error persists, contact Bio-Rad Technical Service.
	The pressure is too high when running gradients (Separation).	133208	1. Replace prefilter. 2. If error persists, replace cartridge. 3. If error persists, contact Bio-Rad Technical Service.
	Varies	Varies	Contact Bio-Rad Technical Service.
System error; touch Reset or Run and try again	A Sysmex rack in process was removed by the user before it was moved to the rack output area.	133057	1. Remove rack from the shuttle. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Transient sensor malfunction		
	Varies	Varies	1. Touch **Reset** or **Run**. 2. If error persists, contact Bio-Rad Technical Service.
Tube spin failure; contact Technical Service	Failed to spin a tube in the rack.	132781	Contact Bio-Rad Technical Service.
Warming up failed; please try again	Normal operating conditions were not achieved within the expected warm-up time.	131700, 131701, 131703	1. Touch **Reset** or **Run**. 2. If error persists, contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
Warning: a sample was skipped	Unable to access the sample tube. Either something prevents the robot module from moving or it needs to be recalibrated.	131222	1. Check Activity Log for sample location. 2. Rerun sample. 3. If error persists, contact Bio-Rad Technical Service.
	Bottom of sample tube was not found.	131404	
Warning: Pressure low; call Technical Service	Pressure lower than lower limit.	132547	Contact Bio-Rad Technical Service for guidance to check for leaks. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Warning: Rack handling fault; stat samples only	The Rack Handling driver failed to initialize.	132776	1. Contact Bio-Rad Technical Service. 2. You may continue to run samples in the Stat Area until Service resolves the rack handler fault.
Warning: Stat area fault; rack handling only	The Stat Area driver failed to initialize.	132777	1. Contact Bio-Rad Technical Service. 2. You may continue to run samples in the Sysmex racks until Service resolves the Stat Area fault.
Warning: Temperature high; call Technical Service	Cartridge temperature higher than upper limit.	132544	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Warning: Temperature low; call Technical Service	Temperature lower than lower limit.	132545	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Wash empty; replace bottle and touch Run or Reset	The process failed because there is not enough Diluent to complete.	131210	1. Replace the Wash Solution bottle(s). 2. Touch **Run** or **Reset**.

Error Message	Probable Cause	Event Code	Recommended Solution
Waste error; check waste and touch Run or Reset	The process failed because there is no waste bottle or lab drain ready to collect waste from the instrument.	131207	1. Ensure at least one empty waste bottle is available and correctly installed (Section 7.1). 2. Touch **Run** or **Reset**. 3. If error persists, contact Bio-Rad Technical Service.
	Failed to drain to waste port 1.	132778	
	Failed to drain to waste port 2.	132779	
	Failed to drain the waste combiner.	132780	
Waste error; contact Technical Service	The waste valve is stuck.	133083	Contact Bio-Rad Technical Service.
Waste full; empty bottle and touch Run or Reset	The process failed because there is not enough space in the waste bottles to collect waste from the instrument.	131211	1. Empty the waste bottle(s). 2. Touch **Run** or **Reset**.

8.4 Chromatography Problems

Problem	Probable Cause	Recommended Solution
Calibrator Pack failure	Barcode error	Verify assigned calibrator values entered correctly in Calibration dialog box (Section 4.5).
	Calibrator Pack placed in wrong position in Stat rack	Ensure Calibrator Pack is in dedicated position in Stat rack.
	Inadequate calibrator volume	Verify microvials contain sufficient volume of calibrator.
	Calibrator Pack reconstituted more than once	Use new Calibrator Pack.
	Air in detector or high-pressure pump(s)	Prime/flush system (Section 7.6).
	Dirty detector	Contact Bio-Rad Technical Service.
	Suspect reagent or cartridge	Replace suspect component.

Problem	Probable Cause	Recommended Solution
Carryover	Sample not capped	1. Rerun all samples with suspected carryover. 2. All primary sample tubes should be capped before loading them onto the system. 3. If cap is missing, predilute sample in microvial prior to analysis.
	System not cleaned as directed	Clean the system as directed in Section 7.8.
	Inadequate wash of sample probe or inadequate drain of dilution well	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
Early retention times	Elevated sample concentration; high total areas.	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
	Cartridge temperature too high	Contact Bio-Rad Technical Service.
	Expired or suspect buffer(s)	Replace buffer(s).
	Suspect cartridge	Replace cartridge.
High Total Area	High hematocrit sample	Manually dilute sample at higher dilution ratio (1:400) and rerun.
	Problem venting sample tubes	Manually dilute sample and rerun.
	Inadequate wash of sample probe or inadequate drain of dilution well	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
Late retention times	Low sample concentration; clotted sample.	Manually dilute sample and rerun.
	Low total areas; sample probe is bent or blocked.	Replace probe (Section 7.9).
	Air in high-pressure pump(s)	Prime/flush system (Section 7.6).
	Leak in flow path	Check for leaks. Contact Bio-Rad Technical Service.
	Cartridge temperature too low	Contact Bio-Rad Technical Service.
	Expired or suspect buffer(s)	Replace buffer(s).
	Suspect cartridge	Replace cartridge.

Problem	Probable Cause	Recommended Solution
Low Total Area	Short sample or clotted sample	Manually dilute sample and rerun.
	Unsupported tube type	
	Low hematocrit sample	Manually dilute sample at lower dilution ratio (1:100) and rerun.
	Prediluted sample not mixed	Remake prediluted sample, mix, and rerun.
	Prediluted sample treated as whole blood	1. Ensure the microvial adapter is properly seated in the rack. 2. If problem persists, discard and replace the adapter.
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
	Leak at dilution well	Contact Bio-Rad Technical Service.
	Sample tubing or probe clogged	Clean system (Section 7.8).
	Sample probe is bent or blocked	Replace probe (Section 7.9).
No HbA_{1c} result	Error occurred during result calculation (Note: Peak integration error)	Rerun sample.
	Very high total area carryover (Note: Automatic repeat - carryover risk)	
	Very high HbA_{1c} carryover (Note: Repeat sample - possible HbA1c carryover)	
Noise spikes appear on chromatogram/drifting baseline	Air in detector or high-pressure pump(s)	Prime/flush system (Section 7.6).
	Dirty detector	Contact Bio-Rad Technical Service.
	Detector board fault	Contact Bio-Rad Technical Service.
No peaks appear on the chromatogram; report shows no data.	Insufficient sample in the tube	Manually dilute sample and rerun.
	Clotted sample	
	Unsupported tube type	
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
	Damaged cartridge	Replace cartridge.
	Sample tubing or probe clogged	Clean system (Section 7.8).
	Sample probe is bent or blocked	Replace probe (Section 7.9).
Poor peak shape (broad peaks, poor EMG fit, tailing)	Expired or suspect prefilter	Replace prefilter.
	Expired or suspect cartridge	Replace cartridge.
	Expired or suspect buffer(s)	Replace buffer(s).
	Dirty detector	Contact Bio-Rad Technical Service.
	Coeluting variant peak	Confirm by alternative methods.

Problem	Probable Cause	Recommended Solution
QC out of range	Low and high QC switched or barcoded incorrectly	Verify correct barcode and position in rack.
	Improper reconstitution of QC	Reconstitute new vials of QC.
	Improper predilution of QC	Prepare new manual dilutions of QC.
Third-party calibrator failure	Data entry error	Verify assigned calibrator values entered correctly in Calibration dialog box (Section 4.5).
	Calibrators placed in wrong position in Stat rack	Ensure patient sample “conditioner”, calibrator level 1, and calibrator level 2 are in positions 1–3 respectively in Stat rack.
	Improper predilution of calibrators	Ensure third-party calibrators are manually diluted 1:300 with D-100 Sample Diluent before running (Section 5.9).

8.5 Other Problems

Problem	Probable Cause	Recommended Solution
Advisor flags applied incorrectly	Misconfigured/incorrectly edited rule(s)	Restore the default rules set (Section 4.7.4.2.3). Otherwise, contact Bio-Rad Technical Service for assistance.
Barcode: Sample barcode misread	Instrument damages barcode	Replace damaged barcode or enter barcode manually (Section 5.7.1). If problem persists, contact Bio-Rad Technical Service.
	Incompatible barcode symbology	See Section B.7 for supported barcode symbologies.
	Poor barcode quality	Replace barcode or enter barcode manually (Section 5.7.1).
	Multiple barcodes on tube	Remove barcodes and replace with single barcode or enter barcode manually (Section 5.7.1).
Barcode: Sample barcode not read	Incompatible barcode symbology	See Section B.7 for supported barcode symbologies.
	Incorrect placement of barcode on tube	Replace barcode (see Section B.7 for placement zone) or enter barcode manually (Section 5.7.1).
	Poor barcode quality	Replace barcode or enter barcode manually (Section 5.7.1).

Problem	Probable Cause	Recommended Solution
Calibrator Pack barcode information not read	Barcode label missing	Discard and replace the Calibrator Pack. Contact Bio-Rad Technical Service for a replacement.
	Barcode damaged	If the last barcode on the label is read and the system recognizes it as a Calibrator Pack, enter the human-readable lot number, expiration date, and assigned values manually in the Calibration dialog box (Section 4.5). Otherwise, discard and replace the Calibrator Pack. Contact Bio-Rad Technical Service for a replacement.
	Barcode reader fault	Contact Bio-Rad Technical Service.
Cartridge leaking	Installed incorrectly	Reinstall the cartridge correctly (Section 3.9).
Consumable installed on system is not detected	RFID tag missing	Discard and replace the consumable. Contact Bio-Rad Technical Service for a replacement.
	RFID tag failed	1. Remove and reinstall the consumable. 2. If problem persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
	Electromagnetic interference	Identify and remove the interfering device.
	Consumable incorrectly installed	Remove and reinstall the consumable. If problem persists, contact Bio-Rad Technical Service.

Problem	Probable Cause	Recommended Solution
Export: System fails to export results or information	Connectivity failure	• Verify the "Save to" location is correct. • If the "Save to" location is an "External" USB drive, ensure that it is properly connected to a USB port on the D-100. • If the "Save to" location is on the "Network": 1. Ensure the D-100 is connected to the network (i.e., the cable is connected to the correct network port and the cable is connected to the LAN port on the rear of the D-100). 2. Verify the network location is active (i.e., navigate to that folder from another connected instrument to confirm it is online and available). 3. Verify the network location is not full.
Internal printer paper jam	Paper installed incorrectly	Install the paper as instructed in Section 3.11.
	Incorrect paper used	Use only D-100 Thermal Printer Paper (REF 290-1013).
LIS: System not communicating with LIS	Incorrect LIS settings	Check LIS settings (Section 4.7.5) and correct as needed.
	LIS is not connected to D-100	Ensure LIS is connected to the D-100 serial port via RS-232 cable.
Power: Instrument does not power on when power switch is turned on, or loses power	Soft power button was not pressed	Press the soft power button on the front of the instrument to activate the power-on sequence.
	Power outage	Check main incoming circuit breaker.
	Fuse failure	Replace fuses (Section 8.6).
	Power switch failure	Contact Bio-Rad Technical Service.
Prefilter leaking	Installed incorrectly	Reinstall the prefilter correctly (Section 3.8).
Pressure variations	Air in high-pressure pump(s)	Prime/flush system (Section 7.6).

Problem	Probable Cause	Recommended Solution
Probe breaks	Unsupported tube type	1. Replace probe (Section 7.9). 2. Predilute sample in microvial prior to analysis.
	Probe is misaligned	Replace probe as instructed in Section 7.9, ensuring the probe is properly centered in the tube holder.
	Hardware failure	Contact Bio-Rad Technical Service.
Probe cannot pierce seal	Unsupported tube type	Predilute sample in microvial prior to analysis.
QC: System indicates QC Passed when actually failed or QC Failed when actually passed	Wrong values entered by user	Ensure the correct QC values are entered in the Edit Control Lot dialog box in the **QC/QC Lots** screen.
	Wrong sample used as QC	Ensure the QC samples are correctly labeled when prediluting.
	Low and high QC switched or barcoded incorrectly	Verify correct barcode and position in rack.
	Rules configured incorrectly by user	Verify the settings in the **QC/Rules** screen.
QC: System not importing/ exporting QC status/results from/to Unity	Connectivity/external media failure	See “Export: System fails to export results or information” solutions.
QC: The wrong QC result is excluded from calculations	User error	Clear the **Exclude data point from calculations** checkbox for that QC result in the Levey-Jennings Details screen.
Reagents: Excessive time for pressurization of newly installed reagent	Leaking connector	Replace the newly installed reagent bottle.
Sample Result: Bad result accidentally released	Operator error	If the LIS Connection is **Off** or no LIS is used, the action can be undone by rejecting the result. However, if the LIS Connection is **On**, releasing a result sends it to the LIS; the bad result must be manually rejected at the LIS.
Sample Result: Good result accidentally rejected	Operator error	The action can be undone by releasing the result.
Sample tube cannot spin	Multiple barcodes on tube	Remove barcodes and replace with single barcode or enter barcode manually (Section 5.7.1).
Sample tube cap sticks to tube spinner	Tube spinning incompatible with tubes	Disable tube spinning (Section 4.7.6).

Problem	Probable Cause	Recommended Solution
Sample tube does not fit in rack	Large-diameter or abnormal tube type	Predilute sample in microvial prior to analysis.
Sample tube is broken by tube spinner	Using non-D-100 sample racks that do not have rotary bottoms	Disable tube spinning (Section 4.7.6).
	Tube spinning incompatible with tubes	
Sample tube is loose in rack	Small-diameter tube	Use appropriate size rack insert. If tube is <12 mm in diameter, predilute sample in microvial prior to analysis.
Sample type misidentified	Wrong tube adapter used	Ensure you are using the correct tube adapters for prediluted samples: • Use QC1, QC2, and QC3 barcoded adapters for QC levels 1, 2, and 3, respectively. • Use non-barcoded adapter for prediluted patient samples.
Sysmex Racks: Input area incorrectly loaded	Too many racks in input area	A maximum of 9 racks can be positioned in the rack input area between the stopper pins and the front of the instrument; a 10th rack can be inserted after the run starts and the 1st rack is in the sampling position.
	Incorrect orientation	Remove and reposition the rack(s) so that the rack barcode faces the instrument and the Sysmex logo faces you.
Sysmex Racks: Rack jam	Non-D-100 rack used	1. Remove jammed rack. 2. Transfer sample tubes to a D-100 Sysmex rack, placing it in the rack input area. 3. Restart run.
	User tried to access rack that was being processed	1. Remove jammed rack. 2. Place the rack in the rack input area. 3. If necessary, restart run.

Problem	Probable Cause	Recommended Solution
Sysmex Racks: Racks not transported from one area to other	Rack jam; rack loaded incorrectly.	Remove and reposition the rack(s) in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you.
	Rack not detected in rack input area	Contact Bio-Rad Technical Service.
	Failure of transport mechanism	Contact Bio-Rad Technical Service.
Sysmex Racks: System moves unprocessed rack to rack output area	User stopped the run and rack was ejected from the shuttle	Move rack to input area and start a new run.
	Power outage	After power is restored, move rack(s) to input area and start a new run.
Third Party Calibrator: System cannot process third party calibrators	User incorrectly loads calibrators	Follow the instructions in Section 5.9.
	User does not identify sample properly	
User Access: Unauthorized user access allowed	Improper user management	Edit the user's role (Section 4.7.3.2).
Waste container overflows without warning	Sensor failure	Contact Bio-Rad Technical Service.
Waste: System not discharging waste; waste backup	Kink in waste tubing backs up the waste combiner	Ensure that the waste tubing is not looped or crimped and is sloped downward at all times.
	Blockage	Contact Bio-Rad Technical Service.
	Pump failure	
	Sensor failure	

8.6 Fuse Replacement

Figure 8-1: *Power Switch Turned Off (O)*

1. Turn off the power switch on the rear of the instrument.
2. Remove the power cord.

Figure 8-2: *Removing Fuse Holder*

3. With an appropriate tool (e.g., standard screwdriver), remove the fuse holder.

Figure 8-3: *Fuse in Fuse Holder*

4. Remove both fuses (one on each side of the holder) and install 2 new 10 A/250 V fuses.

Figure 8-4: *Fuse Inserted for 220-240 V* 	5. Reinsert the fuse holder in the correct direction, based on the voltage used. The white arrow to the right of the voltage in use should be pointing to the white rectangle on the bottom.
6. Reinstall the power cord and turn on the power switch.	
7. Press the soft power button on the front of the instrument to activate the power-on sequence.	

Appendix A Replacement Parts

When ordering replacement parts, please refer to the list below for the catalog number, description, and quantity required. Quantities listed below indicate the minimum units available.

REF	Description	Quantity
740	Lyphochek® Diabetes Control Bilevel, 6 x 0.5 mL	1 pkg
740X	Lyphochek® Diabetes Control Bilevel MiniPak, 2 x 0.5 mL	1 pkg
171	Liquichek™ Diabetes Control, Level 1, 6 x 1.0 mL	1 pkg
172	Liquichek™ Diabetes Control, Level 2, 6 x 1.0 mL	1 pkg
173	Liquichek™ Diabetes Control, Level 3, 6 x 1.0 mL	1 pkg
172X	Liquichek™ Diabetes Control, Trilevel MiniPak, 3 x 1.0 mL	1 pkg
12000070	Lyphochek® Hemoglobin A1C Linearity Set (1 each of 6 levels), 6 x 0.5 mL	1 pkg
290-1004	D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack	1 pkg
290-1006	D-100 HbA_{1c} Calibrator Pack	1 ea
290-1007	D-100 HbA_{1c} Prefilters (5 per package)	1 pkg
290-1008	D-100 Cleaning Tube	1 ea
290-1009	D-100 Sample Diluent	1 ea
290-1010	D-100 HbA_{1c} Elution Buffer A	1 ea
290-1011	D-100 HbA_{1c} Elution Buffer B	1 ea
290-1012	D-100 Wash Solution	1 ea
290-1013	D-100 Thermal Printer Paper (10 rolls per package)	1 pkg
12000063	D-100 Operation Manual with Multi-Language CD	1 ea
12000175	Fuses (10 A/250 V)	2 ea
12000182	D-100 Sample Probe	1 ea
12000230	External Waste Bottle	1 ea
12000231	External Waste Tubing	1 ea
12000232	Sysmex Rack	1 ea
12000233	Rack Inserts, 12 mm (10 per package)	1 pkg
12000234	Rack Inserts, 13 mm (10 per package)	1 pkg
12000235	Rack Inserts, 14 mm (10 per package)	1 pkg
12000236	Microvial Adapters (10 per package)	1 pkg
12000237	Sysmex Rack Barcode Labels (1–100)	1 pkg
12000238	Microvial Adapter QC Barcode Labels (3 sheets of 3 levels)	1 pkg
12000243	Sample Vials (polypropylene microvials with pierceable caps), 100 x 1.5 mL	1 pkg

REF	Description	Quantity
12000244	D-100 USB Flash Drive (8 GB)	1 ea
12000296	D-100 Utility Cartridge	1 ea
12000297	D-100 Utility Prefilter	1 ea
196-2051	Hemoglobin Capillary Collection System (HCCS), 5 Tests	1 pkg
196-2052	Hemoglobin Capillary Collection System (HCCS), 100 Tests	1 pkg
196-2053	Hemoglobin Capillary Collection System (HCCS), 5000 Tests	1 pkg

Appendix B Specifications

B.1 D-100 General Specifications

- Dimensions: 660 mm (W) x 650 mm (D) x 725 mm (H)
  25.98 in. (W) x 25.59 in. (D) x 28.54 in. (H)
- Weight uncrated: 103 kg (227 lb) dry; 121 kg (266 lb) wet
- Operating Environment

  Temperature: 15–35 °C

  Humidity: 20–80%, non-condensing

  Altitude: 3048 m (10,000 ft) max

  Heat Generation: 4092 BTU/h
- Storage Conditions

  Temperature: –10 °C to 50 °C

  Humidity: 20–80%

  NOTE: *Failure to store the D-100 under these conditions may result in damage to the system.*
- Power Input Requirements: 100–240 V~, 50–60 Hz
- Power Consumption: 1100 VA max
- Fuses: 10 A/250 V TLAG (2 fuses)
- Sound Level: <70 dBA
- Sample Requirements: Refer to assay IFU
- Sample Throughput: Refer to assay IFU
- User Interface: Integrated LCD touchscreen
- Adapter/Ports: Ethernet on RJ45 port; RS232 on DB9 port; External DVI-I connectors (with VGA); 6 External USB ports
- External Waste Bottle Volume: 5 L x 2 bottles

B.2 Pump Module Specifications

- Type: Pair of dual-piston HPLC pumps
- Flow Rate Range: 0.05–5.00 mL/min
- Maximum Pressure: 350 bar

B.3 Pressure Transducer Specifications

- Construction: Corrosive resistant titanium (6AL4V)
- Pressure Rating: 344.7 bar
- Over Pressure Range: 689.4 bar

B.4 Injection Valve Specifications

- Type: 3-position, 4-port Titan HT
- Loop Size: 5 µL x 2 internal injection loops

B.5 Sample Handling Specifications

- Sample Rack: Sysmex, 10 positions
- Sample Capacity: 10 racks in input area, 10 racks in output area
- Primary Tubes: width 12–16 mm, height 75–100 mm
- Sample Vials: 1.5-mL microvials
- Rack Inserts: 12 mm, 13 mm, 14 mm
- Microvial Adapters for 1.5-mL microvials
- Sample Probe: Stainless steel 316L, 143 mm
- Sample Dilution

  Dilution capability: 1:300 in 2 steps

  Dilution well volume: 405 µL

  Sample pickup volume: 10 µL

  Syringe volume: 978 µL

  Diluted sample pickup volume: 220 µL

B.6 Stat Area Specifications

- Sample Rack: Customized
- Sample Capacity: 3 positions for primary tubes or microvial adapters, 3 positions for Calibrator Pack

B.7 Sample Identification (Barcode) Specifications

- Barcode Symbologies supported:

Code 39
Code 128
Codabar
Interleaved 2 of 5
EAN
UPC

NOTE: *It is recommended to use a barcode symbology that includes a check digit (checksum character) to reduce the risk of sample misidentification.*

- Number of Characters: 1–22
- Barcode Symbol Dimensions: 10 mm min height, 67 mm max width
- Barcode Symbol Placement Zone: 20 mm above the bottom of the tube to 14 mm below the top of the tube, excluding the cap

B.8 Reagent Compartment Specifications

- Capacity: Accepts 2 bottles of Elution Buffer A, 2 bottles of Elution Buffer B, and 2 bottles of Wash Solution

B.9 External Waste Bottle Specifications

- Capacity: 5 L x 2 bottles
- Material: Polyethylene
- Level Sensor: 3 float switches

B.10 System Controller Specifications

- Central Processing Unit: Onboard PC
- Operating System: Microsoft® Windows® Embedded Standard 7
- Memory: 4 GB DDR3

B.11 Touchscreen Specifications

- Type: Integrated LCD touchscreen
- Dimensions: 282 mm (W) x 109 mm (D) x 377 mm (H)
- Angular Adjustment: 0 to10°
- Pixel format: 1024 horizontal by 768 vertical

B.12 Cartridge Holder Specifications

- Heating Device: Peltier HPE-128-10-05 Multicomp
- Temperature Sensor: PT100 4 wires measurement
- Temperature Accuracy: ±0.3 °C
- Temperature Stability: ±0.5 °C
- Overheating protection: Thermal fuse, 76 °C to 80 °C opening T

B.13 Internal Printer Specifications

- Dimensions: 178 mm (W) x 178 mm (D) x 325 mm (H)
- Paper Width: 76 mm (3 in.)
- Paper Roll: 50 mm
- Power Consumption: 1.5 A max (24 V); 200 mA (5 V)
- Printer Resolution: 8 dots/mm (203 dpi)
- Printer Technology: Thermal Printer
- Signal Connection: USB
- Printer Speed: 200 mm/s max

B.14 External Network Printer Specifications

- Compatible operating system: Microsoft® Windows® 7
- Connectivity: Hi-Speed USB 2.0, Ethernet
- Printing color: Monochrome (black & white)

Appendix C Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
1	Total Area Low*	The Total Area is less than the cutoff.	50000		Low total area	Should not report HbA1c	No	Enabled
2	Total Area High*	Total Area is greater than the cutoff.	350000		High total area	Should not report HbA1c; predilute/rerun	No	Enabled
3	No HbA1c	No HbA1c peak was identified.	NA		No HbA1c peak	No HbA1c result	No	Enabled
4	No HbA0	No HbA0 peak was identified.	NA		No HbA0 peak	No HbA1c result	No	Enabled
5	HbA1c Range*	The HbA1c result is outside the reportable range.	3.5–20.0 (%) **or** 15–195 (mmol/mol)		HbA1c result is out of range	Should not report HbA1c	No	Enabled
6	HbA1c High*	The HbA1c result is greater than the cutoff.	15 (%) **or** 140 (mmol/mol)		High HbA1c	Possible variant interference	No	Enabled
7	E and D Present	Peaks are present in the E-Window and D-Window.	NA		E-Window and D-Window present	Possible variant interference	No	Enabled
8	E and S Present	Peaks are present in the E-Window and S-Window.	NA		E-Window and S-Window present	Possible variant interference	No	Enabled
9	E and C Present	Peaks are present in the E-Window and C-Window.	NA		E-Window and C-Window present	Possible variant interference	No	Enabled
10	D and S Present	Peaks are present in the D-Window and S-Window.	NA		D-Window and S-Window present	Possible variant interference	No	Enabled
11	D and C Present	Peaks are present in the D-Window and C-Window.	NA		D-Window and C-Window present	Possible variant interference	No	Enabled
12	S and C Present	Peaks are present in the S-Window and C-Window.	NA		S-Window and C-Window present	Possible variant interference	No	Enabled

Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
13	Minor Peak(s) > 10%*	The Unknown 1, Unknown 2, Unknown 3, Unknown 4, A1a, A1b, or P3 Area% is greater than the cutoff.	10 (%)		Minor peak(s) > 10%	Possible variant interference	No	Enabled
14	HbS Cutoff	The S-Window Area% is greater than the cutoff.	60 (%)		Elevated peak in S-Window	Possible variant interference	No	Enabled
15	HbC Cutoff	The C-Window Area% is greater than the cutoff.	60 (%)		Elevated peak in C-Window	Possible variant interference	No	Enabled
16	HbD Cutoff	The D-Window Area% is greater than the cutoff.	43 (%)		Elevated peak in D-Window	Possible variant interference	No	Enabled
17	HbE Cutoff	The E-Window Area% is greater than the cutoff.	39.1 (%)		Elevated peak in E-Window	Possible variant interference	No	Enabled
18	HbF Cutoff*	The F Area% is greater than the cutoff.	30 (%)		Elevated HbF	Should not report HbA1c	No	Enabled
19	S-Window Present	A peak is present in the S-Window.	NA		Peak in S-Window	NA	No	Disabled
20	C-Window Present	A peak is present in the C-Window.	NA		Peak in C-Window	NA	No	Disabled
21	D-Window Present	A peak is present in the D-Window.	NA		Peak in D-Window	NA	No	Disabled
22	E-Window Present	A peak is present in the E-Window.	NA		Peak in E-Window	NA	No	Disabled
23	Unread Barcode	A sample tube or microvial barcode was not read.	NA		NA	Unread barcode	No	Disabled
24	LA1c Cutoff	The LA1c Area% is greater than the cutoff.	7 (%)		NA	High LA1c (info only)	No	Disabled
25	Baseline Slope	The A1c Slope-To-Area Ratio is outside the acceptable range.	0.00–0.90	No	NA	Baseline slope outside range (info only)	No	Disabled

Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
26	A1c Sigma	The A1c Sigma is outside the acceptable range.	0.30–0.90	No	NA	A1c sigma outside range (info only)	No	Disabled
27	A1c Tau	The A1c Tau is outside the acceptable range.	0.168–2.200	No	NA	A1c tau outside range (info only)	No	Disabled
28	A1c Tau/Sigma	The A1c Tau/Sigma Ratio is outside the acceptable range.	0.00–3.90	No	NA	A1c tau/sigma outside range (info only)	No	Disabled
29	A1c Fit Crest Time	The A1c Fit Crest Time Diff is outside the acceptable range.	1.40–3.20	No	NA	A1c fit crest time outside range (info only)	No	Disabled

***NOTE:** *The cutoff values for these rules correspond to the performance claims in the assay Instructions For Use.*

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Appendix D:

DRAFT Labeling

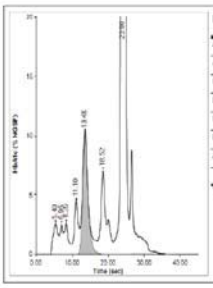
Quick Guide

D-100™ HbA1c

Result Examples

Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: 10.2 %



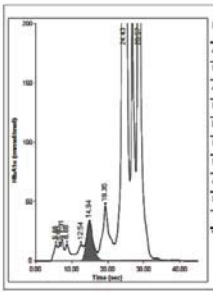
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1.88	---
A1b	6.06	1573.43	1.05	---
F	8.29	2612.14	1.74	---
LA1c	11.10	4492.47	2.99	---
HbA1c	13.48	12431.12	---	10.2
P3	18.52	9123.63	6.06	---
A0	23.95	117434.53	78.03	---

Total Area: 150490

Status: Held

Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

HbA1c: 32 mmol/mol 5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	---
Unknown	6.60	391.10	0.23	---
A1b	7.31	994.53	0.59	---
F	8.66	1222.50	0.73	---
LA1c	12.54	1345.98	0.80	---
HbA1c	14.94	3705.06	---	32
P3	19.36	6726.02	3.42	---
A0	24.43	83432.11	49.81	---
S-Window	28.57	69897.36	41.61	---

Total Area: 167509

Status: Held

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Result Review

Any results that do not meet the following criteria should not be reported.

Item	Criteria
Total Area range	50,000–350,000
Quality Control	Values should be in range
HbA_{1c} reportable range	• NGSP: 3.5–20.0% • IFCC: 15–195 mmol/mol • Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.
HbF	≤30%
Labile A_{1c} (LA1c)	No interference
Carbamylated hemoglobin (CHb)	• No interference • CHb elutes in the LA1c window
P3 peak	≤10%
Heterozygous hemoglobins E, D, S, and C	No interference
E, D, S, and/or C windows	Combined area <50%
"Unknown" peaks	"Unknown" peak ≤10%

Test Components

USE	REF	Symbol	Description
290-1004		ANLT CRTR CAL PACK	Analytical Cartridge/Calibrator Pack
290-1006		CAL PACK	Calibrator Pack
290-1007		PRE FIL	Prefilters (5 per package)
290-1008		CLN TUBE	Cleaning Tube
290-1009		SAMP DIL	Sample Diluent
290-1010		BUF A	Elution Buffer A
290-1011		BUF B	Elution Buffer B
290-1012		WSH SOLN	Wash Solution

D-100™ HbA_{1c} Quick Guide

CE IVD

- *This Quick Guide is for reference use only; for detailed information, see the Instructions For Use and Operation Manual.*
- *Consider any materials of human origin as infectious and handle them using appropriate biosafety procedures.*
- *Wear personal protective equipment while handling all reagents and samples and while operating the D-100 system.*

Sample Preparation

HbA_{1c} Calibrator Pack:

- Contains Conditioner, Calibrator Level 1, and Calibrator Level 2.
- No manual preparation required. Can be used immediately after removing from refrigerator.
- Once reconstituted by system, stable for 24 hours at 2–8 °C; do not freeze.

Liquichek™ Diabetes Controls:

- After opening vial, stable for 14 days at 2–8 °C.
- Dilute 1:200 prior to analysis (5 µL of control in 1.0 mL of Sample Diluent).

Lyphochek® Diabetes Controls:

- Reconstitute each vial with 0.5 mL of DI water.
- Allow to stand for 5–10 minutes; swirl gently to dissolve.
- Stable for 7 days at 2–8 °C.
- Dilute 1:300 prior to analysis (5 µL of control in 1.5 mL of Sample Diluent).

Whole blood samples:

- Samples should be collected in vacuum collection tubes containing anti-coagulant. For detailed information, see the *Instructions For Use*.
- No sample preparation required.
- If abnormal tube type or height of sample is ≤1 cm, sample may need to be prediluted 1:300 prior to analysis (5 µL of sample in 1.5 mL of Sample Diluent).

Replacing the Reagents

After installing the bottles, the reagents are stable for 90 days at 15–35 °C. A reagent bottle can be removed at any time, except when the bottle is "In Use" in Running state.

1. Remove the empty bottle.
   NOTE: *If the bottle is not empty, touch the reagent indicator in the consumables panel, then touch* ***Remove*** *to depressurize the bottle.*
2. Install a new bottle. The reagent information is automatically updated.

Replacing the Prefilter

Replace the prefilter at 90 days or 2000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the prefilter holder door.
3. Remove the old prefilter.
4. Insert the new prefilter.
5. Close the prefilter holder door. The prefilter information is automatically updated.

Replacing the Analytical Cartridge

Replace the analytical cartridge at 90 days or 10,000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the cartridge holder door.
3. Remove the old cartridge.
4. Insert the new cartridge.
5. Close the cartridge holder door. Test parameters are automatically updated.

Calibration

Calibration must be performed once, following the installation of every new analytical cartridge.

1. Ensure the instrument is in Sleeping or Standby state.
2. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
3. Insert Diabetes Controls (in barcoded adapters) in positions 1–3 and insert the D-100 Calibrator Pack in the dedicated position, with the barcodes facing you.
4. Touch ⏏ **Load**.
5. Touch Calibrate Now.

NOTE: *Racks containing patient samples can be placed in the input area to be automatically processed after calibration has passed.*

6. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
7. Remove the samples from the Stat rack.

Routine Sample Run

1. Check the message panel for red or yellow messages and address as needed.
2. Insert Diabetes Controls (in barcoded adapters) followed by patient samples in racks.
   NOTE: *At least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested.*
3. Place the rack(s) in the input area.
4. Touch Run ►.
5. Load additional racks of samples during the run as needed.
6. Remove the processed racks from the output area.

NOTE: *When the run is complete, the instrument goes to Standby state for approximately 2 hours.*

Running Stat Samples

The D-100 System will analyze Stat samples after completing the last aspirated sample from the routine run.

1. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
2. Insert the sample(s) (i.e., primary sample tubes or prediluted samples in microvial adapters) in positions 1–3 of the Stat rack with the barcode(s) facing you.
3. Touch ⏏ **Load** to process the Stat samples.
4. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
5. Remove the samples from the Stat rack.

NOTE: *If the instrument is not in Running state, Stat samples can be run by loading the Stat area, touching* ⏏ ***Load****, and touching* Run ►.

Viewing Results

1. Touch the **Results** tab.
2. To view a sample result in detail, touch the result row.
3. To filter the results in the table, use the Filter buttons.
   - The filter currently in use is indicated to the right of the ⏬ button.
   - Flagged results can be accessed from the **Home** screen by touching Flagged.

All : Displays results for All processed samples.

: Displays results for Flagged samples only.

★ : Displays results for samples meeting the Favorite filter criteria only.

⏬ : Opens the Filter dialog box for more filtering options.

Releasing or Rejecting Results

To release or reject results in the Results screen:

1. Select the corresponding checkbox for each sample result you want to release.
2. Touch **Release** at the bottom of the screen. The result(s) status indicates .
3. Select the corresponding checkbox for each sample result you want to reject.
4. Touch **Reject** at the bottom of the screen. The result(s) status indicates .

To release or reject results in the Result Details screen:

1. In the **Results** table, touch the sample result row to open the Result Details screen.
2. After reviewing the details, touch **Release** to release the result or **Reject** to reject the result.
3. The system updates the result status (Released or Rejected) accordingly and displays the next sample result.

Appendix E:

DRAFT Labeling

Box/Bottle Labels

D-100™ HbA1c

Label List

Label Name	Label Number
D-100 Testing System Instrument Crate Label	LB000327, revB
Accessory box	LB0003280, rev A
D-100 Sample Diluent	16000XXX, rev B
D-100 HbA1c Elution Buffer A (Box Label)	16000348, rev B
D-100 HbA1c Elution Buffer B (Box Label)	12000939, rev B
Wash Buffer (Box Label)	16000XXX, rev B
D-100 HbA1c Elution Buffer A (Bottle Label)	LB0003180, rev A
D-100 HbA1c Elution Buffer B (Bottle Label)	LB0003190, rev A
Wash Buffer (Bottle Label)	LB0003200, rev A
D-100 HbA1c Calibrator/Analytical Cartridge Pack	16000347, rev B
D-100 HbA1c Calibrator Pack	16000346, rev B
D-100 HbA1c Analytical Cartridge	LB000332, rev B
Prefilters (1 X 5)	LB000903, rev A
Cleaning Tube	LB0003250, rev A

Label PIN: LB000327revB
Size: 127 x 279 mm
PMS: 347, 2965, Black

BIO-RAD

REF 290-1000

D-100™ Hemoglobin Testing System

LB000327revB

EN: Contains: D-100 Instrument and Accessory Kit
DE: Inhalt: D-100-Gerät und Zubehör-Kit
FR: Contient : un instrument D-100 et un kit d'accessoires
ES: Contiene: Instrumento y kit de accesorios de D-100
IT: Contenuto: strumento D-100 e kit accessori
SE: Innehåller: D-100 instrument och tillbehörssats
DK: Indeholder: D-100 instrument og tilbehørskit
NO: Inneholder: D-100-instrument og tilbehørssett

UDI-DI 00847817023095

SN

CE IVD

−10°C 50°C

20% 80%

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547
FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette

Made in France

Label PIN: LB0003280revA
Size: 119 x 167 mm
PMS: 347, 2965, Black

BIO-RAD

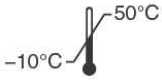
LB0003280revA

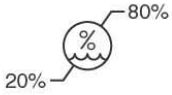
D-100™ Hemoglobin Testing System

Accessory Kit

USE REF 290-1000

CE IVD



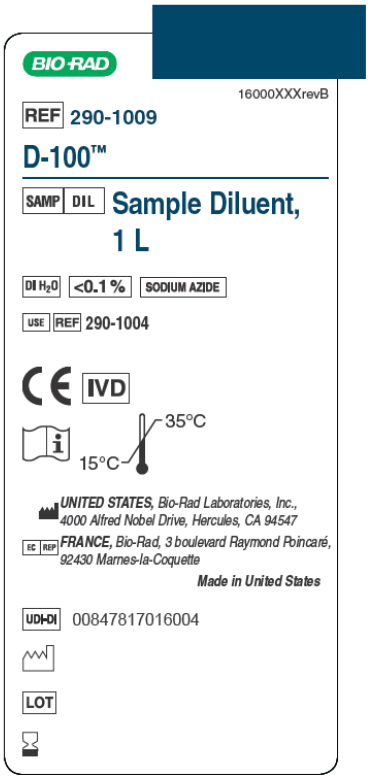


UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in France

LPN: 16000XXXrevB
Size: 2.25" x 5"
PMS: 347, 2965, Black



Label PIN: 16000348revB
Size: 3.625” x 7.5”
PMS: 347, 2965, Black

BIO-RAD

REF 290-1010

D-100™ HbA_{1c} BUF A Elution Buffer A, 2600 mL

16000348revB

CE IVD

15°C 35°C

US: Rx Only

USE REF 290-1004

UDI-DI 00847817025679

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood
Contains Sodium Perchlorate, Succinate Buffer

DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut
Enthält Natriumperchlorat, Succinatpufferr

FR: Pour le dosage de l’hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain
Contient un tampon de succinate et de perchlorate de sodium

ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana
Contiene tampón de perclorato de sodio y succinato sódico.

IT: Per la determinazione quantitativa dell’HbA_{1c} nel sangue intero umano
Contiene un tampone di perclorato e succinato di sodio

SE: För kvantitativ bestämning av HbA_{1c} i humant helblod
Innehåller natriumperklorat- och natriumsuccinatbuffert

DK: Til kvantitativ bestemmelse af HbA_{1c} i humant fuldblod
Indeholder en natriumperchlorat- og succinatbuffer

NO: For kvantifiserende bestemmelse av HbA_{1c} i humant fullblod
Inneholder natriumperklorat, suksinatbuffer

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

Label PIN: 12000939revB
Size: 3.625" x 7.5"
PMS: 347, 2965, Black

BIO-RAD

REF 290-1011

D-100™ HbA_{1c} BUF B Elution Buffer B, 1400 mL

12000939revB

CE IVD

15°C – 35°C

US: Rx Only

USE REF 290-1004

UDI-DI 00847817016028

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood
Contains Sodium Perchlorate, Succinate Buffer

DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut
Enthält Natriumperchlorat, Succinatpufferr

FR: Pour le dosage de l'hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain
Contient un tampon de succinate et de perchlorate de sodium

ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana
Contiene tampón de perclorato de sodio y succinato sódico.

IT: Per la determinazione quantitativa dell'HbA_{1c} nel sangue intero umano
Contiene un tampone di perclorato e succinato di sodio

SE: För kvantitativ bestämning av HbA_{1c} i humant helblod
Innehåller natriumperklorat- och natriumsuccinatbuffert

DK: Til kvantitativ bestemmelse af HbA_{1c} i humant fuldblod
Indeholder en natriumperchlorat- og succinatbuffer

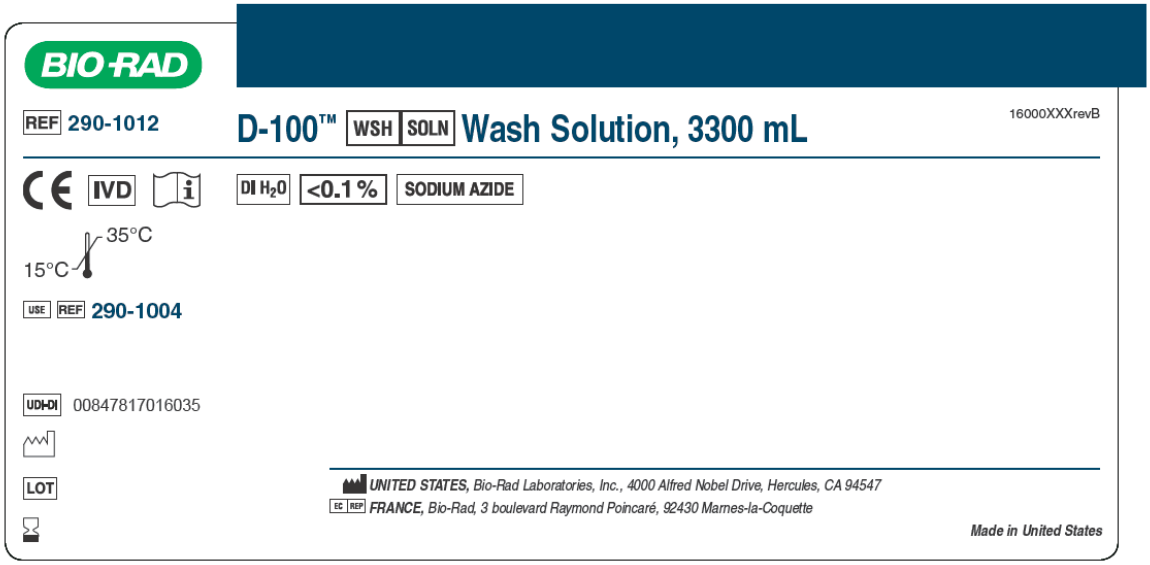
NO: For kvantifiserende bestemmelse av HbA_{1c} i humant fullblod
Inneholder natriumperklorat, suksinatbuffer

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

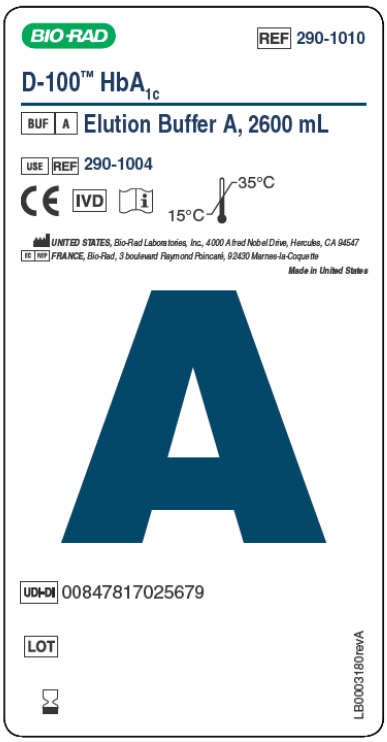
EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

Label PIN: 16000XXXrevB
Size: 3.625” x 7.5”
PMS: 347, 2965, Black



Label PIN: LB0003180revA
Size: 125 x 65 mm
PMS: 2965, 347, Black



Label PIN: LB0003190revA
Size: 125 x 47 mm
PMS: 2965, 347, Black

Label PIN: LB0003200revA
Size: 125 x 88 mm
PMS: 2965, 347, Black

Label PIN: 16000347revB
Size: 5” x 6”
PMS: 347, 2965, Black

BIO-RAD

16000347revB

REF 290-1004

D-100™ HbA_{1c}

ANLT | CRTR | CAL | PACK

Analytical Cartridge/Calibrator Pack

CE IVD

Σ 10000

2°C – 8°C

LATEX

US: Rx Only

UDI-DI 00847817015953

LOT

EN: For the quantitative determination of HbA_{1c} in human whole blood / DE: Für die quantitative Bestimmung von HbA_{1c} in menschlichem Vollblut / FR: Pour le dosage de l'hémoglobine A_{1c} (HbA_{1c}) dans le sang total humain / ES: Para la determinación cuantitativa de HbA_{1c} en sangre humana / IT: Per la determinazione quantitativa dell'HbA_{1c} nel sangue intero umano / SE: För kvantitativ bestämning av HbA1c i humant helblod / DK: Til kvantitativ bestemmelse af HbA1c i humant fuldblod / NO: For kvantifiserende bestemmelse av HbA1c i humant fullblod

1 x 1

ANLT | CRTR 1 x 1

CAL | PACK 1 x 1

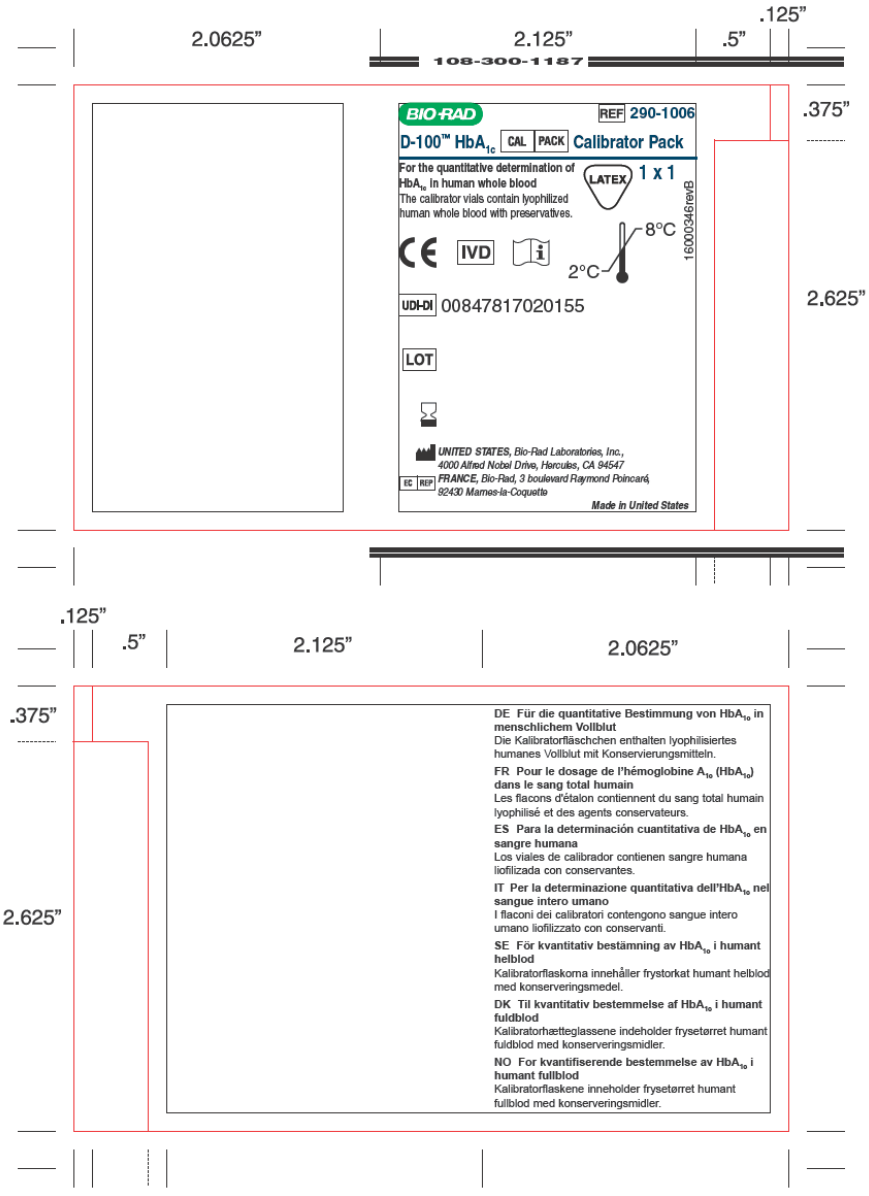
EN: The calibrator vials contain lyophilized human whole blood with preservatives. / DE: Die Kalibratorfläschchen enthalten lyophilisiertes humanes Vollblut mit Konservierungsmitteln. / FR: Les flacons d'étalon contiennent du sang total humain lyophilisé et des agents conservateurs. / ES: Los viales de calibrador contienen sangre humana liofilizada con conservantes. / IT: I flaconi dei calibratori contengono sangue intero umano liofilizzato con conservanti. / SE: Kalibratorrören innehåller frystorkat humant helblod med konserveringsmedel. / DK: Kalibratorhætteglassene indeholder frysetørret humant fuldblod med konserveringsmidler. / NO: Kalibratorflaskene inneholder frysetørret humant fullblod med konserveringsmidler.

UNITED STATES, Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547

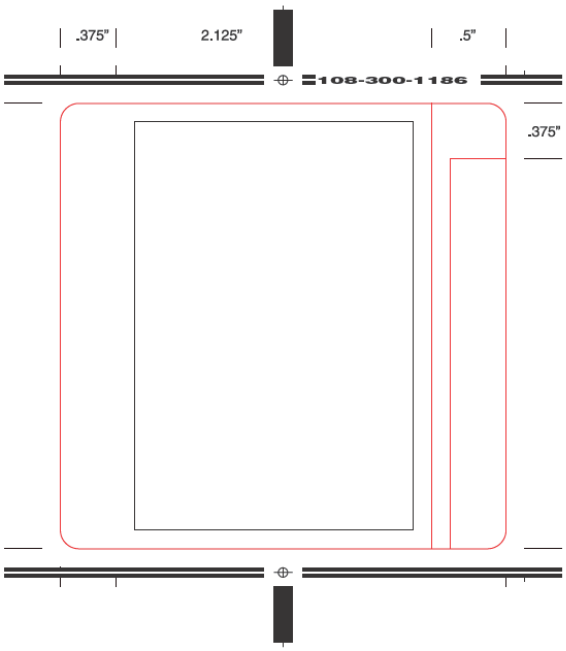
EC REP *FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette*

Made in United States

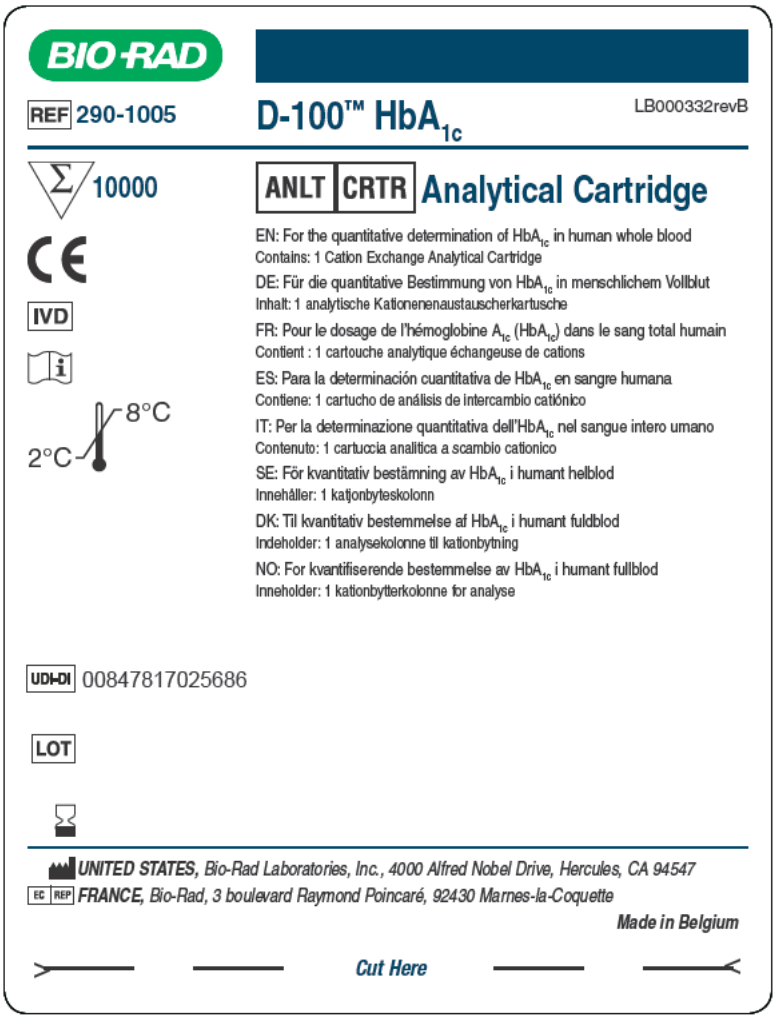
OVERALL SIZE: 4.8125" WIDE X 3.0" HIGH



Label PIN: 16000346revB
Size: 3" x 3"
PMS: 347, 2965, Black



LPN: LB000332revB
Size: 4” x 5.25”
PMS: 347, 2965, Black



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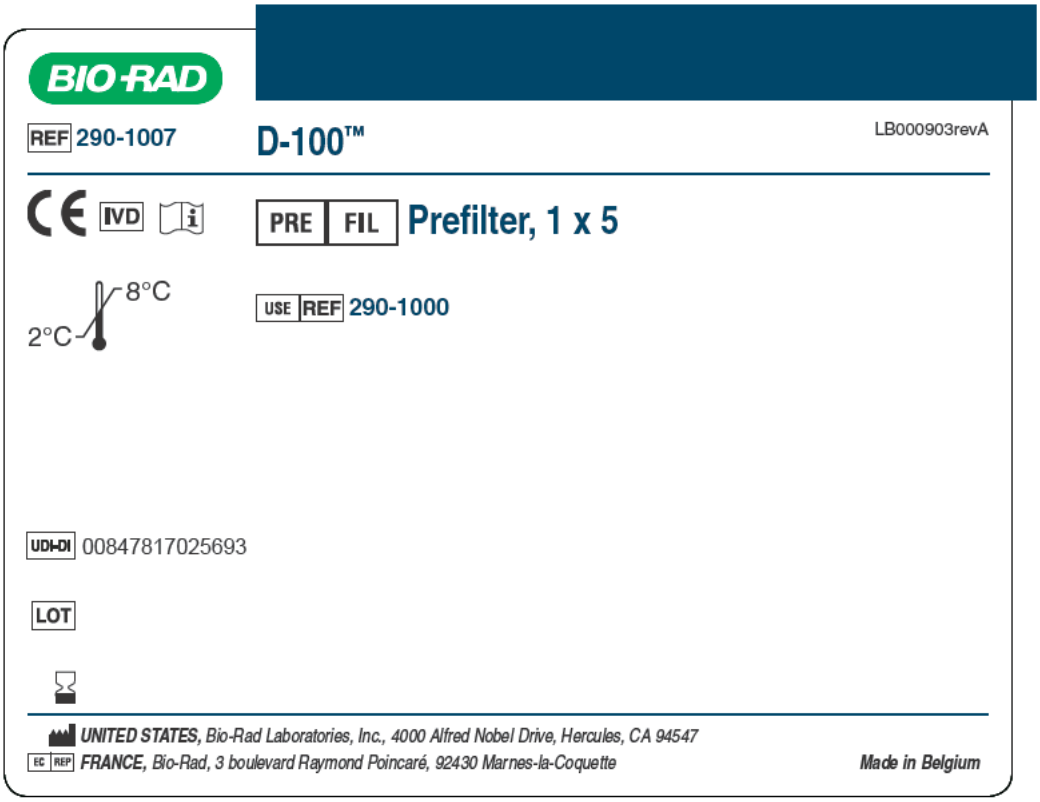
PMS: 347, 2965, Black



Label PIN: LB0003250revA
Size: 3" x 3"
PMS: 347, 2965, 032, Black

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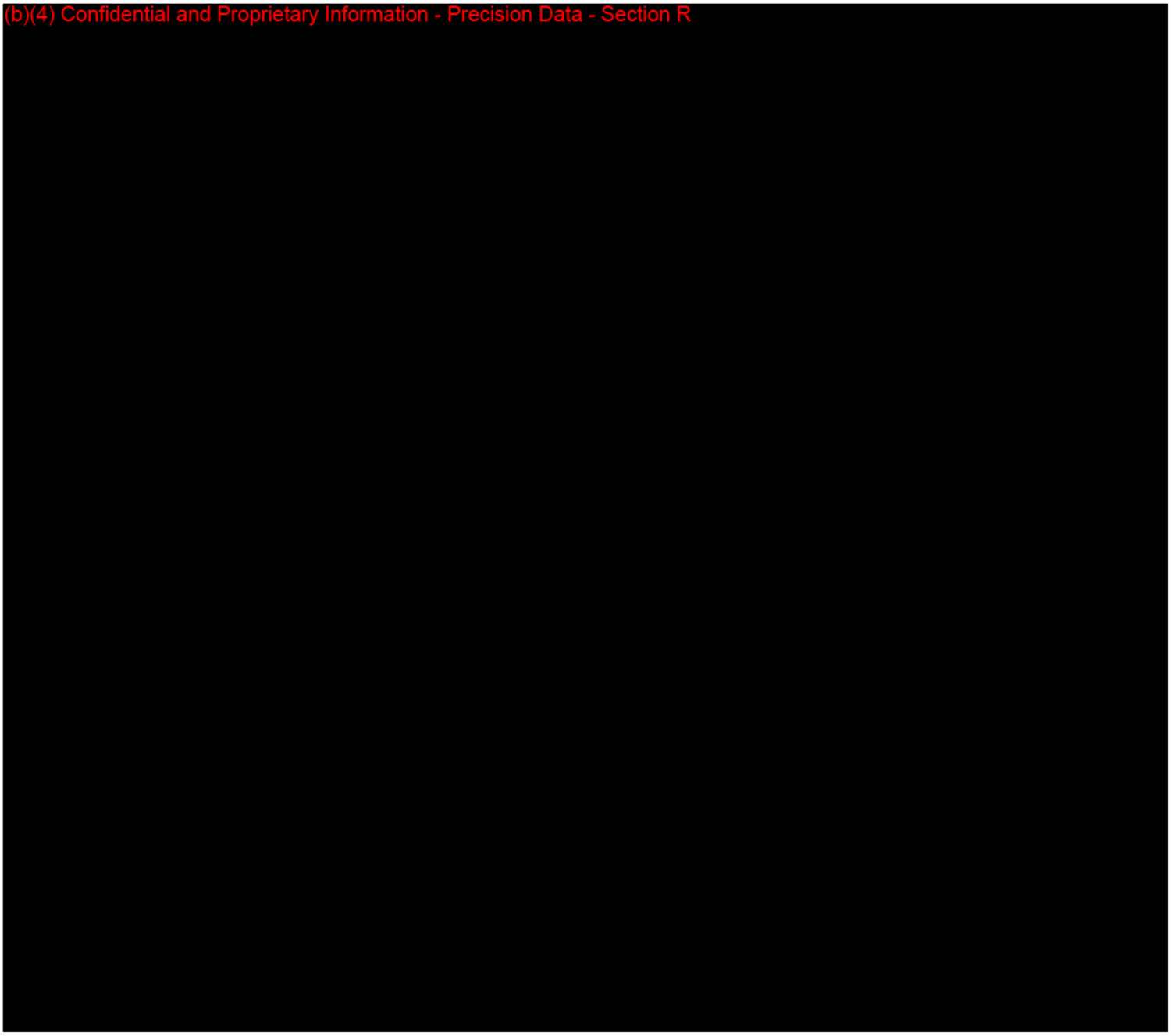
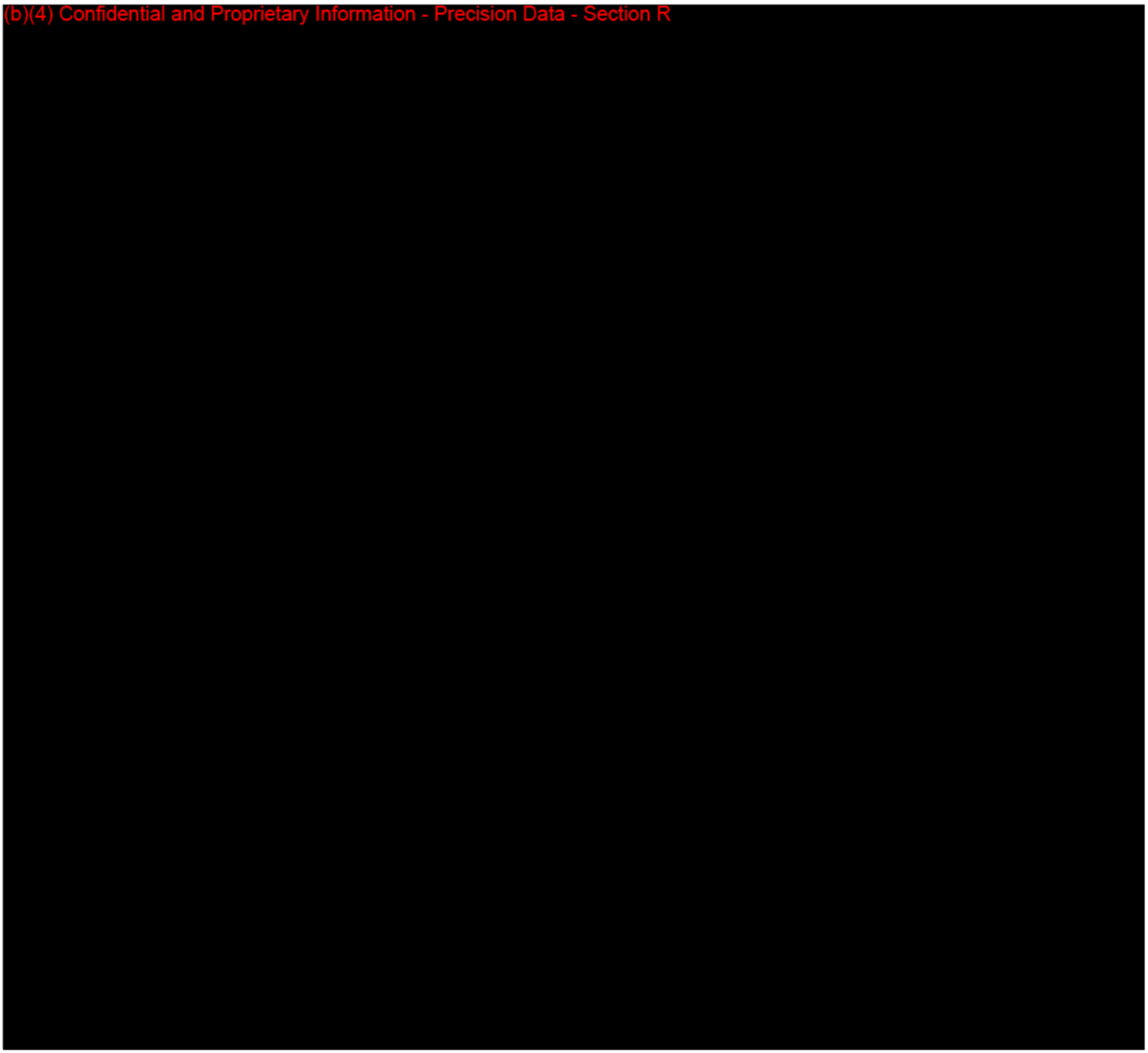
Appendix R:

Printed Precision Data

D-100™ HbA1c





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Software Level of Concern

D-100™ HbA1c and

D-100™ Hemoglobin Testing System

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Appendix U:

Device Hazard Analysis Documents

Risk Management Report

Software Risk Management Report

Software Traceability Report

Cybersecurity Summary

D-100™ Hemoglobin Testing System

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Appendix V:

Software Requirements Specification

D-100™ Hemoglobin Testing System

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Appendix W:

Architecture Design Chart

D-100™ Hemoglobin Testing System

(b)(4) Confidential and Proprietary Information - Architecture Design Chart

(b)(4) Confidential and Proprietary Information - Architecture Design Chart

(b)(4) Confidential and Proprietary Information - Architecture Design Chart

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Appendix X:

Software Design Specification

D-100™ Hemoglobin Testing System

(b)(4) Confidential and Proprietary Information - Software Design Specification

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Appendix Y:

Software Traceability Matrix

D-100™ Hemoglobin Testing System

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Appendix Z:

Software Development Environment Description

D-100™ Hemoglobin Testing System

(b)(4) Confidential and Proprietary Information - Software Development Environment Description

(b)(4) Confidential and Proprietary Information - Software Development Environment Description

Appendix AA:

Verification and Validation Documentation

Verification and Validation Plan
Verification and Validation Report
V&V Test Procedure: Hazard Control (Unexecuted)
V&V Test Procedure: Hazard Control (Executed)

D-100™ Hemoglobin Testing System

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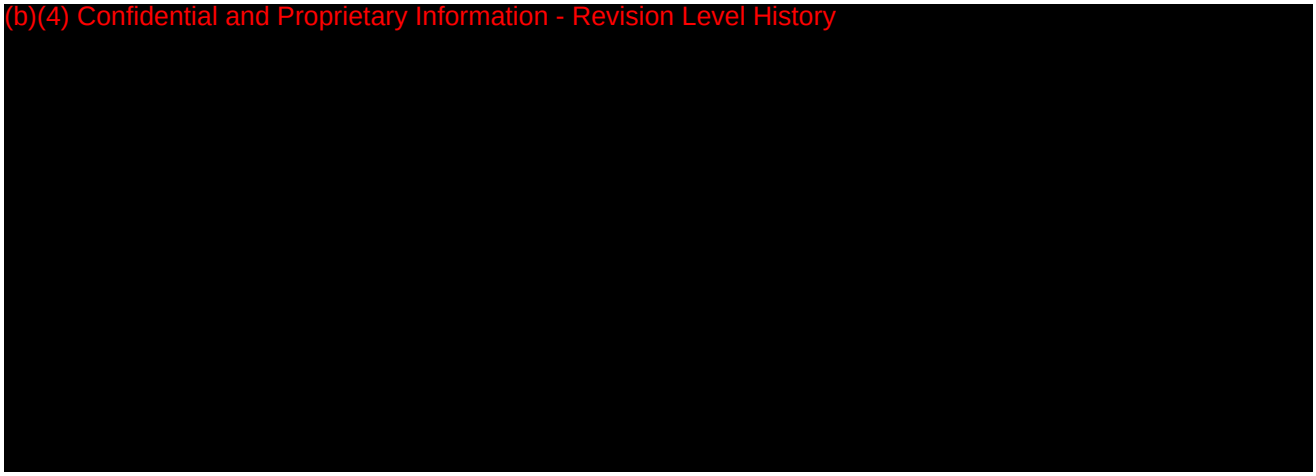
Appendix BB:

Revision Level History

D-100™ Hemoglobin Testing System

Revision Level History



Appendix BB-2

Appendix CC:

Unresolved Anomalies

Customer Notification

D-100™ Hemoglobin Testing System

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Appendix DD:

D-100™ 61010-1 Certificate

D-100™ Hemoglobin Testing System

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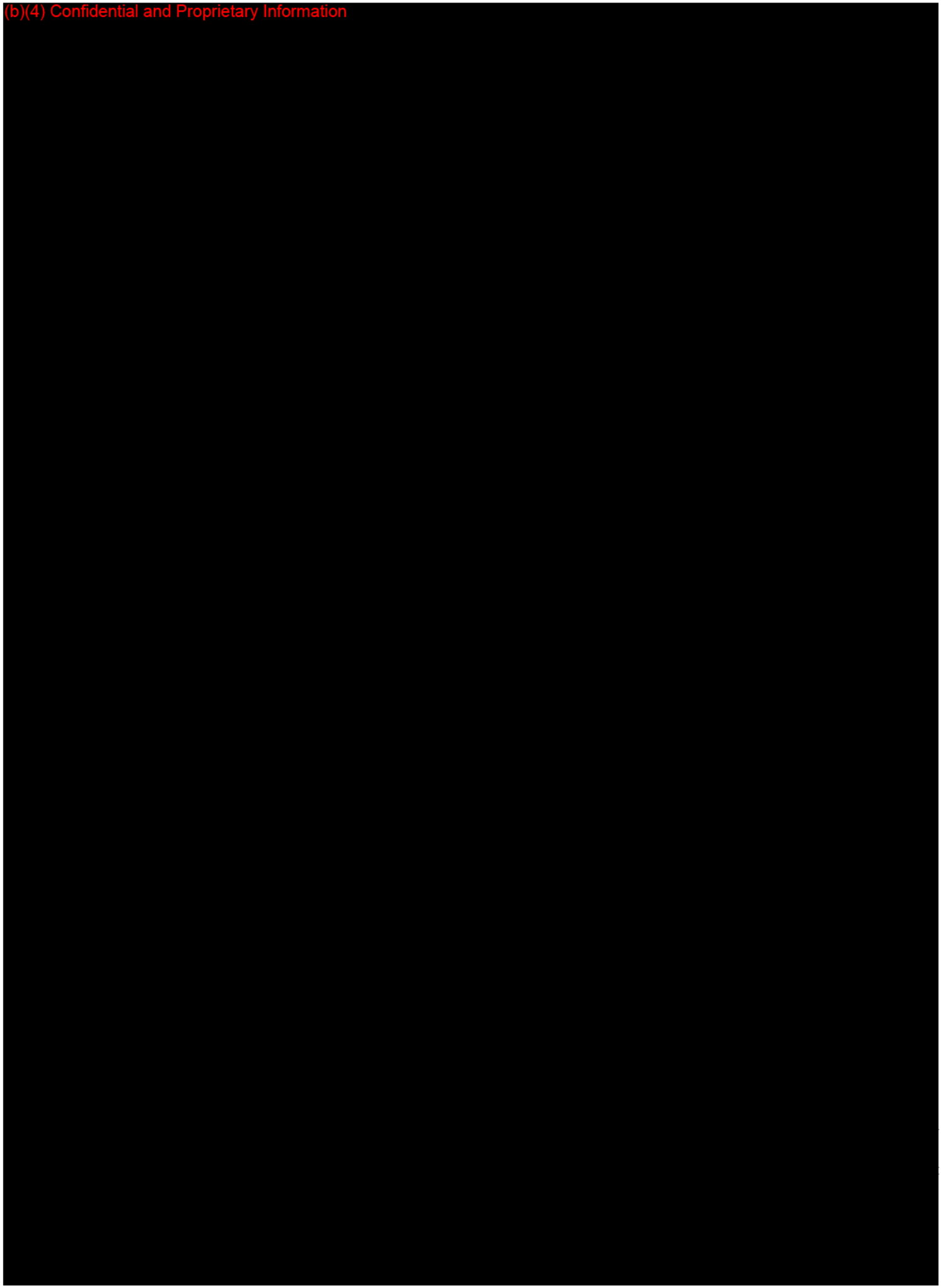

Appendix EE:

D-100™ 61010-1 Report

D-100™ Hemoglobin Testing System

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Appendix FF:

D-100™ EMC Report

D-100™ Hemoglobin Testing System

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K151321/S001

BIO-RAD

Bio-Rad Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

Nov. 6, 2015

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

VIA FEDERAL EXPRESS

Attention: OIR – Chemistry and Toxicology Devices (DCTD) - Chemistry Panel 75
Response to Deficiencies to K151321 in ecopy

Dear Dr. Alain Silke:

(b)(4) Confidential and Proprietary Information - Deficiencies

[redacted]

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Bio-Rad Laboratories

Diagnostics Group
4000 Alfred Nobel Dr.
Hercules, CA 94547 – 1803
Telephone 510 724 7000
Fax 510 741 5824

Nov. 6, 2015

VIA FEDERAL EXPRESS

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Attention: OIR – Chemistry and Toxicology Devices (DCTD) - Chemistry Panel 75
Response to Deficiencies to K151321 in ecopy

Dear Dr. Alain Silke:

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[redacted]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151321

Device Name
D-100™ HbA1c
D-100™ Hemoglobin Testing System
D-100™ HbA1c Calibrator Pack

Indications for Use *(Describe)*

The D-100™ HbA1c test is intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A1c measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100™ HbA1c test is intended for Professional Use Only.

Calibrators:
The D-100™ HbA1c Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A1c(HbA1c) in human whole blood.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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BIO-RAD

D-100™ HbA_{1c}

Instructions For Use

USE REF

290-1004
290-1006
290-1007
290-1008
290-1009
290-1010
290-1011
290-1012

Σ 10000

CE

IVD

US: Rx Only

November 2015
16000328revB

UNITED STATES, *Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, CA 94547, 510-724-7000*

EC REP **FRANCE**, *Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000*

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Prescription Use Only	UDI-DI Unique Device Identification-Device Identifier	LATEX Contains Latex
<0.1 % <0.1%	ANLT CRTR Analytical Cartridge	ANLT CRTR CAL PACK Analytical Cartridge/Calibrator Pack
DI H_2O Deionized Water	BUF A Elution Buffer A	BUF B Elution Buffer B
CAL PACK Calibrator Pack	CLN TUBE Cleaning Tube	IFCC IFCC Value (International Federation of Clinical Chemistry)
NGSP NGSP Value (National Glycohemoglobin Standardization Program)	PRE FIL Prefilter	SAMP DIL Sample Diluent
SAMP VIAL Sample Vials	SODIUM AZIDE Sodium Azide	WSH SOLN Wash Solution

PRODUCT SAFETY INFORMATION

Use caution when handling the following reagent:

CLN TUBE **Cleaning Tube** Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1) **Danger**	
H314	Causes severe skin burns and eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P311	If exposed or if you feel unwell: call a poison center or doctor/physician.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Table of Contents

INTENDED USE

Reagents

The D-100™ HbA_{1c} test is intended for the quantitative determination of hemoglobin A_{1c} (IFCC mmol/mol and NGSP %) in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) on the D-100 Hemoglobin Testing System.

Hemoglobin A_{1c} measurements are used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The D-100 HbA_{1c} test is intended for Professional Use Only.

Calibrators

The D-100 HbA_{1c} Calibrator Pack is for the calibration of the D-100 Hemoglobin Testing System used for the quantitative determination of hemoglobin A_{1c} (HbA_{1c}) in human whole blood.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects >8% of the world population.[3]

HbA_{1c} testing has been recommended for the diagnosis of Type 2 diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the World Health Organization (WHO), which recommend a diagnostic threshold of ≥6.5% (≥48 mmol/mol) HbA_{1c}.[4–6] HbA_{1c} testing has also been recommended for the identification of individuals at increased risk for developing diabetes (pre-diabetic). The ADA has defined the HbA_{1c} range for pre-diabetes as 5.7–6.4% (39–47 mmol/mol).[4] Detection and treatment of pre-diabetes may reduce or eliminate the risk of developing Type 2 diabetes and related complications.

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[7,8] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[9,10] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[11]

The D-100 HbA_{1c} test is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* for more information. The D-100 HbA_{1c} test also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of 45 seconds per sample.

PRINCIPLE OF THE PROCEDURE

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

The D-100 HbA_{1c} test is for use only with the D-100 Hemoglobin Testing System.

TEST COMPONENTS

The components are used in combination to perform the D-100 HbA_{1c} test. Components are available for individual sale.

REF	*Quantity*	*Description*
290-1004	1 each	**D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack.** One pack consisting of: • Cation exchange cartridge. 10,000 tests each. • Calibrator Pack: 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1006	1 each	**D-100 HbA_{1c} Calibrator Pack**. One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.
290-1007	1 each	**D-100 Prefilters**. 2000 tests each. Package of 5.
290-1008	1 each	**D-100 Cleaning Tube**. One microvial containing 1.5 mL of a liquid cleaning solution.
290-1009	1 each	**D-100 Sample Diluent**. Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
290-1010	1 each	**D-100 HbA_{1c} Elution Buffer A**. Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1011	1 each	**D-100 HbA_{1c} Elution Buffer B**. Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
290-1012	1 each	**D-100 Wash Solution**. Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

ADDITIONAL ITEMS AVAILABLE FROM BIO-RAD

REF	*Description*
12000243	**Sample Vials**. 100 polypropylene microvials with pierceable caps, 1.5 mL.
171	**Liquichek™ Diabetes Control, Level 1**. 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2**. 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3**. 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak**. 3 x 1.0 mL
740	**Lyphochek® Diabetes Control Bilevel**. 6 x 0.5 mL
740X	**Lyphochek® Diabetes Control Bilevel MiniPak**. 2 x 0.5 mL
12000070	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 6 levels)**. 6 x 0.5 mL

ADDITIONAL REQUIRED ITEMS NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 1.5 mL

Deionized Water

PRECAUTIONS/WARNINGS

- For in vitro diagnostic use.
- For complete details on safe reagent handling, refer to the Safety Data Sheets (SDS) available at www.bio-rad.com.
- Consider any materials of human origin as infectious and handle them using typical biosafety procedures.
- Wear personal protective equipment while handling all reagents and samples and while operating the system.
- Dispose of all waste in accordance with applicable national and/or local regulations.
- Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
- All waste material (including used cartridges and prefilters) containing patient samples or biological products should be considered biohazardous when disposing or treating.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
- Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
- Each unit of whole blood used in the manufacture of the calibrators and conditioner was tested by FDA-accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and conditioner with the same precautions used with patient specimens.
- Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
- The Calibrator Pack stoppers contain dry natural rubber.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood and capillary blood.

Specimen Additives, Preservatives

The whole blood specimens should be collected in vacuum collection tubes containing K2-EDTA, K3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin, or lithium heparin.

A matrix comparison study was performed to evaluate blood collection tube anticoagulants/preservatives for use with the test. Whole blood samples from 48 patients with HbA_{1c} concentrations spanning the reportable range of the test were collected in the evaluation tube types and the comparative tube type (K3-EDTA). The results of the matrix comparison study are summarized in Table 1.

Anticoagulant/Preservative	Slope	Intercept	R^2
K2-EDTA	0.9929	0.0270	0.9995
Potassium Oxalate/Sodium Fluoride	0.9927	0.0761	0.9994
Sodium Citrate	1.0084	–0.0429	0.9996
Sodium Heparin	0.9972	0.0131	0.9996
Lithium Heparin	0.9959	0.0103	0.9997

Table 1: *Regression Analysis of % HbA_{1c} for Evaluation Tubes vs K3-EDTA Tubes*

Specimen Storage

- Whole blood specimens may be stored as follows:

Anticoagulant/Preservative	Days at:			Months at:
	15–35 °C	2–8 °C	–20 °C	–70 °C
K2-EDTA	1	7	7	6
K3-EDTA	1	7	7	6
Potassium Oxalate/Sodium Fluoride	1	7	7	6
Sodium Citrate	1	7	7	6
Sodium Heparin	1	7	7	6
Lithium Heparin	1	7	7	6

- Prediluted samples are stable for 3 hours at system operating temperature (15–35 °C).

Specimen Preparation

- No sample preparation is required. Mixing the sample tubes before loading is not necessary.
- If the height of the sample in the tube appears to be ≤1 cm, then the sample may need to be prediluted 1:300 prior to analysis:
  1. Before pipetting, thoroughly mix the sample by gently inverting the tube.
  2. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the whole blood sample.
  3. Cap the microvial and mix thoroughly.

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Analytical Cartridge

The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.

Prefilter

The Prefilter is stable until the expiration date when stored at 2–8 °C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15–35 °C.

Elution Buffers and Wash Solution

- The Elution Buffers and Wash Solution are stable until the expiration date when stored unopened at 15–35 °C. After installing the bottles on the instrument, these reagents are stable for 90 days at 15–35 °C.
- The Elution Buffers are interchangeable within cartridge resin lots. All lots of Wash Solution are interchangeable.

Sample Diluent

The Sample Diluent is stable until the expiration date when stored unopened at 15–35 °C. After opening, the Sample Diluent is stable for 90 days when stored at 15–35 °C.

Calibrator Pack

- The Calibrator Pack is stable until the expiration date when stored unopened at 2–8 °C. The Calibrator Pack can be used immediately after removing from refrigerator.
- Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2–8 °C. The Calibrator Pack may be used for a second calibration within this period.

Cleaning Tube

The Cleaning Tube is stable until the expiration date when stored unopened at 15–35 °C. See *Product Safety Information* for hazards and precautions.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the control. Cap each microvial and mix thoroughly.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the reconstituted control. Cap each microvial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If Elution Buffers, Wash Solution, or Sample Diluent were frozen during shipment, allow them to reach room temperature (15–35 °C) and mix each bottle by gently inverting before use.
- Do not use any reagents that show signs of external leakage.

PROCEDURE

For more information, refer to the *D-100 Operation Manual*.

Replacing the Analytical Cartridge

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the cartridge holder door and pull it open.
3. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.
5. Close the cartridge holder door and the cartridge/prefilter compartment door.

NOTE: *Test parameters are automatically updated when the RFID is read.*

Replacing the Prefilter

1. Ensure the instrument is in Sleeping state.
2. Open the cartridge/prefilter compartment door. Grasp the handle of the prefilter holder door and pull it open.
3. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
4. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.
5. Close the prefilter holder door and the cartridge/prefilter compartment door.

NOTE: *The prefilter information is automatically updated when the RFID is read.*

Replacing an Empty Reagent Bottle

1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.
   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

Calibration

- Calibration must be performed once, following the installation of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.
- See the *D-100 Operation Manual* or *Quick Guide* for instructions on running the Calibrator Pack.

QC Requirements

In keeping with good laboratory practice, at least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested. Each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

Routine Sample Run

Once calibration is completed, patient samples can be run. See the *D-100 Operation Manual* or *Quick Guide* for instructions on running patient samples.

Running Stat Samples

See the *D-100 Operation Manual* or *Quick Guide* for instructions on running Stat samples.

Certification/Traceability to Reference Material and Method

The D-100 HbA_{1c} test is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-100 HbA_{1c} test is certified by the NGSP as having documented traceability to the reference method of the Diabetes Control and Complications Trial (DCCT), where the relationship between mean blood glucose and risk for vascular complications was established.[12]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure used as the analytical anchor for traceability of HbA_{1c}.[13] This reference method is used to assign IFCC values to secondary reference materials that are used by manufacturers to assign product calibrator values.[14]

In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[15]

The master equations for conversion between IFCC and NGSP[13] and examples of patient results are as follows:

NGSP = (0.09148 × IFCC) + 2.152
IFCC = (10.93 × NGSP) – 23.50

IFCC	NGSP
39 mmol/mol	5.7%
48 mmol/mol	6.5%
64 mmol/mol	8.0%
108 mmol/mol	12.0%

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to ensure acceptable results. For information on reviewing results and troubleshooting chromatography, refer to the *D-100 Operation Manual*.

1. The D-100 must pass calibration.
2. Quality Control values should be in range.
3. Total area of each analysis must range from 50,000–350,000 units. Results should not be reported if the area is outside this range.
4. The peaks HbA1c and A0 must be correctly identified.

5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.5–20.0
IFCC mmol/mol HbA_{1c}	15–195

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[16]
7. Any sample with a combined area of ≥50% in the E, D, S, and/or C windows should be suspected of having a homozygous or double-heterozygous variant, or a variant–β-thalassemia phenotype.[17,18] The HbA_{1c} result should not be reported for these samples.
8. For diagnosis purposes, results should be interpreted in conjunction with the patient's medical history and clinical findings.

Interpretation of "Unknown" and P3 Peaks

Several minor components of hemoglobin A may be resolved and listed as "Unknown" peaks in the sample report. The number of minor "Unknown" peaks and their integrated area will vary from sample to sample. The largest minor components of hemoglobin A are given designated peak window P3.[19] Whole blood specimens that have been stored or shipped outside of manufacturer guidelines may exhibit an increase in the P3 peak area. In all cases, all components of hemoglobin A (e.g., P3, Unknown) are appropriately included in the total area to accurately determine the relative percent of HbA_{1c}. However, any sample with an Unknown and/or P3 peak >10% should be suspected of having a hemoglobin variant.[20]

LIMITATIONS OF THE PROCEDURE

Sample Dilution

The required total area range for the D-100 HbA_{1c} test is 50,000–350,000 units.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines. If the sample area is still outside of the expected range, the sample should be rediluted and rerun to achieve values within the 50,000–350,000 total area count range.

Special Considerations

- The HbA_{1c} test is not intended for analysis of samples collected from newborns.
- The HbA_{1c} test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.
- In cases of rapidly evolving Type 1 diabetes, the increase of HbA_{1c} values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.
- The HbA_{1c} test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA_{1c} reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

- The HbA_{1c} test should not be used to diagnose diabetes in patients with the following conditions:
  - Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA_{1c} values
  - Malignancies or severe chronic hepatic and renal disease.[16,21–23]

Hemoglobin Variants

The most common heterozygous hemoglobin variants (i.e., HbAS, HbAC, HbAD, and HbAE) do not interfere with the test.

In the homozygous and double-heterozygous forms of variant hemoglobins (e.g., SS, CC, SC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

The effect of common hemoglobin variants on the HbA_{1c} result was evaluated based on the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". The relative % bias to the comparative method is summarized in Table 2.

Hemoglobin Variant	Relative % Bias to Comparative Method	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ~9.0%
HbS	–0.6 (± 4.1)	–1.5 (± 1.2)
HbC	–1.3 (± 1.9)	–3.9 (± 2.2)
HbD	–4.7 (± 1.6)	–4.4 (± 2.7)
HbE	–2.7 (± 2.2)	–1.3 (± 1.0)

Table 2: *Results of Hemoglobin Variants Interference Study*

Other abnormal hemoglobin variants have not been evaluated on the D-100 HbA_{1c} test. For the confirmation of any particular hemoglobin variant, alternative methods are required.

Interfering Substances

Interference studies were conducted in accordance with the CLSI EP07-A2 guideline, "Interference Testing in Clinical Chemistry". Each potentially interfering substance was evaluated using specimens with hemoglobin concentrations of approximately 6.5% (48 mmol/mol) and ≥8.0% (≥64 mmol/mol). The following are the results of the interference studies.

- Hemoglobin F concentrations up to 30% do not interfere with the test. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.[24]
- β-thalassemia trait, as indicated by increased HbA_2 concentrations, does not interfere with the test.

Hemoglobin	Relative % Bias to Comparative Method	
	Relative % Bias (StDev) for HbA_{1c} ~6.5%	Relative % Bias (StDev) for HbA_{1c} ~9.0%
HbF	–2.3 (± 0.9)	–3.5 (± 0.6)
HbA_2	–1.3 (± 0.6)	3.4 (± 0.9)

- At physiologically occurring concentrations, there is no interference from labile A_{1c}, carbamylated hemoglobin, or acetylated hemoglobin.[25]
- Common drugs at therapeutic concentrations do not interfere with the test.[25]

- No significant interference is observed from the following endogenous substances up to the stated concentrations:

Endogenous Substance	Concentration	
	Conventional (US) Units	SI Units
Lipemia (Intralipid®)	6000 mg/dL	60 g/L
Conjugated bilirubin	60 mg/dL	712 µmol/L
Unconjugated bilirubin	60 mg/dL	1026 µmol/L
Glucose	2000 mg/dL	111 mmol/L
Rheumatoid factor	750 IU/mL	750 kIU/L
Total protein	21 g/dL	210 g/L

EXPECTED VALUES/REFERENCE RANGE

Diagnosis of Diabetes

The following HbA_{1c} ranges recommended by the American Diabetes Association (ADA) may be used as an aid in the diagnosis of diabetes mellitus.

Hemoglobin A_{1c}		Suggested Diagnosis
NGSP %	IFCC mmol/mol	
≥6.5	≥48	Diabetic[4–6]
5.7–6.4	39–47	Pre-Diabetic[4]
<5.7	<39	Non-Diabetic

Monitoring HbA_{1c} in Diabetic Patients

The following HbA_{1c} ranges may be used for interpretation of results; however, factors such as duration of diabetes, adherence to therapy, and the age of the patient should also be considered in assessing the degree of blood glucose control. These values are for nonpregnant adults.

Hemoglobin A_{1c}		Glycemic Goal[23]
NGSP %	IFCC mmol/mol	
<8	<64	Less Stringent Goal*
<7	<53	General Goal†
<6.5	<48	More Stringent Goal‡

* *May be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, and those with longstanding diabetes in whom the general goal is difficult to attain.*

† *Shown to reduce microvascular and neuropathic complications and, if implemented soon after diagnosis of diabetes, is associated with long-term reduction in macrovascular disease.*

‡ *May be appropriate for selected patients (e.g., those with short duration of diabetes, long life expectancy, and no significant cardiovascular disease) if this can be achieved without significant hypoglycemia or other adverse effects of treatment.*

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-100 HbA_{1c} test was evaluated based on the CLSI EP05-A2 guideline, "Evaluation of Precision Performance of Quantitative Measurement Methods" using a modified study design. HbA_{1c} results were obtained for a series of samples across the clinical range of the test by analyzing each sample in duplicate in 2 runs per day on 3 instruments for 20 days. The study was repeated using 3 different lots of reagents, yielding a total of 720 results per sample over a 60-day period. The results of the precision study are summarized in Tables 3a (NGSP %) and 3b (IFCC mmol/mol).

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.7%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.7	0.7	0.7	0.6	0.7	0.7
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.3	0.2	0.1	0.3	0.6	0.2
Between-Lot	1.3	1.1	1.0	0.6	1.2	0.8
Total Precision	1.5	1.3	1.2	0.9	1.5	1.1
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.8	0.9	1.0	0.9	1.1	0.9
Between-Run	0.0	0.0	0.2	0.0	0.0	0.3
Between-Day	0.5	0.5	0.3	0.3	0.6	0.2
Between-Lot	1.5	0.6	0.0	0.3	1.2	0.2
Total Precision	1.8	1.2	1.0	1.0	1.7	1.0
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (5.1%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.4%)	Control 2 (9.4%)
Repeatability	1.0	1.1	0.9	0.9	1.0	1.0
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.4	0.5	0.5	0.4	0.6	0.5
Between-Lot	1.5	1.5	1.2	1.1	1.6	1.0
Total Precision	1.9	2.0	1.6	1.5	1.9	1.4
Variation Source	Combined % CV by Sample					
	Patient 1 (5.2%)	Patient 2 (6.6%)	Patient 3 (8.1%)	Patient 4 (12.0%)	Control 1 (5.5%)	Control 2 (9.4%)
Repeatability	0.9	0.9	0.9	0.8	0.9	0.9
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.4	0.4	0.4	0.3	0.6	0.3
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	1.5	1.1	0.9	0.7	1.4	0.7
Total Precision	1.7	1.5	1.3	1.2	1.7	1.2

Table 3a: *Results of Precision Study (NGSP %)*

Variation Source	Instrument 1 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (37 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.2	1.0	0.9	0.8	1.2	0.9
Between-Run	0.0	0.0	0.0	0.0	0.0	0.1
Between-Day	0.5	0.4	0.2	0.4	0.9	0.3
Between-Lot	2.2	1.6	1.3	0.7	2.0	1.1
Total Precision	2.5	1.9	1.6	1.1	2.5	1.4
Variation Source	Instrument 2 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.4	1.4	1.3	1.1	1.7	1.2
Between-Run	0.0	0.0	0.3	0.0	0.0	0.4
Between-Day	0.8	0.7	0.4	0.4	1.0	0.3
Between-Lot	2.6	1.0	0.0	0.4	2.1	0.3
Total Precision	3.1	1.8	1.4	1.2	2.9	1.4
Variation Source	Instrument 3 % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.8	1.7	1.3	1.1	1.6	1.2
Between-Run	0.0	0.0	0.0	0.0	0.0	0.0
Between-Day	0.8	0.8	0.7	0.5	1.0	0.6
Between-Lot	2.6	2.3	1.6	1.3	2.6	1.2
Total Precision	3.3	2.9	2.1	1.8	3.2	1.8
Variation Source	Combined % CV by Sample					
	Patient 1 (33 mmol/mol)	Patient 2 (49 mmol/mol)	Patient 3 (65 mmol/mol)	Patient 4 (108 mmol/mol)	Control 1 (36 mmol/mol)	Control 2 (79 mmol/mol)
Repeatability	1.5	1.4	1.2	1.0	1.5	1.1
Between-Run	0.0	0.0	0.1	0.0	0.0	0.0
Between-Day	0.7	0.6	0.5	0.4	1.0	0.4
Between-Instrument	0.0	0.0	0.0	0.0	0.0	0.0
Between-Lot	2.5	1.7	1.2	0.9	2.2	1.0
Total Precision	3.0	2.3	1.7	1.4	2.9	1.5

Table 3b: *Results of Precision Study (IFCC mmol/mol)*

Accuracy

The D-100 HbA_{1c} test was compared to the NGSP Secondary Reference Laboratory (SRL) method in a study based on the CLSI EP09-A2 guideline, "Method Comparison and Bias Estimation Using Patient Samples". The samples were analyzed in singlicate over 4 days using 1 instrument. The range of values on the D-100 HbA_{1c} test was 3.4–19.2% (14–187 mmol/mol) HbA_{1c}. The results of the method comparison are presented in Figures 1a (NGSP %) and 1b (IFCC mmol/mol). The D-100 HbA_{1c} estimated bias compared to the NGSP SRL Method is presented in Table 4.

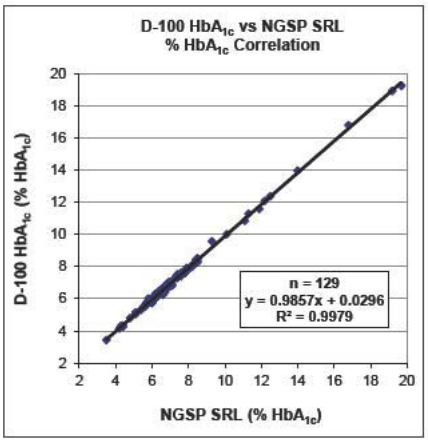


Figure 1a: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (NGSP %)

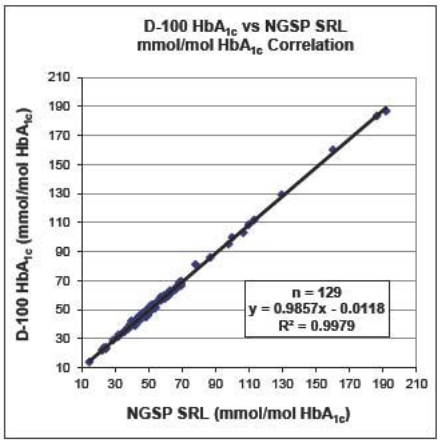


Figure 1b: Correlation of D-100 HbA_{1c} vs NGSP SRL Method (IFCC mmol/mol)

% HbA_{1c}	Bias (% HbA_{1c})	% Bias
5.0	–0.05	–0.85
6.5	–0.07	–0.98
8.0	–0.09	–1.11
12.0	–0.19	–1.57

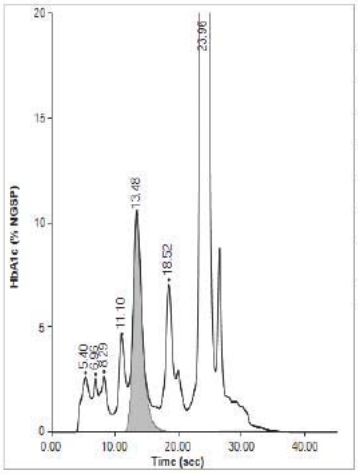
Table 4: D-100 HbA_{1c} Estimated Bias

Linearity

To demonstrate the linearity of the HbA_{1c} measurement throughout the reportable range, a normal and a diabetic HbA_{1c} whole blood patient sample were used to prepare dilutions, and the diluted samples were analyzed with the D-100 HbA_{1c} test. The linearity was assessed following the CLSI EP06-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". The results of the study demonstrate HbA_{1c} linearity from 3.5–20.0% (15–195 mmol/mol) within a maximum measured difference of ± 0.09% (or ± 1.0 mmol/mol) in this interval.

RESULT EXAMPLES

HbA1c: **10.2 %**



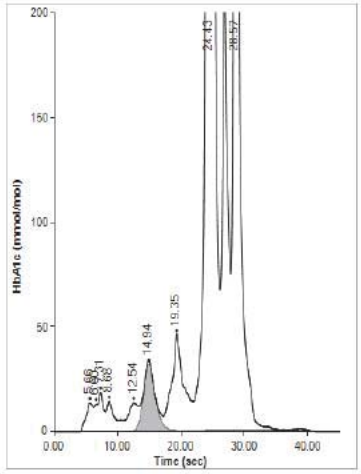
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1 88	—
A1b	6.96	1573.43	1 05	—
F	8.29	2612.14	1.74	—
LA1c	11.10	4492.47	2 99	—
HbA1c	13.48	12431.12	—	10 2
P3	18.52	9123.63	6 06	—
A0	23.96	117434.53	78 03	—

Total Area: 150490

Status: **Held**

Figure 2: Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: **32 mmol/mol**
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	—
Unknown	6.60	391.10	0.23	—
A1b	7.31	994.53	0.59	—
F	8.68	1222.50	0.73	—
LA1c	12.54	1340.98	0.80	—
HbA1c	14.94	3765.08	—	32
P3	19.35	5725.02	3.42	—
A0	24.43	83432.11	49.81	—
S-Window	28.57	69697.38	41.61	—

Total Area: 167509

Status: **Held**

Figure 3: Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

TRADEMARK INFORMATION

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25. Data on file at Bio-Rad Laboratories, Inc.

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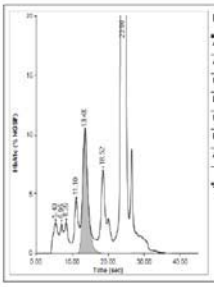
Result Examples

Diabetic Result with an Elevated HbA_{1c} Level

HbA1c: 10.2 %



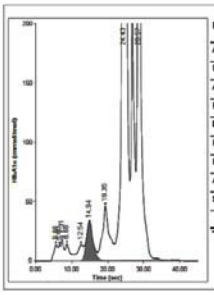
Peak Name	RT	Area	Area%	Concentration (% NGSP)
A1a	5.40	2822.46	1.88	---
A1b	6.06	1573.43	1.05	---
F	8.29	2612.14	1.74	---
LA1c	11.10	4492.47	2.99	---
HbA1c	13.48	12431.12	---	10.2
P3	18.52	9123.63	6.06	---
A0	23.95	117434.53	78.03	---

Total Area: 150490

Status: Held

Non-Diabetic Result with Hemoglobin S Trait, or Sickle Cell Trait (AS)

HbA1c: 32 mmol/mol
5.1 % NGSP



Peak Name	RT	Area	Area%	Concentration (mmol/mol)
A1a	5.66	940.39	0.56	---
Unknown	6.60	391.10	0.23	---
A1b	7.31	994.53	0.59	---
F	8.66	1222.50	0.73	---
LA1c	12.54	1345.96	0.80	---
HbA1c	14.94	3705.06	---	32
P3	19.36	5726.02	3.42	---
A0	24.43	83432.11	49.81	---
S-Window	28.57	69897.36	41.61	---

Total Area: 167509

Status: Held

BIO-RAD Bio-Rad Laboratories

16000920revB
November 2015

Result Review

Any results that do not meet the following criteria should not be reported.

Item	Criteria
Total Area range	50,000–350,000
Quality Control	Values should be in range
HbA_{1c} reportable range	• NGSP: 3.5–20.0% • IFCC: 15–195 mmol/mol • Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.
HbF	≤30%
Labile A_{1c} (LA1c)	No interference
Carbamylated hemoglobin (CHb)	• No interference • CHb elutes in the LA1c window
P3 peak	≤10%
Heterozygous hemoglobins E, D, S, and C	No interference
E, D, S, and/or C windows	Combined area <50%
"Unknown" peaks	"Unknown" peak ≤10%

Test Components

USE	REF	Symbol	Description
290-1004		ANLT CRTR CAL PACK	Analytical Cartridge/Calibrator Pack
290-1006		CAL PACK	Calibrator Pack
290-1007		PRE FIL	Prefilters (5 per package)
290-1008		CLN TUBE	Cleaning Tube
290-1009		SAMP DIL	Sample Diluent
290-1010		BUF A	Elution Buffer A
290-1011		BUF B	Elution Buffer B
290-1012		WSH SOLN	Wash Solution

D-100™ HbA_{1c} Quick Guide

CE IVD

US: Rx Only

- *This Quick Guide is for reference use only; for detailed information, see the Instructions For Use and Operation Manual.*
- *Consider any materials of human origin as infectious and handle them using appropriate biosafety procedures.*
- *Wear personal protective equipment while handling all reagents and samples and while operating the D-100 system.*

Sample Preparation

HbA_{1c} Calibrator Pack:

- Contains Conditioner, Calibrator Level 1, and Calibrator Level 2.
- No manual preparation required. Can be used immediately after removing from refrigerator.
- Once reconstituted by system, stable for 24 hours at 2–8 °C; do not freeze.

Liquichek™ Diabetes Controls:

- After opening vial, stable for 14 days at 2–8 °C.
- Dilute 1:200 prior to analysis (5 µL of control in 1.0 mL of Sample Diluent).

Lyphochek® Diabetes Controls:

- Reconstitute each vial with 0.5 mL of DI water.
- Allow to stand for 5–10 minutes; swirl gently to dissolve.
- Stable for 7 days at 2–8 °C.
- Dilute 1:300 prior to analysis (5 µL of control in 1.5 mL of Sample Diluent).

Whole blood samples:

- Samples should be collected in vacuum collection tubes containing anti-coagulant. For detailed information, see the *Instructions For Use*.
- No sample preparation required.
- If abnormal tube type or height of sample is ≤1 cm, sample may need to be prediluted 1:300 prior to analysis (5 µL of sample in 1.5 mL of Sample Diluent).

Replacing the Reagents

After installing the bottles, the reagents are stable for 90 days at 15–35 °C. A reagent bottle can be removed at any time, except when the bottle is "In Use" in Running state.

1. Remove the empty bottle.
   NOTE: *If the bottle is not empty, touch the reagent indicator in the consumables panel, then touch* ***Remove*** *to depressurize the bottle.*
2. Install a new bottle. The reagent information is automatically updated.

Replacing the Prefilter

Replace the prefilter at 90 days or 2000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the prefilter holder door.
3. Remove the old prefilter.
4. Insert the new prefilter.
5. Close the prefilter holder door. The prefilter information is automatically updated.

Replacing the Analytical Cartridge

Replace the analytical cartridge at 90 days or 10,000 tests.

1. Ensure the instrument is in Sleeping state (**Utilities/Manual Operations/General/Sleep**).
2. Open the cartridge holder door.
3. Remove the old cartridge.
4. Insert the new cartridge.
5. Close the cartridge holder door. Test parameters are automatically updated.

Calibration

Calibration must be performed once, following the installation of every new analytical cartridge.

1. Ensure the instrument is in Sleeping or Standby state.
2. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
3. Insert Diabetes Controls (in barcoded adapters) in positions 1–3 and insert the D-100 Calibrator Pack in the dedicated position, with the barcodes facing you.
4. Touch ⏏ **Load**.
5. Touch Calibrate Now.

NOTE: *Racks containing patient samples can be placed in the input area to be automatically processed after calibration has passed.*

6. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
7. Remove the samples from the Stat rack.

Routine Sample Run

1. Check the message panel for red or yellow messages and address as needed.
2. Insert Diabetes Controls (in barcoded adapters) followed by patient samples in racks.
   NOTE: *At least one diabetic and one non-diabetic control specimen should be tested each day patient samples are tested.*
3. Place the rack(s) in the input area.
4. Touch Run ►.
5. Load additional racks of samples during the run as needed.
6. Remove the processed racks from the output area.

NOTE: *When the run is complete, the instrument goes to Standby state for approximately 2 hours.*

Running Stat Samples

The D-100 System will analyze Stat samples after completing the last aspirated sample from the routine run.

1. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen.
2. Insert the sample(s) (i.e., primary sample tubes or prediluted samples in microvial adapters) in positions 1–3 of the Stat rack with the barcode(s) facing you.
3. Touch ⏏ **Load** to process the Stat samples.
4. Touch ⏏ **Open** after all samples in the Stat Area have been processed.
5. Remove the samples from the Stat rack.

NOTE: *If the instrument is not in Running state, Stat samples can be run by loading the Stat area, touching* ⏏ ***Load****, and touching* Run ►.

Viewing Results

1. Touch the **Results** tab.
2. To view a sample result in detail, touch the result row.
3. To filter the results in the table, use the Filter buttons.
   - The filter currently in use is indicated to the right of the ⏷ button.
   - Flagged results can be accessed from the **Home** screen by touching Flagged.

All : Displays results for All processed samples.

⚑ : Displays results for Flagged samples only.

★ : Displays results for samples meeting the Favorite filter criteria only.

⏷ : Opens the Filter dialog box for more filtering options.

Releasing or Rejecting Results

To release or reject results in the Results screen:

1. Select the corresponding checkbox for each sample result you want to release.
2. Touch ⇗ **Release** at the bottom of the screen. The result(s) status indicates ⇗.
3. Select the corresponding checkbox for each sample result you want to reject.
4. Touch ⊘ **Reject** at the bottom of the screen. The result(s) status indicates ⊘.

To release or reject results in the Result Details screen:

1. In the **Results** table, touch the sample result row to open the Result Details screen.
2. After reviewing the details, touch ⇗ **Release** to release the result or ⊘ **Reject** to reject the result.
3. The system updates the result status (Released or Rejected) accordingly and displays the next sample result.

D-100™ Hemoglobin Testing System

REF 290-1000

BIO-RAD

D-100™ Hemoglobin Testing System

Operation Manual

USE D-100™ Software Version 1.0

CE

IVD

US: Rx Only

October 2015
LB000341revB

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EC REP FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430 Marnes-la-Coquette, 33-1-4795-6000

Translations

Product documents are provided in additional languages on electronic media.

Symbols Lexicon

CE European Conformity	EC REP Authorized Representative in the European Union	Manufacturer	Consult Instructions for Use
IVD For In Vitro Diagnostic Use	REF Catalog Number	Date of Manufacture	Temperature Limit
SN Serial Number	USE For use with	Rx Only Prescription Use Only	UDI-DI Unique Device Identifica ion-Device Identifier
Biohazard	Caution	Fuse	Sharp Biohazard
Protective Conductor Terminal	Alternating Current (AC)	Ground Terminal	Power On
Power Off	Humidity Range	Moving Parts	Waste from Electrical and Electronic Equipment
ANLT CRTR Analy ical Cartridge	PRE FIL Prefilter		

Bio-Rad Laboratories, Inc.
Clinical Diagnostics Group
4000 Alfred Nobel Drive
Hercules, California 94547 USA

Technical Assistance

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

Outside the USA: Contact your regional Bio-Rad office. Go to www.bio-rad.com for contact information.

Trademark Information

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Lyphochek and Unity Real Time are registered trademarks of Bio-Rad Laboratories, Inc.

All other trademarks are the property of their respective companies.

Naming Conventions

Abbreviated text is used in place of full names or descriptions. The following is a list of naming conventions used in this manual and/or in the user interface.

CONVENTION	DESCRIPTION
D-100	D-100 Hemoglobin Testing System
A1c	Hemoglobin A_{1c}

Graphic Conventions

Throughout the text, icons and signal words appear where the information warrants special attention. The following conventions are used in this manual.

CONVENTION	DESCRIPTION
NOTE:	Note statements alert you to important information relevant to the current subject matter.
	**Warning:** This icon directs you to follow specified instructions where safety is involved.
	**Caution:** This icon directs you to follow specified instructions to prevent electrical shock.
	**Biohazard:** This icon alerts you to a potentially biohazardous condition.
	**Sharp Biohazard:** This icon alerts you to a potentially biohazardous sharp condition.

GENERAL SAFETY INFORMATION

- The D-100 System was designed, tested, and certified to meet various safety standards.
- These safety certifications do not extend to other equipment or accessories not similarly certified, even when connected to the D-100 System.
- This system is safe to use when operated in accordance with the instructions in this operation manual.
- Unauthorized modification or alteration of this system voids the warranty, voids the certifications, and creates a potential safety hazard for the operator.
- Read through and familiarize yourself with the contents of this operation manual before using the system for the first time.

HAZARDS

- The D-100 System is designed to operate safely and effectively when used in the manner prescribed by the manufacturer.
- If the D-100 or any of its associated components are used in a manner not specified by the manufacturer, the inherent protection provided by the equipment may be impaired.
- Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by the use of this system for purposes other than for which it is intended or by unauthorized modifications of the system.
- Service of the D-100 System should be performed only by qualified Bio-Rad personnel.
- The D-100 System should be moved only by qualified Bio-Rad personnel.
- Although the D-100 System provides some inherent protection against hazards, special precautions should be taken to avoid harm to the operator or equipment.

Biohazards

The following activities may expose the operator to biohazardous conditions:

- Handling samples, calibrator packs, and controls
- Cleaning spills
- Handling and disposing of solid and liquid waste
- Performing maintenance procedures
- Replacing system parts

Biohazard General Precautions

To protect yourself from potentially biohazardous materials, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location:

- Always wear laboratory gloves, coat, and goggles or safety glasses with side shields.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials. Seal wounds with waterproof bandages under protective clothing (e.g., gloves, sleeves). Individuals with cuts or abrasions that cannot be completely sealed under protective clothing should not handle any potentially infectious materials.

- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Remove wristwatches and jewelry before working at the bench to facilitate hand washing and prevent puncturing of gloves.
- Store all potentially infectious materials in unbreakable, leakproof containers.
- Before leaving the laboratory or clean-up room for non-laboratory areas, remove protective clothing and leave it in the laboratory or clean-up room; wash your hands thoroughly.
- Do not use a gloved hand to write, answer the telephone, turn on a light, or touch anything that other people may touch without gloves.
- Synthetic gloves, such as nitrile, neoprene, and vinyl, are recommended because they are effective and contain no natural latex ingredients associated with latex glove allergic reaction.
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.
- Keep only materials needed for the day's procedures in the work area to prevent contamination of non-laboratory materials.
- Materials that cannot be properly decontaminated should not be exposed to potentially infectious material.
- Upon completion of the operations involving biohazardous materials, decontaminate the work area with an appropriate disinfectant (e.g., 1:10 dilution of household bleach).
- If material becomes contaminated with dried blood or other potential biohazard, decontaminate it and clean up any solid material before completing decontamination. The dried blood should be wetted and softened with diluted bleach (1:10 dilution) or detergent disinfectant. Carefully remove to prevent scattering potentially infectious material. After removal, disinfect the cleaned surfaces.

Biohazard Specific Precautions

The following precautions are necessary when handling and disposing of potentially biohazardous material:

- Solid and liquid waste from the D-100 System should always be considered potentially biohazardous and should be handled accordingly.
- All patient samples are potentially biohazardous and should be handled accordingly, using Universal Precautions.
- Liquid waste should be decontaminated on site if possible. It is recommended to mix liquid waste with household bleach (5% sodium hypochlorite) at a ratio of 1 part bleach to 10 parts waste. Let the mixture stand for at least 30 minutes before emptying.
- Treat all calibrator packs and controls as potentially biohazardous materials and handle accordingly.

Disposal of Biohazardous Materials

Dispose of the following potentially contaminated materials in accordance with local, regional, and national laboratory regulations:

- Clinical samples
- Reagents
- Calibrator packs
- Controls
- Used sample vials and other consumables (e.g., cartridges, prefilters) that may be contaminated

Sharp Biohazard

The sample probe is very sharp. Use caution when handling to avoid injury. The used sample probe should be considered potentially biohazardous; discard according to the laboratory standard operating procedures for biohazardous sharps.

Chemical Hazards

D-100 assay components may contain potentially harmful chemical materials. Follow all instructions for handling, storage, and disposal as described in the applicable assay Instructions For Use.

- Consult the Safety Data Sheets (SDS) for specific safety information.
- Do not smoke, eat, or drink in areas where reagents are handled.
- Wear personal protective equipment while handling all reagents.
- Chemical reagents should be handled in accordance with Good Laboratory Practices.
- Use caution when handling the following reagent:

CLN **TUBE** **Cleaning Tube**

Contains 2-aminoethanol (141-43-5), Subtilisin (9014-01-1)

Danger

H314	Causes severe skin burns and eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P311	If exposed or if you feel unwell: call a poison center or doctor/physician.
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

Electrical Hazards

NOTE: *The main power cord at the rear of the instrument serves as the primary power disconnect. Do not position the system where it is difficult to disconnect the main power cord.*

- Do not remove instrument covers. There are no user-serviceable parts inside. Refer all servicing to Bio-Rad service personnel.
- Always follow basic safety precautions when using this instrument to reduce the risk of injury, fire, or electrical shock.
- Do not perform Hipot Testing (i.e., Dielectric Withstand Testing) on the instrument without contacting Bio-Rad Technical Service for assistance. The instrument power supply requires a temporary modification for this test to prevent damage to the unit.

Electrical and Electronic Waste Hazards

The Waste Electrical and Electronic Equipment (WEEE) Regulations implement provisions of the European Parliament and Council Directive 2002/96/EC aimed at reducing the amount of EEE waste going for final disposal. As the producer, Bio-Rad Laboratories, Inc. has specific instructions for the recovery of this instrument at the time of end of use. Please refer to the **EU Recycle Program** at www.bio-rad.com for the process applicable to your region.

Environmental Hazards

In the event of a flood or other natural disaster, contact Bio-Rad Technical Service before resuming use of the instrument.

Warning Regarding Cybersecurity on the D-100 System

Please observe the following precautions to protect the integrity of your D-100 System:

1. Do not attempt to install any third-party software.
2. Do not attempt to access the World Wide Web.
3. Before using any removable media (e.g., USB flash drive), ensure the media is free of malware.
4. If your D-100 System is connected to a network, it is your laboratory's responsibility to monitor and protect the network.

EQUIPMENT AUTHORIZATION AND REGULATORY COMPLIANCE

Federal Communications Commission (FCC) Compliance

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

This device complies with FCC RF radiation exposure limits set forth for general population (uncontrolled exposure). This device must be installed to provide a separation distance of at least 20 cm from all persons and must not be colocated or operating in conjunction with any other antenna or transmitter.

CAUTION: This equipment may not be modified, altered, or changed in any way without signed written permission from BIO-RAD Laboratories. Unauthorized modification may void the equipment authorization from the FCC and will void the BIO-RAD Laboratories warranty.

Industry Canada (IC) Compliance

WARNING TO USERS IN CANADA

This device complies with Industry Canada license-exempt RSS standard(s), and with ICES-003 (Class B) for its non-RF parts. Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.

Under Industry Canada regulations, this radio transmitter may only operate using an antenna of a type and maximum (or lesser) gain approved for the transmitter by Industry Canada. To reduce potential radio interference to other users, the antenna type and its gain should be so chosen that the equivalent isotropically radiated power (e.i.r.p.) is not more than that necessary for successful communication.

This device complies with Industry Canada RF radiation exposure limits set forth for general population (uncontrolled exposure). This device must be installed to provide a separation distance of at least 20 cm from all persons and must not be colocated or operating in conjunction with any other antenna or transmitter.

Contents

1 Introduction

1.1 General Description

The Bio-Rad D-100™ Hemoglobin Testing System performs automated analysis of whole blood samples within the clinical laboratory. With advanced automation and high-throughput operation, the D-100 generates high-quality results using high-performance liquid chromatography (HPLC) technology with an efficient and flexible testing workflow.

The D-100 System consists of a fully-integrated standalone workstation of automated HPLC instrumentation that includes an onboard computer. The software operating the instrumentation is controlled through a graphical user interface via an onboard touchscreen.

The D-100 is intended for use only with Bio-Rad D-100 assays. It must be operated by trained laboratory staff within a conventional laboratory environment.

Figure 1-1: *D-100 Hemoglobin Testing System*

Figure 1-2: *D-100 with Optional Internal Printer*

1.2 Principles of Operation

The D-100 uses the principles of high-performance liquid chromatography (HPLC). A high-pressure pumping system delivers a buffer solution to an analytical cartridge and detector. Whole blood samples undergo an automatic hemolysis and dilution process before being introduced into the analytical flow path. Prediluted samples are identified based upon the use of a microvial adapter in the sample rack, and the automatic dilution step is omitted.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge where the hemoglobin species are separated based upon their ionic interactions with the cartridge material and the buffer gradient. The separated hemoglobin species then pass through the flow cell where changes in the absorbance are measured at 415 nm and recorded as a digital chromatogram.

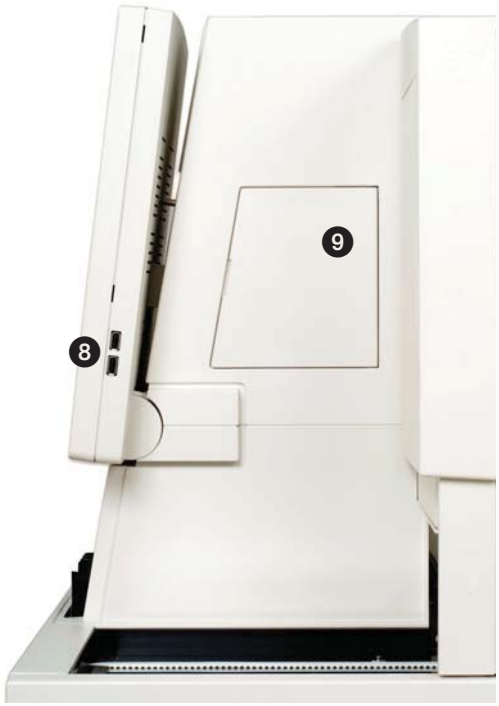
The software performs an analysis of the hemoglobin peaks in the chromatogram, recording information including retention time, peak area, and relative area percent. Any peaks that are identified as the target analyte(s) are calibrated before generating a sample report and chromatogram for each sample. The software includes an optional feature (Advisor) that compares the sample report against a set of rules that are programmed to take user-specified actions.

2 System Description

2.1 D-100 System Components

Figure 2-1: *D-100 Front View*

Figure 2-2: *D-100 Right Side View*



No.	Name	Function
1	Touchscreen	The liquid crystal display (LCD) detects human touch to interact with the instrument software. To optimize visibility, the angle of the touchscreen can be adjusted by tilting the top of the screen forward or back. See Chapter 4 for a detailed description of the user interface.
2	Internal Printer	The optional thermal printer prints sample results and reports. See Section 2.8.
3	Soft Power Button 	The soft power button activates the instrument from the standby power mode to full operating power. The button LED is green when activated.
4	Cartridge/Prefilter Compartment	This compartment houses the sample analysis components. See Section 2.7.
5	Rack Handler	The rack handler consists of the Sysmex® rack processing components. See Section 2.4.
6	Stat Area	The Stat Area includes components to process the Calibrator Pack and other special samples. See Section 2.2.
7	Reagent Compartment	This compartment houses the assay reagents. See Section 2.3.

No.	Name	Function
❽	USB Ports	2 ports accommodate USB storage devices for exporting/storing data.
❾	Probe Compartment	This compartment houses the sample probe and supporting components. See Section 2.5.
❿	Low-Pressure Filter Compartment (not shown)	This compartment houses the sample dilution components and low-pressure filter. See Section 2.6.

2.2 Stat Area

Figure 2-3: *Stat Area with Stat Rack in the Loading Position*

Figure 2-4: *Stat Rack with Controls and Calibrator Pack in the Loading Position*

The Stat Area is used to run the Calibrator Pack, Controls, urgent patient samples, and the Cleaning Tube via the Stat rack. The Stat rack holds the Calibrator Pack and/or up to 3 samples in microvials or primary tubes. The Stat rack is anchored to the instrument, but can be removed for cleaning. See Section 3.6 for more information regarding the Stat rack.

The Stat Area has its own barcode reader to scan the barcodes on the adapters/tubes in the Stat rack for identification purposes. See Section 3.5 for information regarding barcode labels.

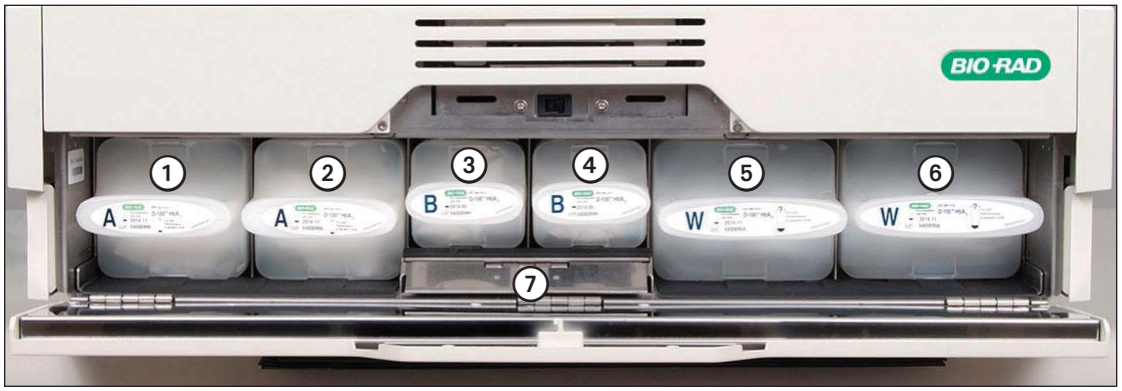
See Chapter 5 for instructions on running the Calibrator Pack (Section 5.3), Controls (Section 5.4), and Stat samples (Section 5.6). See Section 7.8 for instructions regarding running the Cleaning Tube.

2.3 Reagent Compartment

The reagent compartment houses the assay buffers (used to form the elution gradient) and wash solution (used for diluting samples and rinsing the sample probe). The system uses positive pressure to fill each bottle with air, squeezing the reagent from the interior bag.

The D-100 holds 2 bottles of each reagent; however, only one bottle is in use at a time. The system automatically switches to the second bottle when the first is empty, allowing the empty bottle to be removed without stopping the run.

Figure 2-5: *Reagent Compartment (Door Open)*

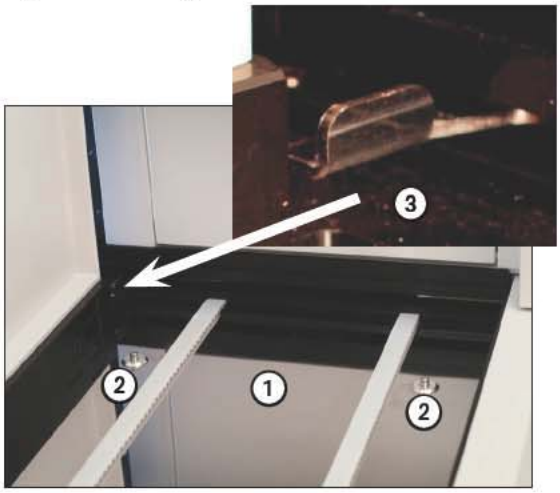


No.	Name	Function
1	Buffer A Bottle 1 Compartment	Holds 1 bottle of Elution Buffer A.
2	Buffer A Bottle 2 Compartment	Holds 1 bottle of Elution Buffer A.
3	Buffer B Bottle 1 Compartment	Holds 1 bottle of Elution Buffer B.
4	Buffer B Bottle 2 Compartment	Holds 1 bottle of Elution Buffer B.
5	Wash Bottle 1 Compartment	Holds 1 bottle of Wash Solution.
6	Wash Bottle 2 Compartment	Holds 1 bottle of Wash Solution.
7	Leak Tray Compartment	Holds tray to collect any fluid leaking from the system.

2.4 Rack Handler

Figure 2-6: *Rack Input and Output Areas*

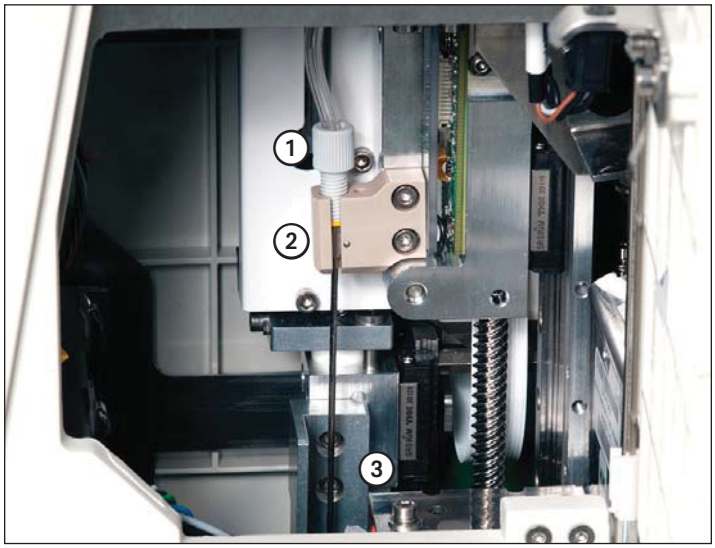
Figure 2-7: *Stopper Pins and Shuttle*



No.	Name	Function
1	Rack Input Area	Sysmex racks are placed in the right rack input area; the conveying belts move the racks to the sampling area.
2	Stopper Pins	Racks are placed anywhere between the pins and the front of the instrument. The stopper pins retract to release the rack into the shuttle.
3	Shuttle	The shuttle moves the rack into and out of the sampling area.
4	Tube Spinner (not shown)	The tube spinner rotates sample tubes and microvials in the rack to check for barcodes.
5	Barcode Reader (not shown)	The barcode reader scans the barcodes from the racks, sample tubes, microvials, and/or microvial adapters. The information is included with the sample result for identification purposes. See Section 3.5 for information regarding barcode labels.
6	Rack Output Area	The forward pusher moves the Sysmex racks away from the sampling area to the left rack output area after processing is complete.

2.5 Probe Compartment

Figure 2-8: *Probe Compartment (Door Open)*

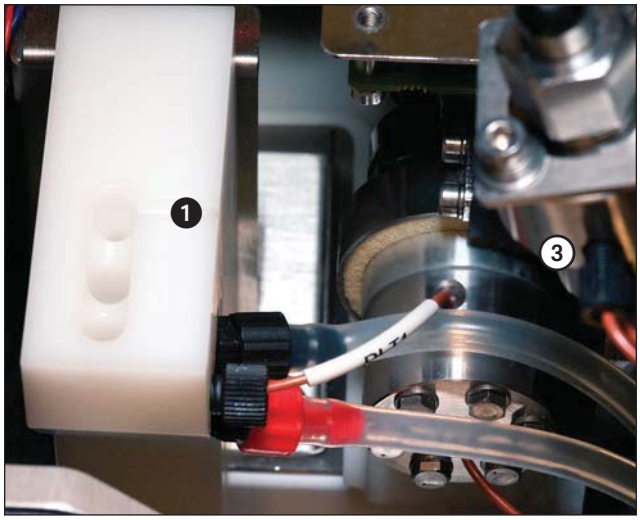


NOTE: *The probe door is interlocked. When open, the interlock switch shuts off power to the probe assembly.*

No.	Name	Function
❶	Sample Probe	The sample probe pierces each primary tube to release the vacuum and to aspirate sample from the tube. The probe then dispenses the sample with a measured amount of diluent (Wash Solution) into the dilution well. The sample probe and dilution well are rinsed between samples to prevent cross-contamination. The sample probe also pierces the microvials to aspirate sample.
❷	Probe Carrier	The probe carrier secures and moves the probe.
❸	Tube Holder	The tube holder detects the sample tube/adapter in the rack, aligns it, and stabilizes the tube/microvial during probe piercing.

2.6 Low-Pressure Filter Compartment

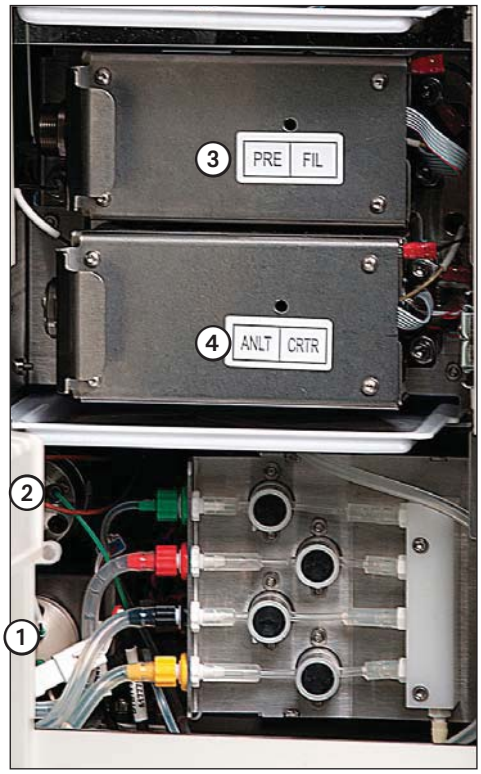
Figure 2-9: *Low-Pressure Filter Compartment (instrument cover removed for visibility)*



No.	Name	Function
❶	Dilution Manifold	The dilution manifold includes the dilution well (front), overflow well (center), and wash well (rear). The dilution well is where the sample is diluted before introduction into the analytical path. The wash well is where the sample probe is rinsed. The dilution manifold is not user-accessible.
❷	Dilution Pump (not shown)	The dilution pump consists of 2 syringes. The sample is aspirated from the tube/microvial via the sample probe using the first syringe. The sample is dispensed into the dilution well where it is diluted to the required concentration.* The second syringe introduces the diluted sample into the fluid circuit through the low-pressure filter. ***NOTE:** *The dilution step is omitted for prediluted samples in microvials.*
❸	Low-Pressure Filter	The low-pressure filter protects the downstream valves from sample particulates. This filter is replaced only by Bio-Rad service personnel.

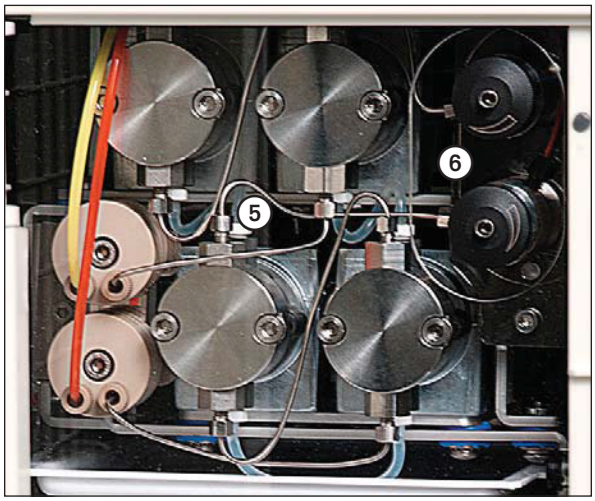
2.7 Cartridge/Prefilter Compartment

Figure 2-10: *Cartridge/Prefilter Compartment (Door Open)*



No.	Name	Function
❶	Rotary Valve	The rotary valve directs the flow of the sample into the fluid circuit and into the sample loop. It has two possible positions: • The first position creates a fluidic path between the second syringe and the dilution well through the low-pressure filter. • The second position creates a fluidic path between the second syringe and the injection valve.
❷	Injection Valve	The injection valve contains two 5-µL internal sample loops and switches from load position to inject position. The diluted sample is loaded into one sample loop and then injected into the analytical flow path. While the sample is being injected, the second loop is flushed with Wash Solution and loaded with the next sample.
❸	Prefilter Holder	The prefilter holder houses the prefilter, which protects the analytical cartridge from sample particulates. The LED indicates whether the prefilter holder door is open (green) or closed (red).
❹	Cartridge Holder	The cartridge holder houses the analytical cartridge, which is responsible for the hemoglobin separation. An attached Peltier device provides temperature control. The LED indicates whether the cartridge holder door is open (green) or closed (red).

Figure 2-11: *High-Pressure Pumps*



No.	Name	Function
❺	High-Pressure Pumps	There are two dual-piston reciprocating pumps that generate the buffer gradient. • Pump A (upper) delivers Elution Buffer A. • Pump B (lower) delivers Elution Buffer B.
❻	Purge Valves	Each pump has a purge valve that allows manual priming of the buffer inlet line. • Purge valve A (upper) is used to prime Elution Buffer A. • Purge valve B (lower) is used to prime Elution Buffer B.
❼	Detector (not shown)	The detector measures the absorbance of the sample constituents at 415 nm.

2.8 Internal Printer

Figure 2-12: *Printer (Door Open)*

No.	Name	Function
1	Paper Feed Button	Press this button to advance the paper.
2	Printer Settings Button	For Service use only.
3	Printer Power LED	Green light indicates the printer is on.
4	Printer Door LED	Red light indicates the printer paper door is open.
5	Printer Error LED	Red light indicates a printer problem. • 1 flash = Printer memory is full and cannot receive additional data. • 2 flashes = Printer head temperature too high. • 3 flashes = Entering configuration mode. • Permanent blink = No paper or paper door is open. • 1 long flash and 1 short flash = Printer head failure.
6	Printer Paper Door Latch	Push down the latch to open the printer paper door.
7	Printer Paper Input Well	The paper roll is inserted in the input well.
8	Printer Paper Output Well	The printout rolls up inside the output well.

2.9 D-100 Rear Components

Figure 2-13: *D-100 Left Rear View*

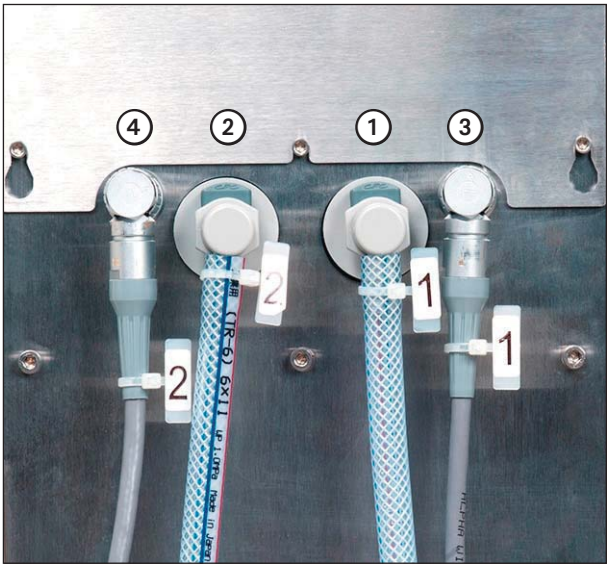
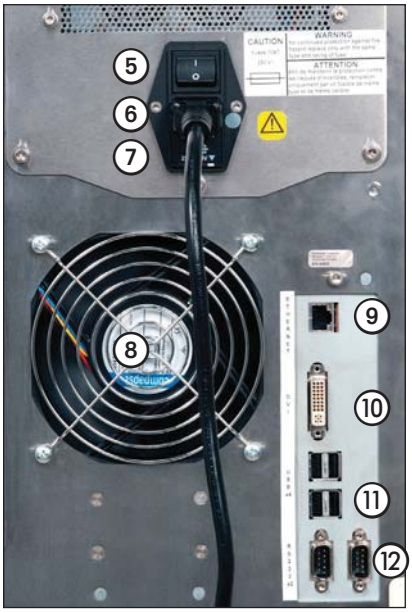


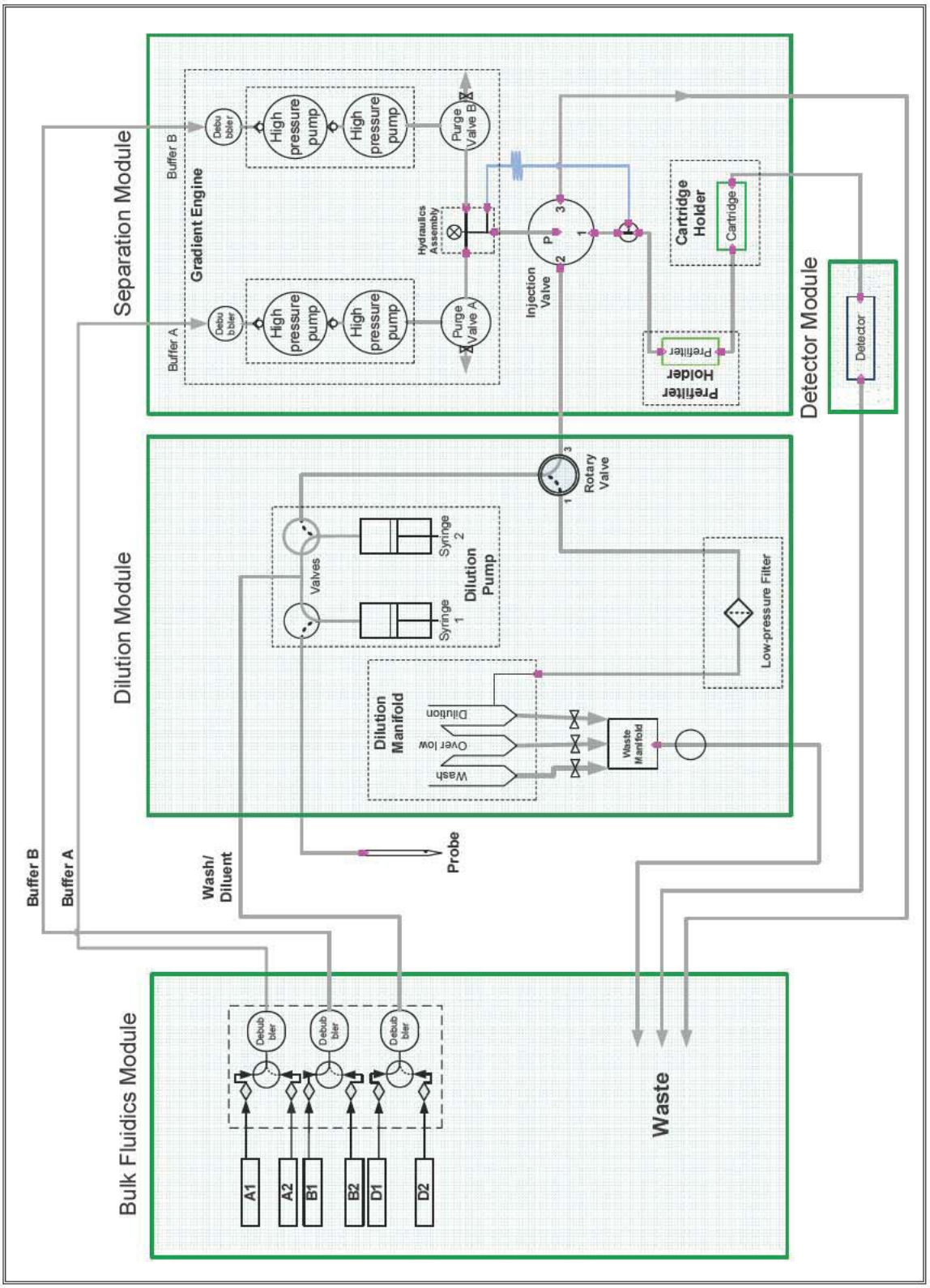
Figure 2-14: *D-100 Right Rear View*



No.	Name	Function
❶	Waste Port 1	This port is for connection of the external waste tubing to Waste Bottle 1 or to a drain.
❷	Waste Port 2	This port is for connection of the external waste tubing to Waste Bottle 2 or to a drain.
❸	Waste Sensor Port 1	This port is for connection of the level sensor cable to Waste Bottle 1, or a waste sensor connector when the lab drain is used.
❹	Waste Sensor Port 2	This port is for connection of the level sensor cable to Waste Bottle 2, or a waste sensor connector when the lab drain is used.
❺	Power Switch	The power switch controls power to all system components.
❻	AC Power Input	The power input allows connection of a 3-conductor modular cord with ground to a suitable power source (110 VAC or 220 VAC).
❼	Fuse Holder	The 2 main power fuses provide over-current protection.
❽	Main Fan	The fan expels warm air away from the components.
❾	LAN Port	This port is for connection to a Local Area Network.
❿	DVI Port	Digital visual interface (DVI) port for connection to an external display.
⓫	USB Ports	These 4 ports are for connection of USB storage devices, printer, keyboard, or mouse.
⓬	Serial Ports	These 2 ports are for connection of an RS-232 cable to an LIS interface.

2.10 D-100 Fluid System

Figure 2-15: *D-100 Fluidics Diagram*



3 Installation

NOTE:

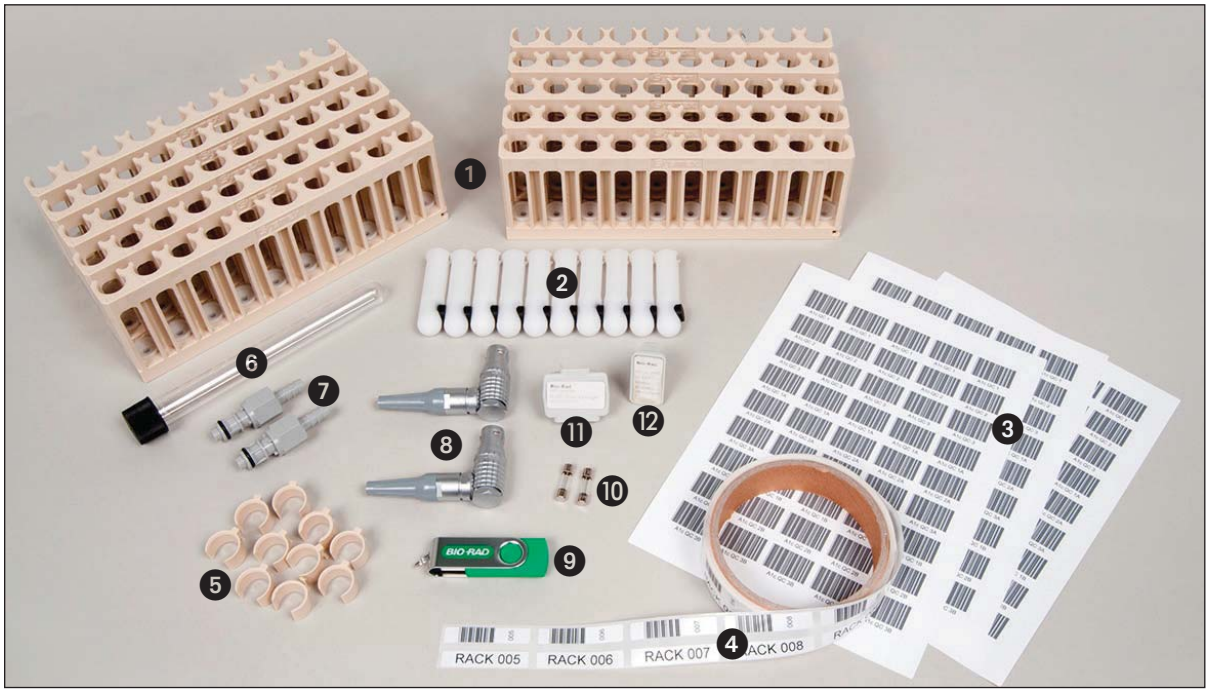
- *Installation of the D-100 System should be performed only by an authorized Bio-Rad representative; installation by any other person invalidates the system warranty.*
- *To move the D-100 System, contact your local Bio-Rad office for assistance.*

3.1 Installation Requirements

1. Choose a location for the system away from direct sunlight and relatively dust-free.
2. Room temperature should be 15–35 °C.
3. The bench or table should have a flat, level surface free from vibrations and capable of supporting 121 kg (266 lb).
4. The system requires a minimum benchtop space (W x D x H) of 66 x 65 x 73 cm (26 x 26 x 29 in.).
5. Maintain a minimum clearance of 13 cm (5 in.) on the left side, right side, and rear of the instrument.
6. A grounded electrical outlet should be within 1.8 m (6 ft) of the system. The maximum power consumption of the system is 1100 VA. See Appendix B for specifications.

3.2 Accessories

Figure 3-1: *D-100 Accessories*



No.	Description	Qty
1	Sysmex Racks	10
2	Microvial Adapters	10
3	Microvial Adapter QC Barcode Labels (3 sheets of 3 levels)	3
4	Sysmex Rack Barcode Labels (1 roll of 100)	1
5	13-mm Sysmex Rack Inserts, preinstalled in racks	100
6	Sample Probe	1
7	Waste Tubing Bottle Connector	2

No.	Description	Qty
8	Waste Sensor Connector	2
9	8GB USB Flash Drive	1
10	Spare Fuses	2
11	Utility Cartridge, shipped installed on instrument	1
12	Utility Prefilter, shipped installed on instrument	1
13	D-100 Operation Manual with Multi-Language CD (not shown)	1

3.3 Waste Bottles

There are 2 external waste bottles included with the D-100 to collect the liquid waste from the instrument. The external waste bottle tubings and level sensor cables are connected to the rear of the instrument (see Figure 2-13). See Section 7.1 for instructions on emptying the waste bottles.

NOTE: *Alternatively, the external waste tubing can be connected to a laboratory drain.*

Figure 3-2: *Waste Bottle*

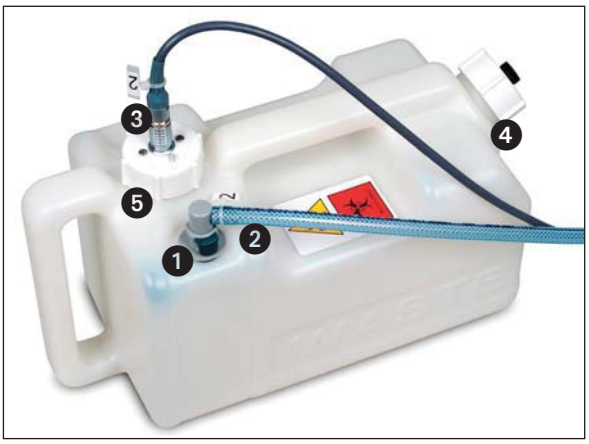
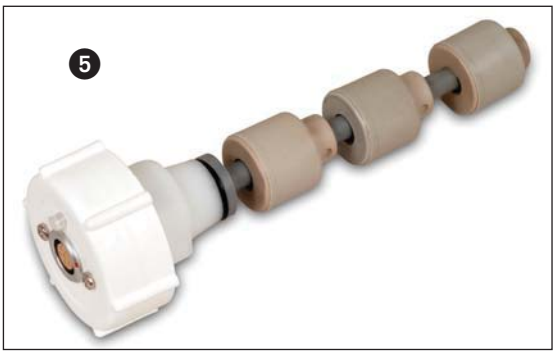


Figure 3-3: *Waste Level Sensor*



1. External Waste Quick-Disconnect
2. External Waste Tubing
3. Level Sensor Connector
4. Waste Bottle Cap
5. Level Sensor

3.4 Sysmex® Racks

Sysmex racks are used to hold all routine samples to be tested by the D-100. The racks include rotary bottoms in each tube position to allow rotation of primary sample tubes for barcode reading. A maximum of 10 samples can be loaded into each rack. Sysmex racks are placed in the rack input area with the Sysmex logo facing you.

Figure 3-4: *Sysmex Rack, Front View*

NOTE: *If you use sample racks from another system that do not include rotary bottoms, tube spinning must be disabled. See Section 4.7.6.*

3.4.1 Sysmex Rack Inserts

The Sysmex racks are designed to hold 16-mm sample tubes. For proper alignment of smaller diameter tubes (i.e., 12 mm, 13 mm, and 14 mm), plastic inserts must be inserted into the racks. 13-mm inserts are included in the D-100 Accessories Kit; other insert sizes can be purchased from Bio-Rad.

Figure 3-5: *Insert for Small Diameter Tubes*

3.4.2 Microvial Adapters

If the sample is in an abnormal size/type tube, or if the height of the sample in the tube appears to be ≤1 cm, then the sample must be prediluted in a 1.5-mL microvial prior to analysis. See the assay Instructions For Use for Specimen Preparation instructions.

For proper alignment of 1.5-mL microvials, microvial adapters must be used in the rack. See Figure 3-6.

Figure 3-6: *Microvial Adapter with Prediluted Sample*

The D-100 recognizes the microvial adapter by sensing the adapter's attached magnet and bypasses the sample dilution process. The microvial adapter must be positioned in the Sysmex rack so that the magnet faces the back of the rack.

Figure 3-7: *Adapters Inserted in Sysmex Rack, Rear View*

3.5 Barcode Labels

To correctly identify the Sysmex racks and samples, barcode labels are required in the proper locations.

NOTE: *A starter set of Sysmex rack and QC microvial adapter barcodes is provided with the system. Additional barcodes can be purchased from Bio-Rad.*

- Sysmex rack barcodes must be affixed on the rear of the rack, between the first and second sample tube positions, higher than 18 mm from the bottom of the rack; see Figure 3-8 for correct label placement. The corresponding human-readable rack number label should be affixed on the front of the rack (see Figure 3-4).

  Figure 3-8: *Rack Barcode Label on Rear of Sysmex Rack*

- QC barcodes must be affixed to the microvial adapters above the magnet with the barcode lines running horizontally, so that when the adapter is in the Sysmex rack, the barcode faces the back of the rack. See Figure 3-7.
- Patient samples may be labeled with your site-specific barcode labels. See Section B.7 for supported barcode symbologies. Barcodes on the primary sample tubes and prediluted sample microvials should be checked for quality to ensure correct identification by the barcode reader; there is a risk of sample misidentification if the barcode print quality is poor. Barcoded primary sample tubes and prediluted sample microvials do not require particular orientation in the Sysmex rack; they are rotated by the tube spinner for barcode reading.

  NOTE: *If you use sample racks from another system that do not include rotary bottoms, tube spinning must be disabled. See Section 4.7.6. The primary sample tubes must be positioned in these racks so that the barcodes face the back of the rack, toward the instrument.*

3.6 Stat Rack

A maximum of 3 samples (in primary tubes or microvials) and/or the Calibrator Pack can be loaded into the Stat rack. Samples can be loaded in positions 1–3 only; the Calibrator Pack can be loaded in positions 4–6 (dedicated Calibrator Pack position) only.

NOTE: *Samples are not rotated in the Stat Area. You must align the barcode labels for proper scanning when loading the Stat rack. Barcode labels must face you so they are visibly displayed through the rack slots.*

Figure 3-9: *Stat Rack with Controls, Patient Sample, and Calibrator Pack*

3.7 Test Component Identification

Radio-Frequency Identification (RFID) technology is used to automatically identify and track most test components. The following test components have an RFID tag attached that is encoded with information specific to the component.

Figure 3-10: *Test Component RFID Tag*

Test Component	RFID Information
Elution Buffers A and B	• Catalog number • Lot number • Expiration date • Open stability • Capacity (volume or number of tests) • Date of first use • Remaining volume or number of tests
Wash Solution	
Prefilter	
Analytical Cartridge	Includes all information listed above, plus the following: • Serial number • Test parameters • Buffer and Calibrator Pack compatibility information

Other test components have barcode labels with information specific to the component:

Test Component	Barcode Information
Calibrator Pack	• Lot number • Expiration date • Assigned calibrator values

NOTE: *Some component labels include an SN (Serial Number) or T# (Tracking Number). These numbers are for internal tracking purposes only.*

3.8 Replacing the Prefilter

NOTE: *When the prefilter is removed, a small amount of liquid may drip. Use a paper towel to absorb any drips. Never leave the instrument without a prefilter installed.*

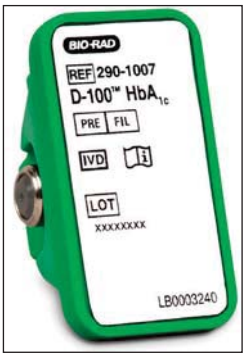
1. The instrument must be in Sleeping state.
2. Open the cartridge/prefilter compartment door to access the prefilter holder.

Figure 3-11: *Prefilter Holder*

3. Grasp the handle of the prefilter holder door and pull it open.

Figure 3-12: *Prefilter Holder (Door Open)*

Figure 3-13: *Prefilter*



4. Grasp the old prefilter with the thumb and forefinger, and pull it forward to remove it from the holder.
5. Remove a new prefilter from its package; remove the black caps.
6. With the label upright and facing you, insert the new prefilter into the holder. Magnets ensure that the prefilter is securely seated.

   NOTE: *The prefilter information is automatically updated when the RFID is read.*
7. Close the prefilter holder door.
8. Close the cartridge/prefilter compartment door.

3.9 Replacing the Analytical Cartridge

NOTE: *When the cartridge is removed, a small amount of liquid may drip. Use a paper towel to absorb any drips. Never leave the instrument without a cartridge installed.*

1. The instrument must be in Sleeping state.
2. Open the cartridge/prefilter compartment door to access the cartridge holder.

Figure 3-14: *Cartridge Holder*

3. Grasp the handle of the cartridge holder door and pull it open.

Figure 3-15: *Cartridge Holder (Door Open)*

Figure 3-16: *Analytical Cartridge*

4. Grasp the old cartridge with the thumb and forefinger, and pull it forward to remove it from the holder.
5. Remove a new cartridge from its package; remove the black caps.
6. With the label upright and facing you, insert the new cartridge into the holder. Magnets ensure that the cartridge is securely seated.

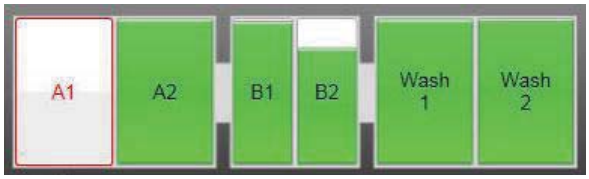
   NOTE: *Test parameters are automatically updated when the RFID is read.*
7. Close the cartridge holder door.
8. Close the cartridge/prefilter compartment door.

3.10 Replacing Reagents

A reagent bottle can be removed and replaced at any time, except when the bottle is "In Use" while the instrument is in Running state (i.e., the Remove button is disabled).

3.10.1 Replacing an Empty Reagent Bottle

Figure 3-17: *Consumables Panel, Bottle A1 Empty*



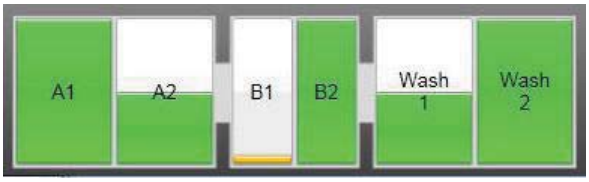
1. Open the reagent compartment door.
2. Remove the empty bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.
3. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.

   NOTE: *The reagent information is automatically updated when the RFID is read.*
4. Close the reagent compartment door.

NOTE: *If you install a bottle of reagent that is not full while the instrument is in Running state, the message panel displays a white message "Partial bottle will be loaded after the run", the reagent indicator appears blank, and the reagent dialog box indicates "Not Ready". When the instrument transitions to Standby after the run, the bottle will be pressurized, and both the indicator and dialog will update.*

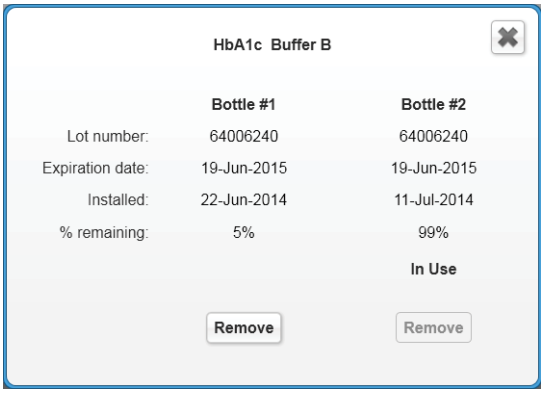
3.10.2 Replacing a Non-Empty Reagent Bottle

Figure 3-18: *Consumables Panel, Bottle B1 Low*



1. Touch the reagent indicator in the consumables panel for the bottle you want to replace.

Figure 3-19: *HbA1c Buffer B Dialog Box, Bottle B1 5% Remaining*



2. In the reagent dialog box, touch Remove to depressurize the bottle.

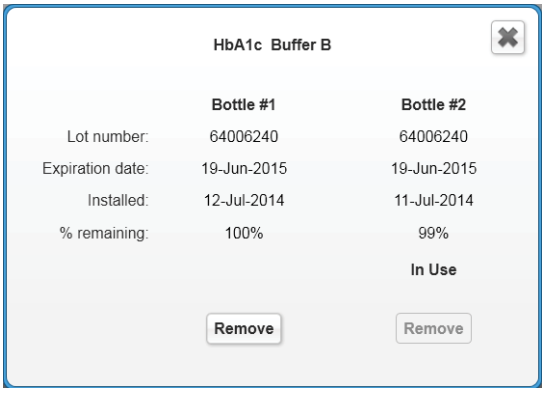
Figure 3-20: *HbA1c Buffer B Dialog Box, Bottle B1 Depressurized*

3. After depressurization, the dialog box indicates "Please Remove".
4. Open the reagent compartment door.
5. Remove the bottle by pushing it in, lifting it up over the ledge, and pulling it out by the handle.

Figure 3-21: *HbA1c Buffer B Dialog Box, Bottle B1 Removed*

6. After removing the bottle, the reagent information is blank.

Figure 3-22: *HbA1c Buffer B Dialog Box, New Bottle B1 Installed*



7. Insert a new bottle of the same reagent, pushing it into the compartment until it latches in place.

   NOTE: *The reagent information is automatically updated when the RFID is read.*
8. Close the compartment door.
9. Touch ✖ to close the dialog box.

3.11 Replacing Internal Printer Paper

1. Open the green printer door.
2. Push down the latch to open the printer paper door.
3. Remove the remaining paper roll and/or core. Ensure the paper well is free from debris, which may cause the printer to jam.
4. Remove the adhesive strip from a new roll of thermal paper.
5. Position the roll in the input well so that the paper exits the top of the roll towards you.

Figure 3-23: *Positioning the Roll of Paper*

6. Pull the paper towards you and close the printer paper door, with the paper exiting through the slot above the door.
7. Press the paper feed button  to feed the paper through the printer.
8. Place the starting edge of the paper in the paper output well so that the printout rolls up neatly inside the well.
9. Close the green printer door.

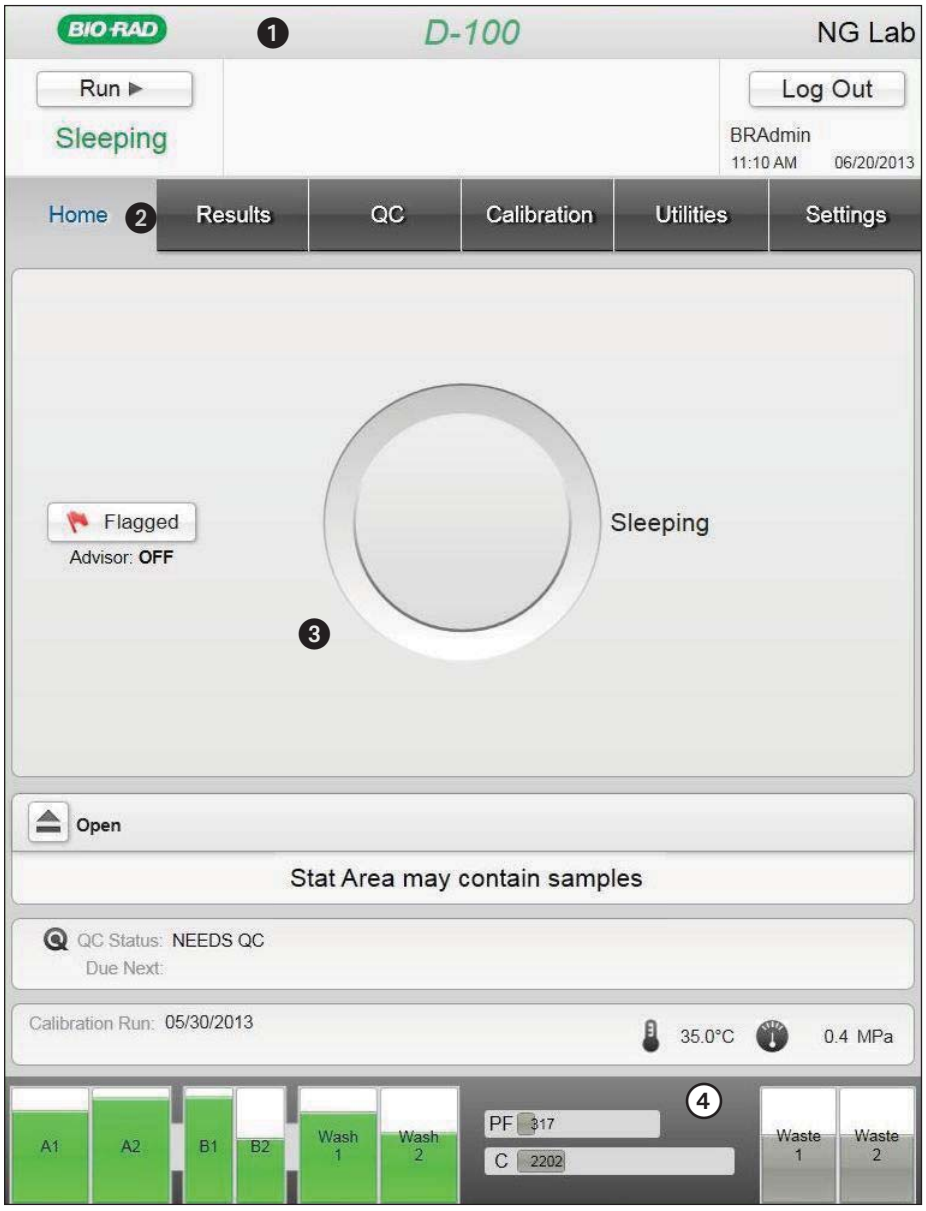
4 Software Overview

4.1 User Interface Layout

The user interface has 4 main areas:

1. Banner
2. Navigation Controls
3. Workspace
4. Consumables Panel

Figure 4-1: *User Interface Layout, Home Tab*



4.1.1 Banner

Figure 4-2: *Banner*

1	System Name (See Section 4.7.1)
2	**Run/Stop/Resume/Reset** button: This toggle button is used to start, stop, and resume runs and to reset the system.
3	Instrument State (See Section 4.1.1.1)
4	***Figure 4-3:*** *Message Panel, Information-Only Message*

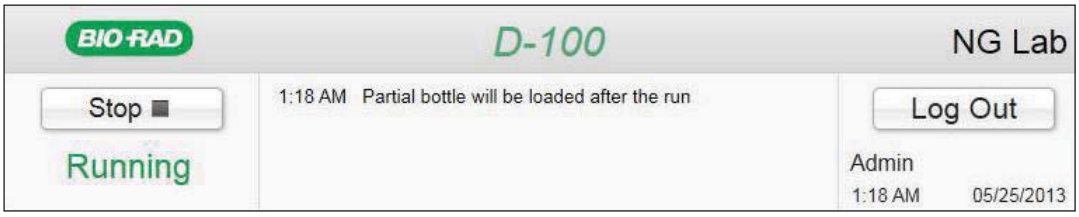


Figure 4-4: *Message Panel, Warning Messages*

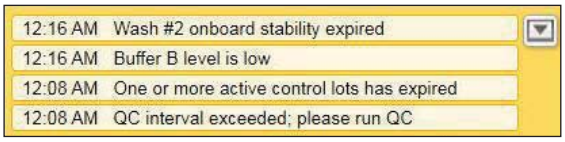
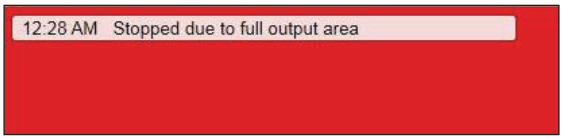


Figure 4-5: *Message Panel, Critical Message*



Message Panel: The instrument generates messages to alert you to important conditions. These messages are displayed in this area of the banner, which is color-coded based on the urgency of the message.

- For information-only messages (e.g., Waiting for cartridge information, Partial bottle will be loaded after the run, etc.), the message panel is white. Information-only messages do not need to be acknowledged.
- For warning messages (e.g., Buffer level is low, lot expired, printer error, etc.), the message panel is yellow. Warning messages require user action soon to remain running, or indicate that an important function is not working. When warning messages require user action, the instrument senses when the action is taken and clears the message.
- For critical messages (e.g., Buffer is empty, Stopped due to full output area, Calibration required-new cartridge installed, etc.), the message panel is red and the run is disabled or stopped. Urgent messages require user action to start or resume the run.

NOTE: *When the message panel contains more messages than fit in the display area, touch* ▼ *to extend the panel to view all messages; touch* ▲ *to condense the panel.*

5 **Log In/Log Out** button: This toggle button is used to log in, log out, switch users, and shut down the system.

Figure 4-6: *Log In Dialog Box*

NOTE: *Touch **Cancel** to close the dialog box without taking action.*

To log in:

1. Touch **Log In**.
2. In the Log In dialog box, touch the User Name field or drop-down arrow to enter your user name.
3. Touch the Password field to type your password.

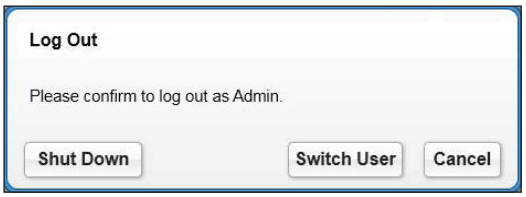
   NOTE: *User names are not case-sensitive; passwords are case-sensitive.*
4. Touch **Log In**.

Figure 4-7a: *Log Out Dialog Box (when user is logged in)*

To log out:

1. Touch **Log Out**.
2. In the Log Out dialog box, touch **Log Out**.

Figure 4-7b: *Log Out Dialog Box (when Default user is enabled)*



To switch the user:

If the **Default user** feature is enabled (see Section 4.7.3), but you want to log in as the user, you can switch the user.

1. Touch **Log Out**.
2. In the Log Out dialog box, touch **Switch User**.
3. The Log In dialog box appears. Continue to log in as instructed above.

To shut down the D-100 System:

1. Touch **Log Out**.
2. In the Log Out dialog box, touch **Shut Down**.

6 User Name: The user currently logged in to the system.

7 Current time and date (See Section 4.7.1 to select format.)

4.1.1.1 Instrument States

Instrument State	Description
Sleeping	The HPLC core (i.e., cartridge heater and detector) is cold and the system is in the low-fluidic mode. The system enters Sleeping state if a run is not initiated within 2 hours. You can access and use features of the user interface while in Sleeping state. Touch Run ► to go to Warming Up state (or Running state if samples are loaded).
Warming Up	This is a transitional state between Sleeping and Standby. During this state, buffer is flushed through the fluid path, the detector LED and cartridge heater warm up, the buffer and waste levels are checked, and the calibration of the system is confirmed.
Standby	The system enters Standby (i.e., ready-to-run) state under the following conditions: • The system has completed an automatic warm-up. • The system is finished warming up but there are no samples available to run. • The system has finished running samples and there are no other samples available to run. The HPLC core is hot and the system is in the low-fluidic mode. Touch Run ► to go to Running state.
Running	The system is processing samples or actively waiting for samples.
Paused	The system enters Paused state under the following conditions: • The system is out of reagents, waste space, or output space. The system remains in this state until you replenish the resource and touch Resume ►.
Stopping	This transitional state occurs under the following conditions: • The user has touched Stop ■ and the system is processing the last samples that have already been queued. Touch Run ► to return to Running state. • The system has encountered an error and is attempting to process samples already aspirated and ejecting all racks from the shuttle. The **Run** button is briefly disabled (appears dimmed) until the shuttle is empty.
Stopped	The system has stopped processing samples and the output area is full. During this state, the **Run** button is briefly disabled (appears dimmed) until the shuttle is empty.
Cooling Down	The system components are turned off and in the process of cooling down before transitioning to Sleeping state.
Maintenance	The system is performing a maintenance operation. After the maintenance operation is completed, the system transitions to Sleeping state.
Fault	The system has encountered a critical error and has stopped all processing. The system remains in this state until you resolve the error and touch Reset ►. The system then transitions to Initialization state.

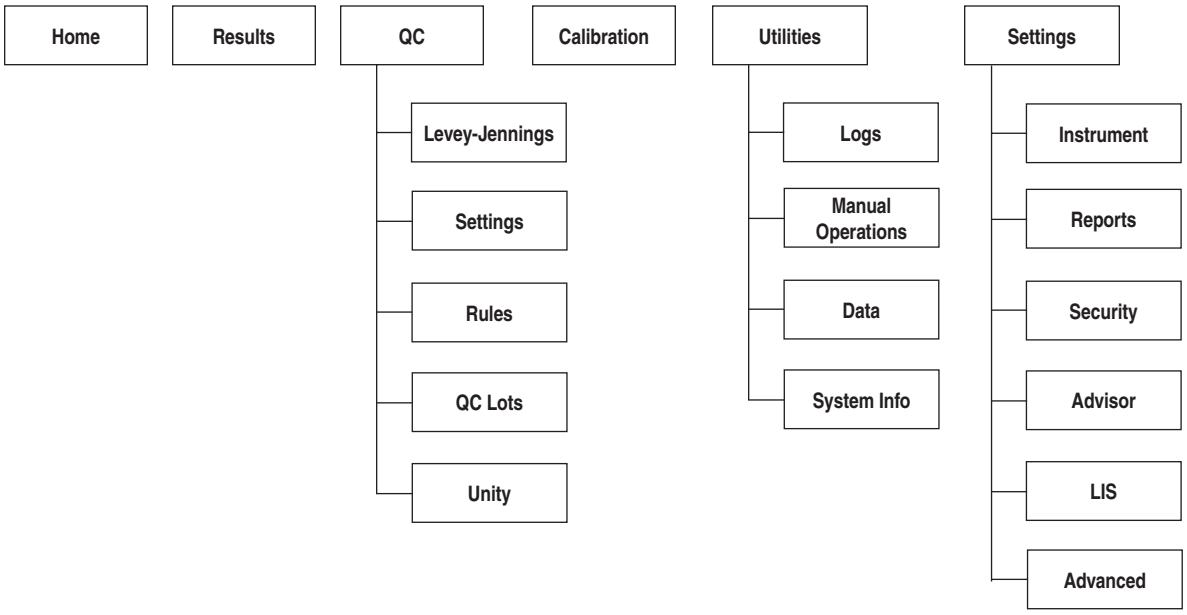
Instrument State	Description
Initialization	This transitional state occurs between powering on the instrument and Sleeping, and between Fault and Sleeping. The system initializes all hardware and software to a functional state.

4.1.2 Navigation Controls

Figure 4-8: *Navigation Controls, QC/Levey-Jennings Selected*

❶	Primary navigation tabs: The **Home, Results, QC, Calibration, Utilities,** and **Settings** tabs are used to move between the main function screens.
❷	Secondary navigation buttons: The **QC, Utilities,** and **Settings** tabs include secondary buttons that are used to move between the subfunction screens.

Figure 4-9: *Screen Navigation Diagram*



For ease of reference in this manual, subscreen names are written with the name of the main screen first, followed by the subscreen name, separated by a forward slash (e.g., **QC/Levey-Jennings**).

The selected tab and button are highlighted to indicate the screen currently displayed.

4.1.3 Workspace

The selected primary tab and secondary button determine what information is displayed in the workspace. This area is where data is entered, displayed, and managed.

Figure 4-10: *Workspace, Home Tab*

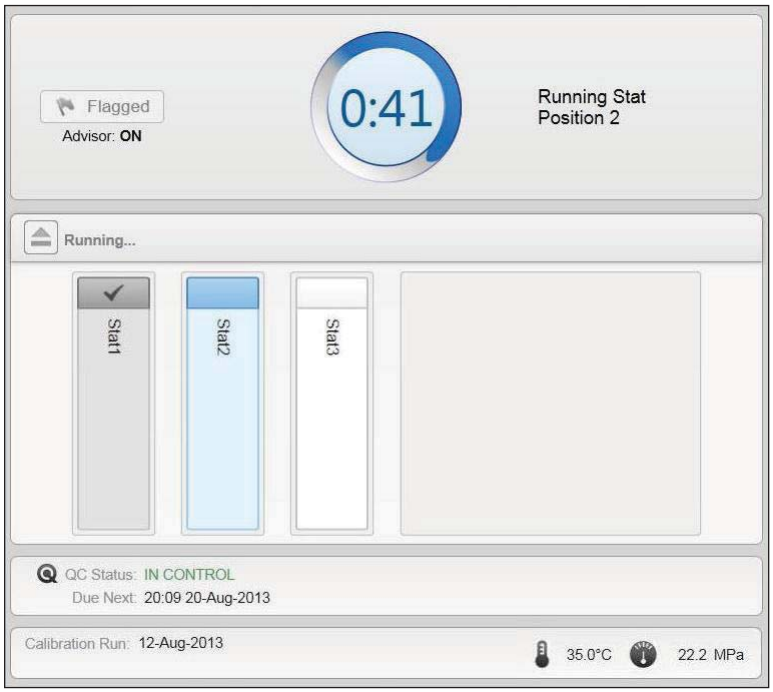


Figure 4-11: *Keyboard*

NOTE: *Touch* ✖ *to close the keyboard without saving the entry.*

The user interface provides on-screen character input devices when needed.

When you touch a text field, a keyboard displays at the bottom of the screen to allow alphanumeric data entry. The text field is replicated above the keyboard so you can see the text entered as you touch the keys.

- Use the **Shift** key to switch from lower case and numbers to upper case and symbols.
- Touch **Enter** to save the entry and close the keyboard.

Figure 4-12: *Number Pad*

NOTE: *Touch* ✖ *to close the number pad without saving the entry.*

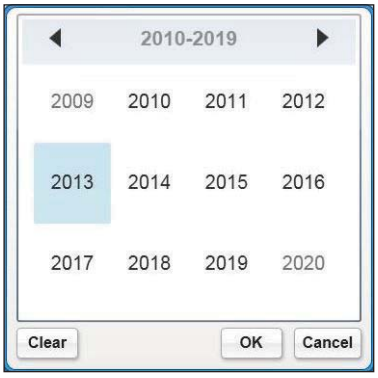
When you touch a value field, a number pad displays at the bottom of the screen to allow numeric data entry. The value field is replicated above the number pad so you can see the number entered as you touch the keys.

- Touch **Enter** to save the entry and close the number pad.

Figure 4-13: *Month/Day Calendar*

Figure 4-14: *Year/Month Calendar*

Figure 4-15: *Year Calendar*



When you touch a date field, a calendar displays on the screen to allow selection of a date.

1. To change the month, use the arrows to scroll backward or forward.
2. To change the year, touch the month/year in the header to open the year/month calendar.
   - Touch the year in the header to open the year calendar and use the arrows to scroll through decades to find the year.
   - Touch the appropriate year, then touch the appropriate month.
3. Touch the appropriate date.

NOTE: *The selected month/year/date are highlighted blue.*

4. Touch **OK** to save the entry and close the calendar.

NOTE: *Touch* ***Cancel*** *to close the calendar without saving the entry.*

4.1.4 Consumables Panel

The consumables panel displays the status of the test consumables.

Figure 4-16: *Consumables Panel*

Remaining level in Buffer A1/A2 bottles.	Remaining level in Buffer B1/B2 bottles.	Remaining level in Wash 1/2 bottles.	Number of tests completed on the Prefilter (PF) and Analytical Cartridge(C).	Level in Waste 1/2 bottles.

NOTE:

- *If a reagent is not installed, the indicator appears blank.*
- *If an empty reagent bottle is installed, the indicator appears blank.*
- *If the Waste tubings are connected to a drain, both Waste indicators appear blank.*

Indicator color-coding helps alert you to the status of each consumable:

Consumable	Color Code			
	Green	Gray	Yellow	Red
Buffer A, Buffer B, Wash	>10–100% remaining	NA	≤10% remaining	Empty
Prefilter	NA	Tests remain	Exceeded recommended number of tests	No Prefilter installed
Cartridge	NA	Tests remain	Exceeded recommended number of tests	No Cartridge installed
Waste	NA	0–90% full	>90% full	Full

In addition to the consumables panel indicators, warning messages will appear in the message panel at the top of the screen, alerting you when the total volume (i.e., bottle 1 + 2) of each reagent is low (yellow message) and empty (red message), when the cartridge or prefilter is past the recommended number of tests (yellow message), and when the waste level is full (red message). See Section 4.1.1.

Figure 4-17: *Consumables Panel, Reagent Dialog Box*

Touching a reagent icon opens a dialog box indicating the lot number, expiration date, date installed, and % remaining. It also indicates if the reagent bottle is "In Use" or "Empty".

See Section 3.10 for more information.

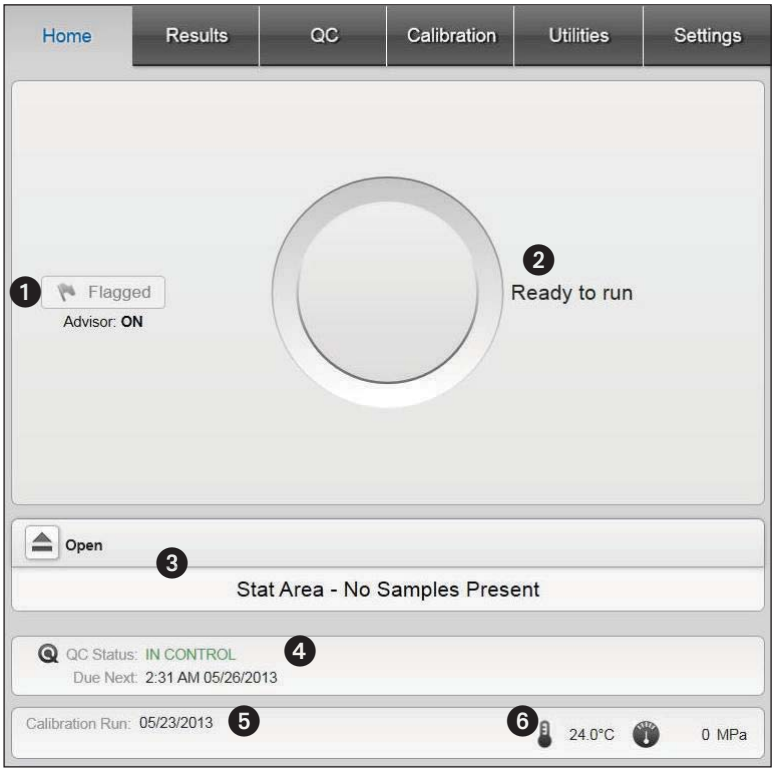
NOTE:

- *If there is no bottle installed, there is no reagent information displayed.*
- *A bottle cannot be removed if it is "In Use" while the instrument is in Running state (i.e., the* Remove *button is disabled).*

4.2 Home Screen

The **Home** screen indicates the status of the calibration, QC, and samples being processed. It also indicates the cartridge temperature and pump pressure. The Stat Area is controlled and displayed on this screen. There are 6 main areas:

Figure 4-18: *Home Screen*



NOTE: *The upper pane of the **Home** screen (which includes the Advisor Status and Instrument Status) expands when the Stat Area contains no samples; the upper pane contracts when the Stat Area is open (i.e., Stat rack is ejected) or contains samples.*

1. Advisor Status
2. Instrument Status
3. Stat Area
4. QC Status
5. Calibration Status
6. Temperature and Pressure

4.2.1 Advisor Status

1	**Flagged** button: This button is enabled (flag is red) when one or more sample results meet the **Flagged** filter criteria defined in the **Results** screen. Touching the button takes you to the **Results** screen, where results have been filtered to display only flagged samples. See Section 4.3.1 for information regarding the **Flagged** filter.
2	Advisor: **ON/OFF**: Indicates whether the Advisor rules processing function is on or off. See Section 4.7.4 for information regarding Advisor.

4.2.2 Instrument Status

The Activity Indicator (large circle) becomes animated when the instrument is active.

Figure 4-19: *Instrument Status: Sleeping State* 	❶	When the instrument is in Sleeping state, the Activity Indicator is gray with no animation; the status is indicated to the right.
Figure 4-20: *Instrument Status: Warming Up State*  ***NOTE:** In specific instances (e.g., after instrument shutdown or after a fault occurs) a longer warm-up (approximately 6 minutes) is performed to ensure the pumps are adequately primed.*	❷	During an automatic warm-up, the instrument transitions to Warming Up state. During this period, the Activity Indicator remains gray, but becomes animated; the status is "Performing Warm-Up". After warming up (approximately 3 minutes*), the instrument transitions to Standby state.
	❸	If there are no samples loaded and the **Run** button is touched in Sleeping state, the instrument transitions to Warming Up state. During this period, the Activity Indicator remains gray, but becomes animated; the status is "Performing Warm-Up". After warming up (approximately 3 minutes*), the instrument transitions to Standby state or, if samples were loaded during warm-up, transitions to Running state.
Figure 4-21: *Instrument Status: Preparing System*  ***Figure 4-22:*** *Instrument Status: Flushing System* 	❹	If there are samples loaded and the **Run** button is touched in Sleeping state, the instrument transitions to Running state. The Activity Indicator turns blue and becomes animated. The status first indicates "Preparing system" (approximately 1 minute*) and then transitions to "Flushing System" (approximately 1 minute). **NOTE:** *When samples are loaded and the **Run** button is touched in Standby state, the status immediately transitions to "Flushing System".*

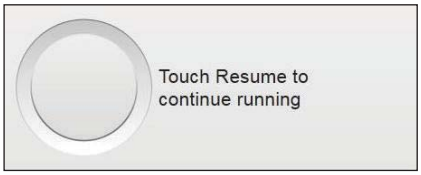
Figure 4-23: *Instrument Status: Rack R1, Tube Position 3 being processed* 	❺	After sample processing begins, a countdown timer appears, indicating the sample's remaining processing time (seconds). As rack samples are processed, the rack number, tube position number, and accession number of the sample in process are displayed. The 10 small circles represent the tube positions in the sample rack; the tube position being processed is colored blue.
Figure 4-24: *Instrument Status: Stat Position 1 being processed* 	❻	When samples in the Stat Area are being processed, the Stat position is displayed.
Figure 4-25: *Instrument Status: Calibrator Pack being processed* 	❼	When the Calibrator Pack is being processed, the status is "Calibrating"; there is no countdown timer. Calibration takes approximately 30 minutes.
Figure 4-26: *Instrument Status: Paused State*  ***Figure 4-27:*** *Instrument Status: Paused State after resource error resolved* 	❽	If the system runs out of a reagent, waste space, or rack output space during a run, it transitions to Paused state. The Activity Indicator is gray with no animation; the status is "Resolve error to continue running". After resolving the resource error, the status changes to "Touch Resume to continue running".

Figure 4-28: *Instrument Status: Standby State* 	❾	After the last sample is processed, the instrument performs end-of-run operations (approximately 5 minutes), then transitions to Standby state. The Activity Indicator is gray with no animation; the status is “Ready to run”. If a run is not started during the Standby period (approximately 2 hours), the instrument transitions to Sleeping state.
Figure 4-29: *Instrument Status: Fault State* 	❿	If the system encounters a critical error, it stops all processing and transitions to Fault state. The Activity Indicator is gray with no animation; the status is “Resolve error to continue running”. After resolving the error, touch **Reset**. The instrument then transitions to Sleeping state.
Figure 4-30: *Instrument Status: Maintenance State* 	⓫	When a maintenance procedure is being performed on the instrument, the instrument transitions to Maintenance state. The Activity Indicator is gray with no animation; the status is “Performing Maintenance…”

4.2.3 Stat Area

This area displays the status and contents of the Stat rack.

NOTE: *The Stat Area display expands when the Stat rack is loaded (i.e., inside the instrument) and contains at least one sample or when the Stat rack is ejected; the Stat Area display contracts when the Stat rack is loaded but empty.*

Figure 4-31: *Stat Area: Stat Rack Loaded but Empty*

Figure 4-32: *Stat Area: Stat Rack Ejected*

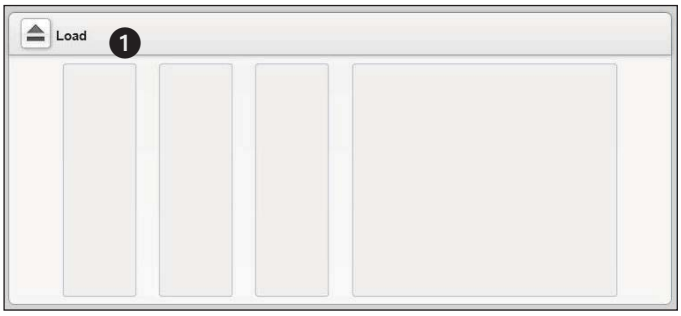
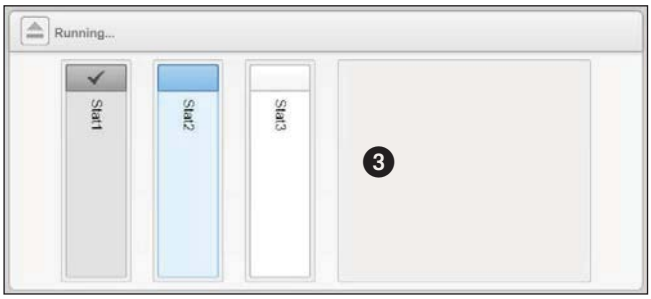


Figure 4-33: *Stat Area: Stat Rack Loaded with Samples in Positions 1–3*



1	**Open/Load** button: This toggle button is used to eject the Stat rack from the Stat Area and load the Stat rack into the Stat Area. • The Stat Rack cannot be ejected when a Stat sample is being processed; the button is disabled and indicates "Running". See Figure 4-33. • When the Stat rack is ejected, the Stat Area display is empty and the **Load** button is enabled. See Figure 4-32.
2	The Stat Area indicates whether or not there are samples present in the Stat rack. • If the Stat rack is empty, it indicates "No Samples Present". • If the Stat rack is loaded and contains samples when the system is shut down, the Stat Area indicates "Stat area may contain samples" when the system is restarted. • The rectangles represent the positions in the rack. • An empty rectangle indicates there is no sample present in that position. • The sample ID is shown for each Stat position that is occupied.
3	Color-coding indicates the status of each Stat position: • Stat positions that have not been processed are white. • The Stat position currently being processed is blue. • Stat positions that are finished being processed are gray and have a checkmark at the top.

The following are examples of the Stat Area when the Stat rack contains the Calibrator Pack, QC samples, and a patient sample. See Section 5.3 for information regarding running the Calibrator Pack.

Figure 4-34a: *Stat Samples Not Processed Yet*

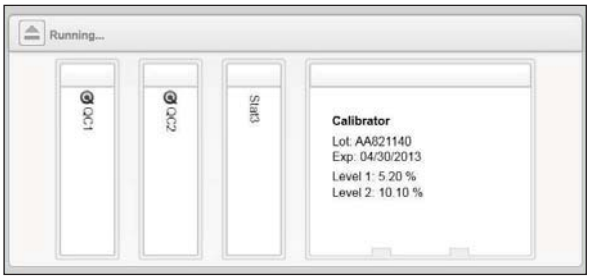


Figure 4-34b: *Calibrator Pack Being Processed*

Figure 4-34c: *Calibrator Pack Finished, QC1 Being Processed*

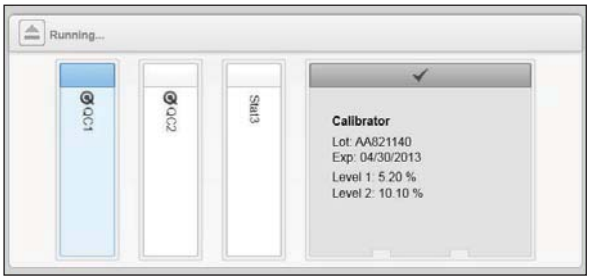
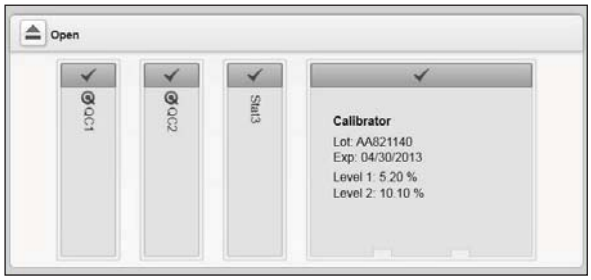
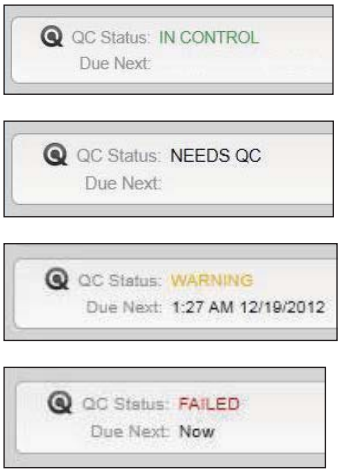


Figure 4-34d: *All Stat Samples Finished*



4.2.4 QC Status

This area displays the status of the active controls. The active lot numbers are indicated in the **QC/QC Lots** screen (see Section 4.4.4).

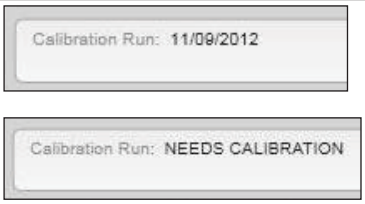


QC Status: There are 4 possible statuses for Quality Control (QC):

- **IN CONTROL:** QC samples have been run within the required time interval and the results are within the acceptable range.
- **NEEDS QC:** QC samples must be run because the system has been recalibrated.
- **WARNING:** The results of one or more QC samples have violated a QC rule.
- **FAILED:** The results of one or more QC samples have failed (i.e., are outside the fixed control range or failed a QC rule).

	Due Next: Indicates the next time QC must be run, either in number of samples or time and date. Once the interval is reached, it will indicate "Now". **NOTE:** *See Section 4.4.2 for information regarding setting the QC Interval. If the QC Interval option is not selected, the Due Next field will be blank.* In addition to the QC Status indicator, a yellow message appears in the message panel at the top of the screen, alerting you that QC is due.

4.2.5 Calibration Status

	Calibration Run: The date the cartridge was last calibrated. "NEEDS CALIBRATION" is displayed after a new cartridge is installed; no samples can be run (i.e., the **Run** button is disabled) until the Calibrator Pack is run. In addition to the Calibration indicator, a red message appears in the message panel at the top of the screen, alerting you that calibration is required.
NOTE: *If a calibrated cartridge is removed from the instrument and later reinstalled, the "Calibration Run" date will display the date that cartridge was last calibrated. The message panel displays a yellow message "Cartridge reloaded – Calibration recommended".* *If the cartridge was stored properly (i.e., tightly capped and refrigerated at 2–8 °C), it can be reinstalled without recalibration; ensure the first run begins with QC samples and that the results are acceptable. If you choose to run without recalibrating, a pop-up message alerts you that the cartridge has not been recalibrated and asks if you want to run anyway. If you select **Yes**, the last calibration of that cartridge will be used to calibrate the run.*	

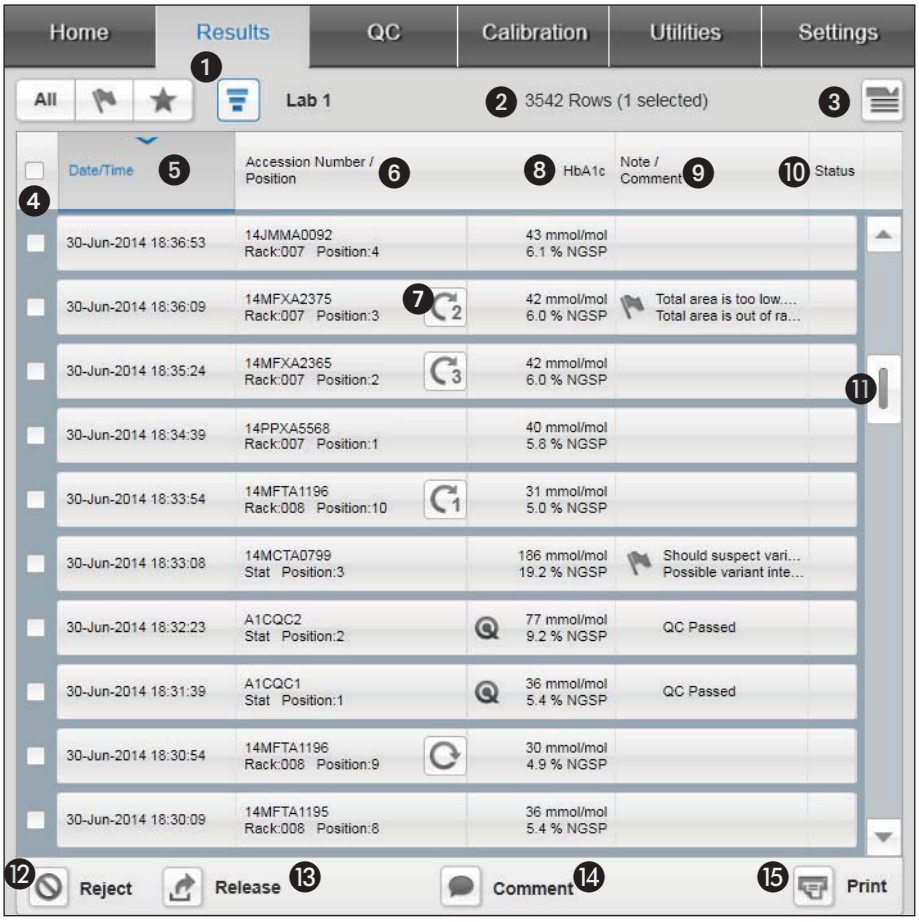
4.2.6 Temperature and Pressure

	❶ The cartridge holder temperature (°C) is displayed. ❷ The system pressure (MPa=megapascal) is displayed. **NOTE:** *If the temperature or pressure is outside the acceptable range, a message appears in the message panel to alert you.*

4.3 Results Screen

The **Results** screen provides access to all sample results in a table format. The table configuration can be customized to meet your laboratory's needs. The results can be filtered for display and searching purposes. The **Results** screen can be accessed at any time with the instrument in any state. Sample results can be released or rejected in this screen.

Figure 4-35: *Results Screen, Default Table Configuration*



1	Filter buttons: These buttons are used to filter the results in the table. • All button: Displays results for All processed samples, or as configured. • button: Displays results for Flagged samples only, or as configured. • button: Displays results for samples meeting the Favorite filter criteria only, or as configured. • button: Opens the Filter dialog box. See Section 4.3.1. The filter currently in use is indicated to the right of the button.
2	The number of rows in the table and the number selected are indicated. Each row represents a sample result.
3	Results Menu button: See Section 4.3.3.

❹	Row checkbox: Each sample result has a checkbox for selection purposes. To take the same action on one or more results, select the corresponding checkbox(es) for the desired sample(s). Selected results are highlighted blue. **NOTE:** *To select all results, touch the checkbox in the column header.*
❺	Date/Time: The date and time the sample result was generated.
❻	Accession Number/Position: The sample ID (i.e., barcode) appears on the first line. The Sysmex rack ID and tube position, or the position in the Stat rack, appears on the second line.
❼	Repeat icon: Displayed when a sample has been processed multiple times (i.e., repeated) within a defined interval. See Section 4.7.1 to set the interval. For example:  indicates the first result of a sample that has been repeated.  indicates the first repeat (i.e., second result) of the sample.  indicates the second repeat (i.e., third result) of the sample. **NOTE:** *When there are >9 repeats, an ellipsis appears instead of a number (i.e.,*  *).*
❽	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s).
❾	Note/Comment: Any notes (associated with rules or QC), or comments entered regarding the sample, appear in this field. •  indicates the sample result has been flagged for violating a rule or failing QC. • A Note provides instruction to the user regarding a flag. Notes are not transmitted to the LIS. • A Comment provides information regarding the sample. Comments can be predefined for Advisor rules or can be entered by the user. Comments can be transmitted to the LIS.
❿	Status: The status of the sample result (i.e., Released  or Rejected ). Results with no icon in the Status field are Held.
⓫	Scroll bar: Use the scroll bar to move up/down the table.
⓬	 **Reject** button: Touch to reject the selected sample result(s).
⓭	 **Release** button: Touch to release the selected sample result(s).
⓮	 **Comment** button: Touch to enter a comment for the selected sample result(s) (maximum: 40 characters).
NOTE: *The **Reject, Release,** and **Comment** buttons are disabled (appear dimmed) if no sample result is selected in the table.*	
⓯	 **Print** button: Touch to access the Print Results dialog box. See Section 4.3.4.
NOTE: • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To view the sample result in detail, touch the result row. See Section 4.3.2.*	

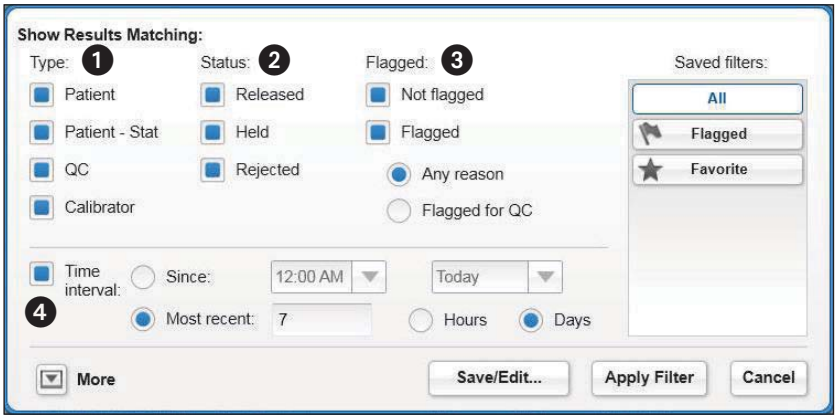
4.3.1 Filtering Results

From the **Results** screen, touch  . The Filter dialog box appears. The saved filters are listed in the right-hand column; the selected filter appears in blue.

There are 3 default filters predefined in the software:

- **All** (See Figure 4-36)
- **Flagged** (See Figure 4-37)
- **Favorite** (See Figure 4-38)

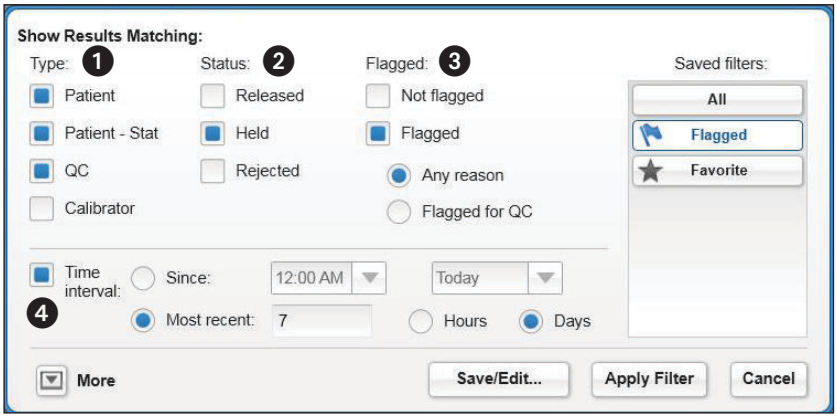
Figure 4-36: *Results Screen, Filter Dialog Box, Default All Filter*



The default **All** filter uses the following sample criteria:

1	Type: **Patient, Patient-Stat, QC**, and **Calibrator**
2	Status: **Released, Held,** and **Rejected**
3	Flagged: **Not flagged** and **Flagged** for **Any reason**
4	Time interval: **Most recent 7 Days**

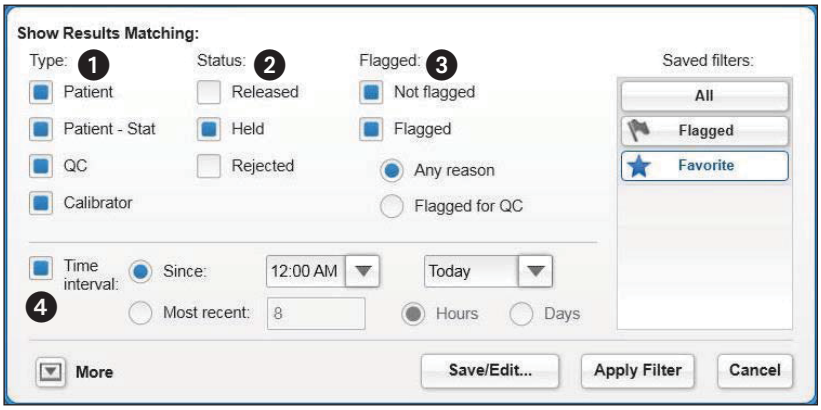
Figure 4-37: *Results Screen, Filter Dialog Box, Default Flagged Filter*



The default **Flagged** filter uses the following sample criteria:

1	Type: **Patient, Patient-Stat** and **QC**
2	Status: **Held**
3	Flagged: **Flagged** for **Any reason**
4	Time interval: **Most recent 7 Days**

Figure 4-38: *Results Screen, Filter Dialog Box, Default Favorite Filter*



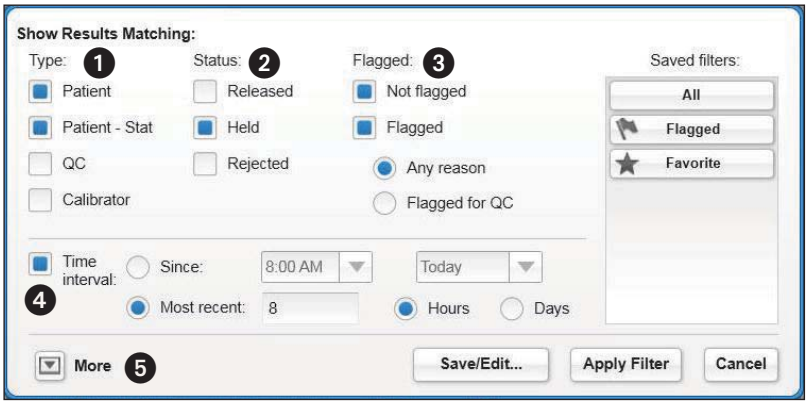
The default **Favorite** filter uses the following sample criteria:

❶	Type: **Patient, Patient-Stat, QC**, and **Calibrator**
❷	Status: **Held**
❸	Flagged: **Not flagged** and **Flagged** for **Any reason**
❹	Time interval: **Since 12:00 AM (or 00:00) Today**

4.3.1.1 Creating Custom Filters

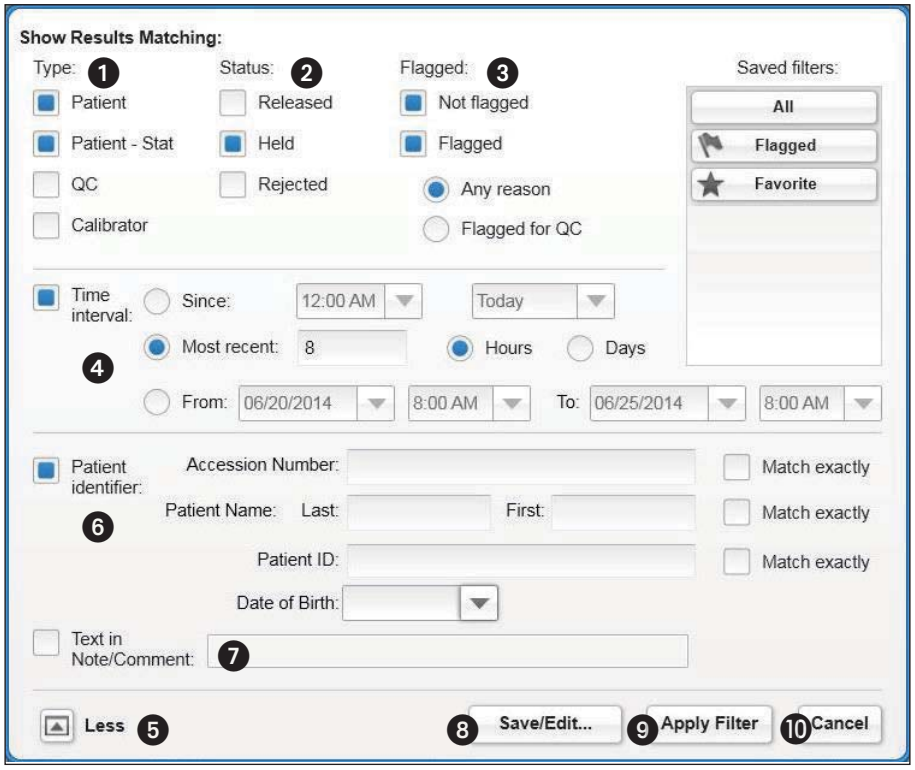
The default filters can be customized for laboratory preferences and workflows. From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-39: *Results Screen, Filter Dialog Box (Condensed)*



To create a custom filter for your lab, select or enter the applicable criteria as follows:

❶	Type: Select **Patient, Patient-Stat, QC**, and/or **Calibrator** **NOTE:** ***Patient-Stat*** *results are those that have a "Stat" (S) priority code in the LIS order.*
❷	Status: Select **Released, Held,** and/or **Rejected**
❸	Flagged: Select **Not flagged** and/or **Flagged**; if **Flagged** is selected, must select **Any reason** or **Flagged for QC**.
❹	To filter by an interval of time, select **Time interval** and then select one of the following options: • **Since**: enter a time and select a day (**Today** or **Yesterday**) from the drop-down list. • **Most recent**: enter a number and select **Hours** or **Days**. • **From/To**: enter a "From" date and time plus a "To" date and time. **NOTE:** *The date format and time format are set in the* ***Settings/Instrument*** *screen. See Section 4.7.1.*

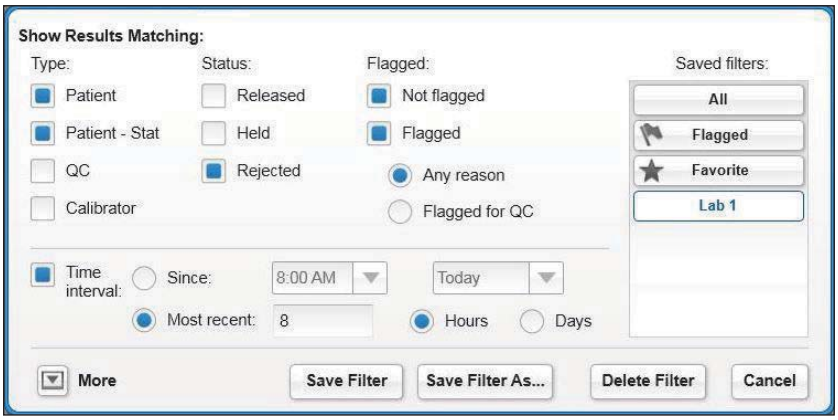
❺	Touch the **More** button to extend the dialog box to view all remaining filter criteria; touch the **Less** button to condense the dialog box. ***Figure 4-40:*** *Results Screen, Filter Dialog Box (Extended)* 
❻	To filter by a patient identifier, select **Patient identifier** and then enter <u>one or more</u> of the following identifiers: • **Accession Number** (i.e., sample ID or barcode) • **Patient Name** (**Last** and/or **First**) • **Patient ID** (i.e., healthcare system's patient identification) • **Date of Birth** If you want to search for the exact number/name/ID entered, select the corresponding **Match exactly** checkbox for the identifier; otherwise, if you enter only part of an identifier, any sample containing that partial information will be included in the filter. **NOTE:** *None of the Patient identifier information is retained when the new filter is saved.*
❼	To filter by the text in the Note/Comment field, select **Text in Note/Comment** and then enter the applicable text.

❽	**Save/Edit** button: After selecting filter criteria, touch this button to save the filter. The **Save Filter As** button appears. Touch this button to open the Save Filter As dialog box. Enter a name (maximum 15 characters) for the filter and touch **Save**. ***Figure 4-41:*** *Save Filter As Dialog Box* 
❾	**Apply Filter** button: To apply the filter without saving it, touch this button. The results table will display the filtered results (under the name "Custom Filter") until you log out of the system or select a new filter.
❿	**Cancel** button: Closes the dialog box without saving filter selections.

4.3.1.2 Editing a Filter

From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-42: *Filter Dialog Box, Saving Edited Filter*



1. In the Saved filters column, ensure the filter you want to edit is selected (appears in blue). If it is not selected, touch the desired filter button (which will display the filtered results), then touch  again.
2. Touch **Save/Edit**.
3. Make your desired edits to the filter criteria.
4. To save the changes under the existing filter name, touch **Save Filter**.
   - To save the changes under a new filter name, touch **Save Filter As**, enter a new name (maximum 15 characters), and touch **Save**.

4.3.1.3 Deleting a Filter

From the **Results** screen, touch  . The Filter dialog box appears.

Figure 4-43: *Filter Dialog Box, Deleting a Filter*

1. In the Saved filters column, ensure the filter you want to delete is selected (appears in blue).
2. Touch **Save/Edit**.
3. Touch **Delete Filter**.
4. A pop-up message appears, prompting you to confirm the deletion. Touch **Yes** to confirm.

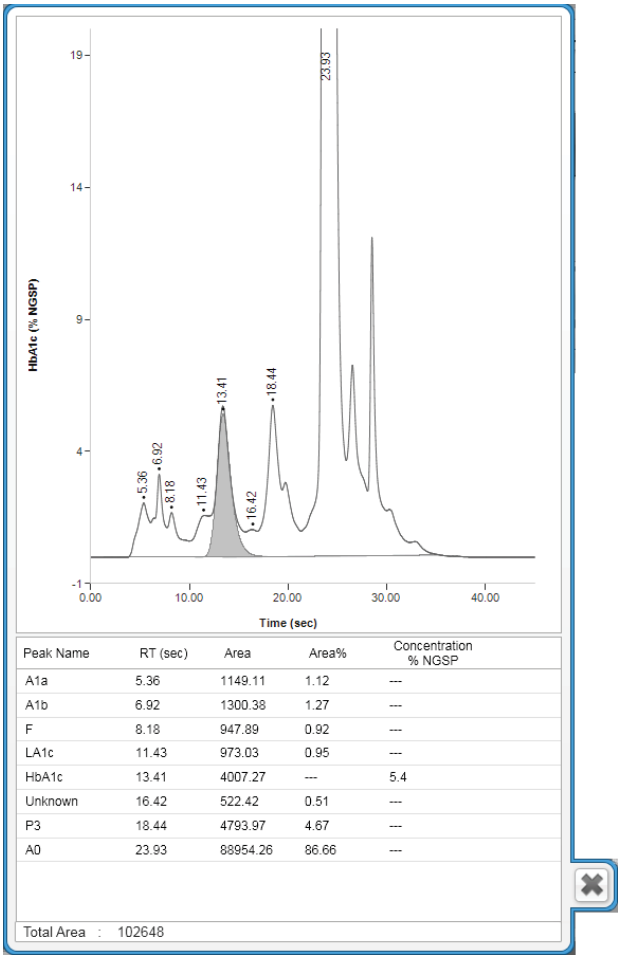
4.3.2 Viewing Result Details

From the **Results** screen, touch the desired sample result row in the table. The Result Details screen appears.

Figure 4-44: *Result Details Screen*

In addition to the sample information already provided in the **Results** screen (described in Section 4.3), the Result Details screen displays the following:

1	Accession Number: The sample ID (i.e., barcode). This field becomes editable if the barcode is missing (e.g., prediluted patient sample) or unread. See Section 5.7.1.
2	Patient ID: The healthcare system's patient identification (optional).
3	Physician: The name of the physician (optional).
4	DOB: The patient's date of birth (optional).
5	Gender: The patient's gender (optional).
6	If the sample has been repeated, all results for the sample will be displayed in table format, exactly as shown in the **Results** screen. The result details displayed are for the result highlighted blue. Touch another result in this table to display the details for that result. **NOTE:** *If there are more than 2 results for the sample, a scroll bar is provided to move up/down the table.*

❼	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s). The primary reporting unit appears first and is bolded.
❽	Note: Any notes associated with rules or QC. •  indicates the sample result has been flagged for violating a rule or failing QC. • Long notes appear truncated; view complete note in the **More Details** window (see Figure 4-46). • Notes are not transmitted to the LIS.
❾	Comment: To enter a comment, edit an existing comment, or view a truncated comment in its entirety, touch this field (no limit to number of characters).
❿	***Figure 4-45:*** *Enlarged Chromatogram and Peak Table*  Chromatogram: graph of detector output vs time. For an enlarged view of the chromatogram and peak table, touch the chromatogram. See Figure 4-45. Touch  to close the enlarged view.
⓫	Peak Table: This table includes the list of detected peaks, retention time (RT) in seconds, area, area % of non-calibrated peaks, and concentration of HbA1c peak. The HbA_{1c} result appears in the primary reporting unit.
⓬	Total Area: The sum of all detected analyte peak areas.

Peak Name	RT (sec)	Area	Area%	Concentration % NGSP
A1a	5.36	1149.11	1.12	---
A1b	6.92	1300.38	1.27	---
F	8.18	947.89	0.92	---
LA1c	11.43	973.03	0.95	---
HbA1c	13.41	4007.27	---	5.4
Unknown	16.42	522.42	0.51	---
P3	18.44	4793.97	4.67	---
A0	23.93	88954.26	86.66	---

Total Area : 102648

⓭ ***Figure 4-46:*** *More Details Pop-Up Window*

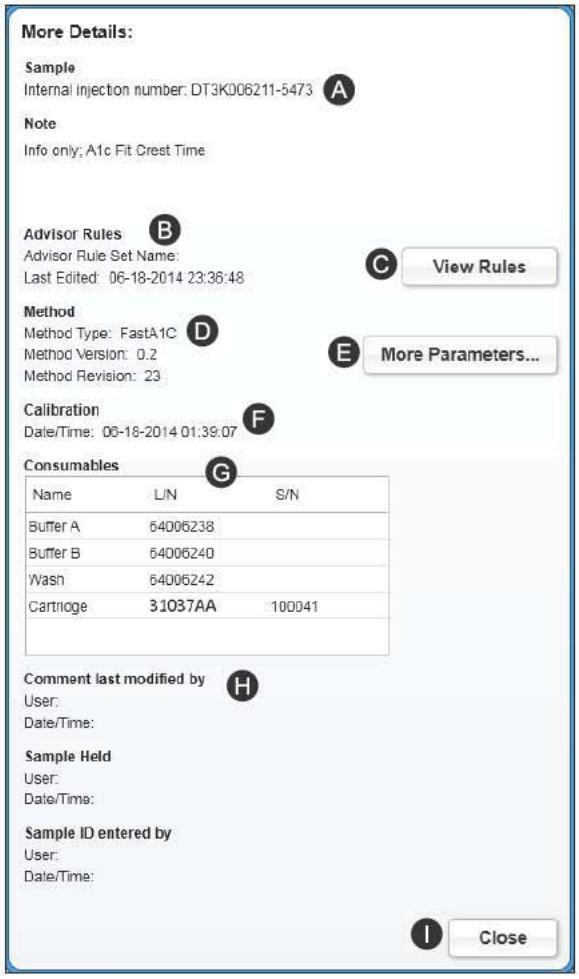
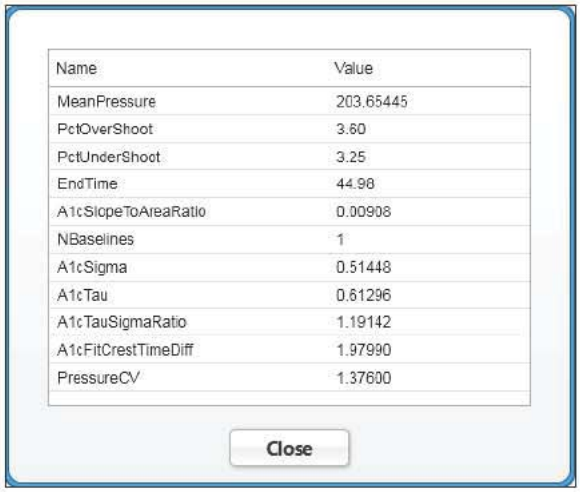


Figure 4-47: *More Details/More Parameters Pop-Up Window*

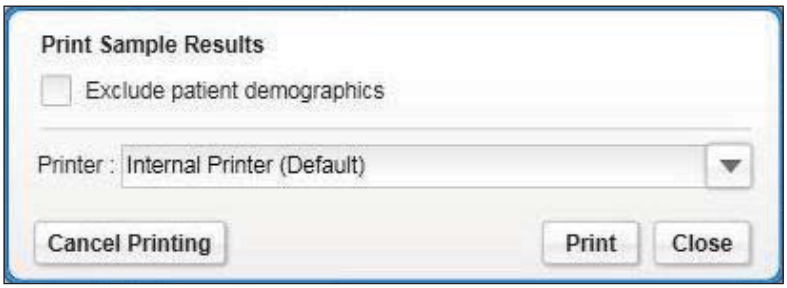


More Details button: to view additional details regarding the sample analysis, touch this button. A pop-up window displays the following:

- Ⓐ Internal injection number
- Ⓑ Advisor Rule Set name and date last edited
- Ⓒ **View Rules** button: To view the complete set of Advisor rules used to process the sample, touch this button.
- Ⓓ Method name, version, and revision.
- Ⓔ **More Parameters** button: To view additional analysis parameters for the sample, touch this button. See Figure 4-47. For more information about these parameters, see Appendix C, rules 25–29.
- Ⓕ Calibration date and time
- Ⓖ Consumables lot numbers and serial numbers
- Ⓗ User name, date, and time of specific user actions.
- Ⓘ Touch **Close** to close the window.

⓮ **Print** button: Touch to print the sample result report.

Figure 4-48: *Print Sample Results Dialog Box*



- **Exclude patient demographics**: Select this checkbox to exclude demographic information from the printed report; if not selected, patient demographics will be included in the report.
- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).

  NOTE: *The default printer is set in the **Settings/Reports** screen. See Section 4.7.2.*

  The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the sample result report (i.e., Patient Report) is "Accession Number-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print results.

  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Result Details screen, then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

⓯ **Reject** button: Touch to reject the sample result.

⓰ **Release** button: Touch to release the sample result. See Section 6.2 for more information regarding releasing results.

NOTE: *After rejecting or releasing a sample result in the Result Details screen, the software immediately displays the next sample result.*

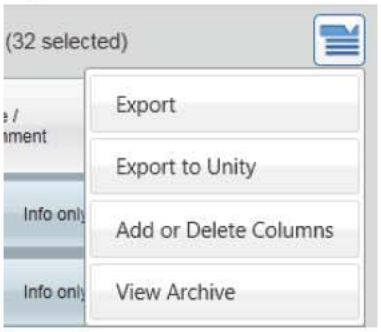
⓱ Touch ▶ to display the next sample result or ◀ to display the previous sample result.

NOTE: *To exit the Result Details screen, touch any primary navigation tab (e.g., touch **Results** to return to the **Results** screen).*

4.3.3 Results Menu

From the **Results** screen, touch . The Results Menu appears. There are 4 functions available:

Figure 4-49: *Results Menu*



- **Export** (See Section 4.3.3.1)
- **Export to Unity** (See Section 4.3.3.2)
- **Add or Delete Columns** (See Section 4.3.3.3)
- **View Archive** (See Section 4.3.3.4)

4.3.3.1 Exporting Results

1. From the **Results** screen, select the corresponding checkbox(es) for the desired sample(s). Selected results are highlighted blue.
2. Touch .
3. From the Results Menu, touch **Export**.

Figure 4-50: *Export Results Dialog Box*

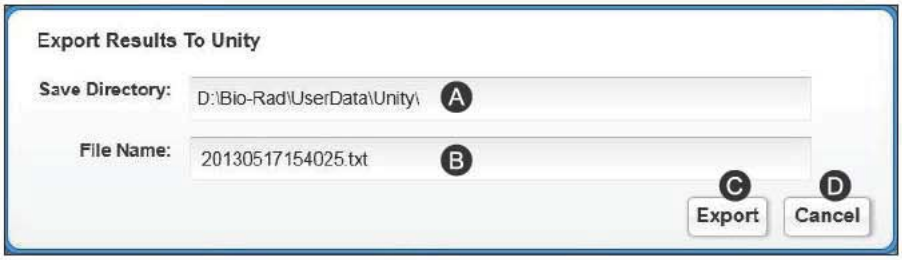
A	**Exclude patient demographics**: Select this checkbox to exclude demographic information from the results; if not selected, patient demographics will be included in the results.
B	Save to: The default location for exported files is the D:\Bio-Rad\UserData\Exports folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
C	File Name: The default file name is "Export_yyyymmddhhmmss.csv". Touch the field to change the file name.
D	**Export** button: Touch this button to export the selected results. A pop-up message confirms that the export was completed successfully. Touch **OK**.
E	**Cancel** button: Closes the dialog box without exporting results.

4.3.3.2 Manually Exporting QC Results to Unity™

QC results can be exported automatically to Unity (see Section 4.4.5). Alternatively, to manually export QC results, follow these instructions.

1. From the **Results** screen, select the corresponding checkbox(es) for the desired QC sample(s). Selected results are highlighted blue.
2. Touch  .
3. From the Results Menu, touch **Export to Unity**.

Figure 4-51: *Export Results to Unity Dialog Box*



A	Save Directory: The default location for manually exported QC Unity files is the D:\Bio-Rad\UserData\Unity folder. The location is set in the **QC/Unity** screen (see Section 4.4.5).
B	File Name: The default file name is simply the export date and time: "yyyymmddhhmmss.txt". A prefix can be added to the file name in the **QC/Unity** screen (see Section 4.4.5).
C	**Export** button: Touch this button to export the selected results. A pop-up message confirms that the export was completed successfully. Touch **OK**.
D	**Cancel** button: Closes the dialog box without exporting results.

4.3.3.3 Adding or Deleting Columns in the Results Table

The results table can be customized to meet your laboratory's needs.

1. From the **Results** screen, touch  .
2. From the Results Menu, touch **Add or Delete Columns**.

Figure 4-52: *Add or Delete Columns Dialog Box*

A	Secondary Identifier: In the default table configuration, the Accession Number appears on the first line of each result as the primary identifier and the Position (i.e., rack ID/tube position) appears on the second line. The **Patient Name, Patient ID,** or **Date of Birth** can be displayed as a secondary identifier below the Accession Number by selecting the desired option; when a secondary identifier is displayed, the Position appears in a separate column in the table.
B	Results: A column for **Total Area** can be added to the table by selecting this option.
C	Peak Results: Columns can be added to the table for up to 3 additional peaks by selecting peak(s) in the list.
NOTE:	*The maximum number of columns that can be added to the table is 3.*
D	**Save** button: Touch this button to save the changes to the table.
E	**Cancel** button: Closes the dialog box without saving any changes to the table.

4.3.3.4 View Archive

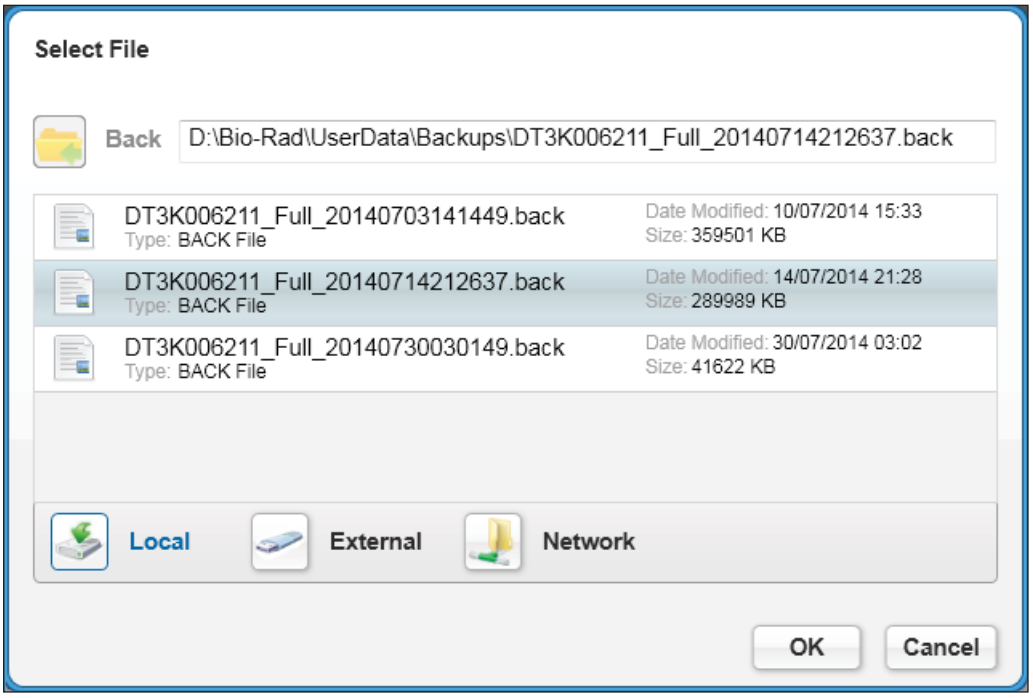
Sample results from a backed-up database can be viewed on the system.

1. From the **Results** screen, touch .
2. From the Results Menu, touch **View Archive**.
3. The View Archive dialog box appears. To locate the database backup file, touch the browse button ...

Figure 4-53: *View Archive Dialog Box*

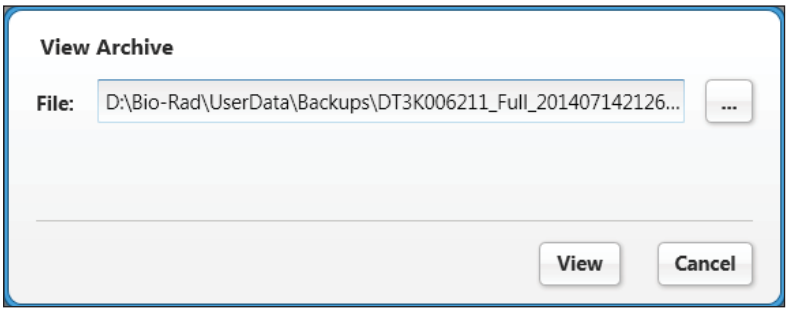
4. In the Select File dialog box, select the file to view from the applicable drive/folder and touch **OK**.

Figure 4-54: *Select File Dialog Box*



5. The file path appears in the File field of the View Archive dialog box. Touch **View** to open the file.

   Figure 4-55: *View Archive Dialog Box after file selected*



   NOTE: *The time to open the file depends on the size of the database and the speed of the drive.*

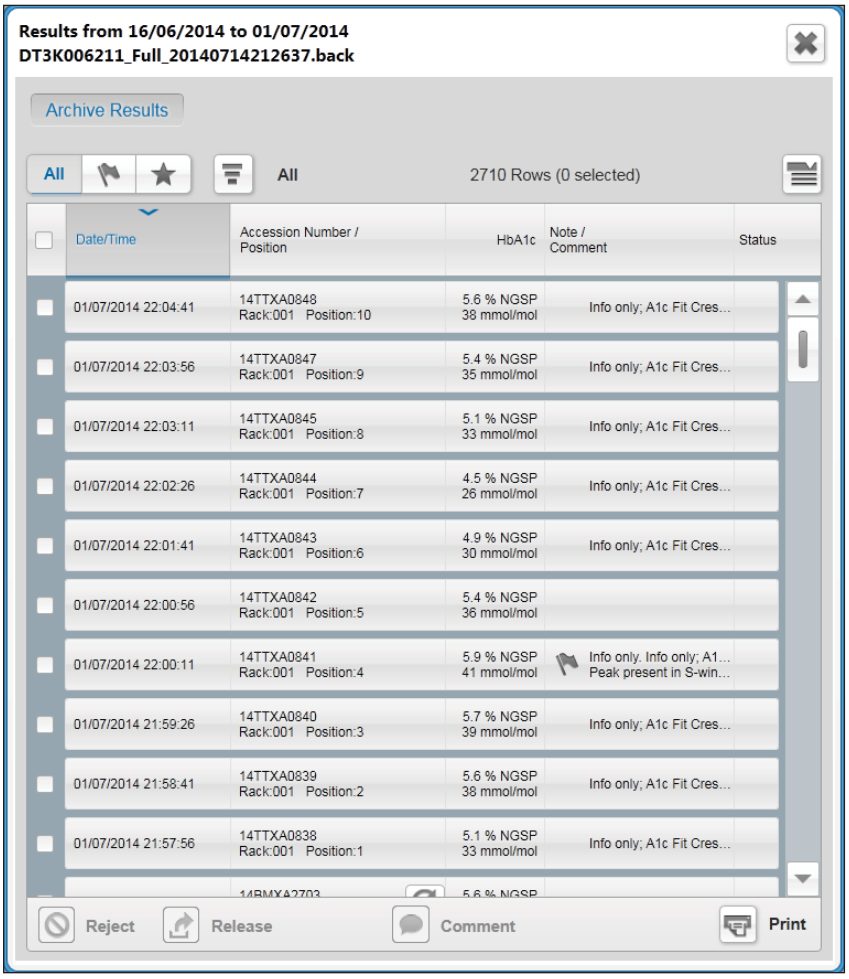
6. The Archive Results window appears. See Figure 4-56. The window header provides the date range of the results and the backup file name. Results are displayed in table format identical to the **Results** screen.

   The Archive Results window has the same functionality as the **Results** screen, with the exception of changing results:

   - Results can be filtered.
   - Results can be exported.
   - Columns can be added or deleted.
   - Result Details can be viewed.

     NOTE: *After viewing the Result Details for an archived sample result, you can return to the results table by touching the* Archive Results *button.*
   - Results can be printed.
   - Comments cannot be changed or entered.
   - Results cannot be Rejected or Released.

Figure 4-56: *Archive Results Window*

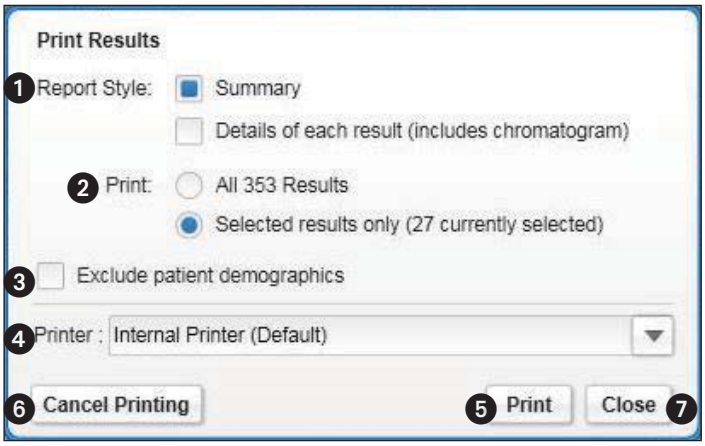


7. After you are finished viewing the archived results, touch  to close the file. A pop-up message asks if you are sure you want to close the archive. Touch **OK**.

4.3.4 Printing Results

From the **Results** screen, touch **Print**.

Figure 4-57: *Results Screen, Print Results Dialog Box*



1	Report Style: Select one or both option(s). • **Summary**: The selected results are printed in table format just like they appear in the **Results** screen; also included is a separate table listing the lot numbers and serial numbers of the consumables used for the analysis. • **Details of each result (includes chromatogram)**: A detailed report is printed for each selected sample, including the chromatogram and peak table.
2	Print: Select option. • **All Results**: Results will be printed for all samples in the results table. • **Selected results only**: Results will be printed for only the selected samples in the results table.
3	**Exclude patient demographics**: Select this checkbox to exclude demographic information from the printed report; if not selected, patient demographics will be included in the report.
4	Printer: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). **NOTE:** *The default printer is set in the **Settings/Reports** screen. See Section 4.7.2.* The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Summary Report is "Summary-System Name-yyyy-mm-dd_hh-mm-ss.pdf". The default file name for the detailed report (i.e., Patient Report) is "Accession Number-yyyy-mm-dd_hh-mm-ss.pdf".
5	**Print** button: After selecting options, touch this button to print results.
6	**NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Results** screen, then touch **Cancel Printing** in the dialog box.*
7	**Close** button: Closes the dialog box without printing.

4.4 QC Tab

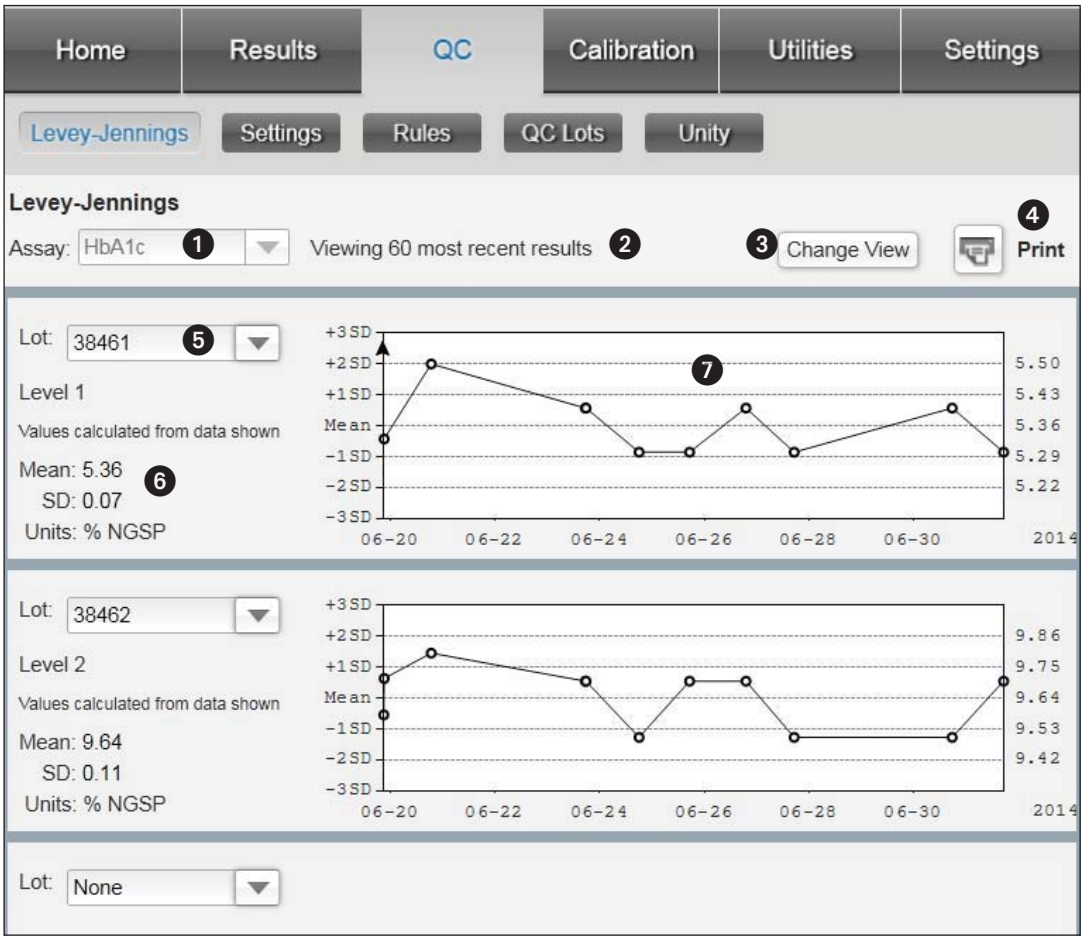
The **QC** tab includes the following secondary navigation buttons:

- **Levey-Jennings** (See Section 4.4.1)
- **Settings** (See Section 4.4.2)
- **Rules** (See Section 4.4.3)
- **QC Lots** (See Section 4.4.4)
- **Unity** (See Section 4.4.5)

4.4.1 QC/Levey-Jennings Screen

QC results are presented in the form of a Levey-Jennings chart. A separate chart is displayed for each control level (up to 3 levels).

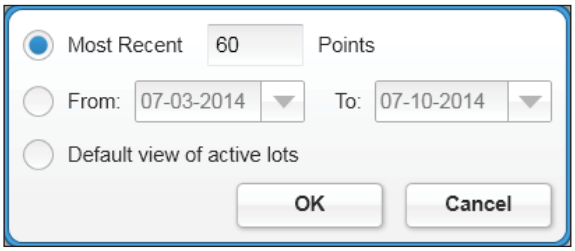
Figure 4-58: *QC/Levey-Jennings Screen*



1	Assay: The assay name (HbA1c).
2	Indicates the results included in the Levey-Jennings chart.

❸ **Change View** button: Opens a dialog box, allowing you to select the control data to be viewed.

Figure 4-59: *Change View Dialog Box*



Select from the following options:

- **Most recent**: Enter a number of data points.
- **From/To**: Enter a "From" date and a "To" date.
- **Default view of active lots**: The most recent 60 points from the control lots in use are included.

Touch **OK** to save the entry and close the dialog box.

❹ **Print** button: Touch to print the QC Summary Report. The report includes the Levey-Jennings charts plus the individual QC results in table format for all control levels.

Figure 4-60: *Print QC Summary Report Dialog Box*

- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the QC Summary Report is "QC-System Name-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print the report.

  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **QC/Levey-Jennings** screen, then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

❺ Lot: The lot number of the control results being displayed is indicated. A different lot can be selected from the drop-down list.

❻ The mean, standard deviation (SD), and primary reporting unit (% NGSP or mmol/mol IFCC) are indicated.

❼ The Levey-Jennings chart plots the dates of analyses along the X-axis and control values on the Y-axis. The mean and standard deviation (SD) limits are also marked on the Y-axis.

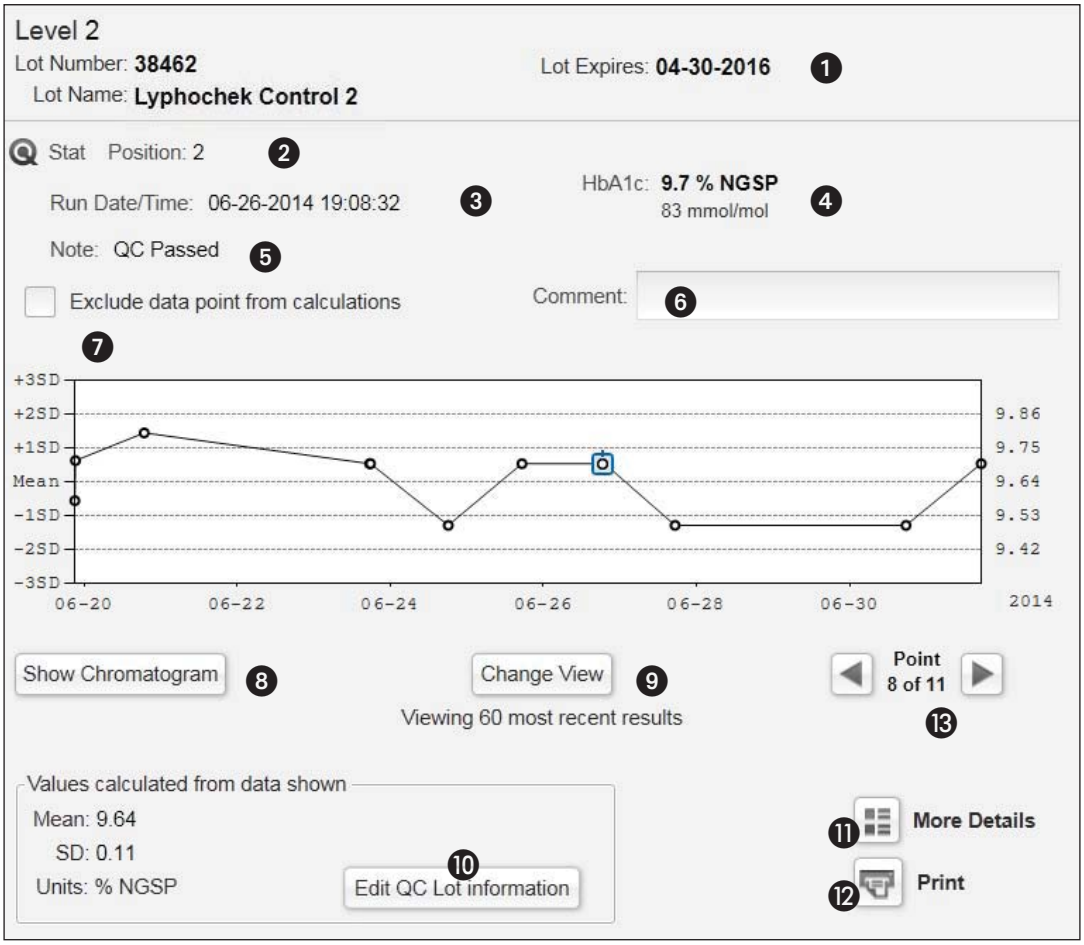
NOTE: *An arrow pointing above or below the chart indicates there is a data point outside ±3 standard deviations (SD) from the mean.*

NOTE: *To view the Levey-Jennings results in detail, touch the Levey-Jennings chart. See Section 4.4.1.1.*

4.4.1.1 Viewing Levey-Jennings Details

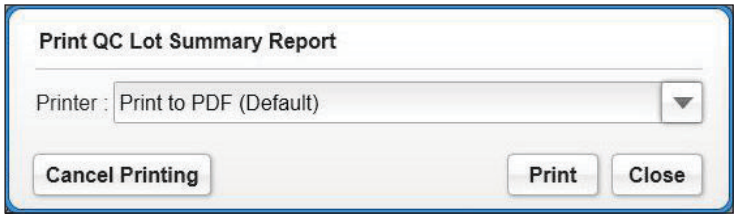
From the **QC/Levey-Jennings** screen, touch the chart. The Levey-Jennings Details screen appears. This screen displays the individual QC results for the specific control lot.

Figure 4-61: *Levey-Jennings Details Screen*



In addition to the information already provided in the **QC/Levey-Jennings** screen (described in Section 4.4.1), the Levey-Jennings Details screen displays the following:

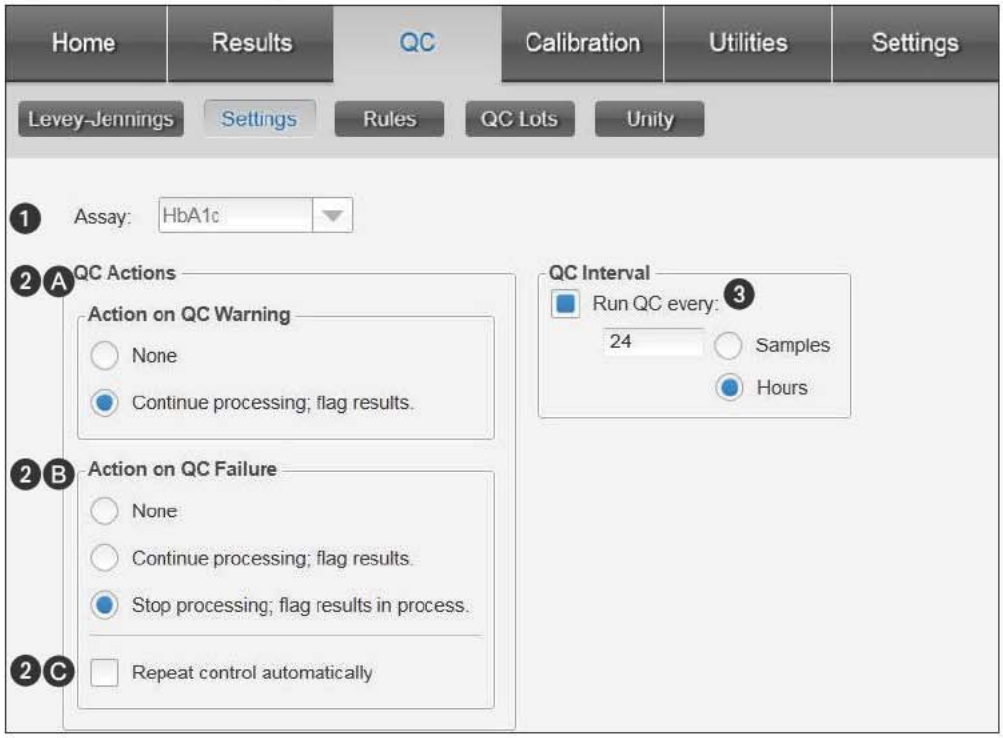
1	Lot Expires: The QC lot expiration date.
2	The Sysmex rack ID and tube position, or the position in the Stat rack.
3	Run Date/Time: The date and time the control result was generated.
4	HbA1c: The HbA_{1c} result is displayed in the selected unit(s). See Section 4.7.6 to select unit(s). The primary reporting unit appears first and is bolded.
5	Note: Any notes associated with QC or Advisor rules (e.g., QC Passed) appear in this field. • indicates the QC result has been flagged for QC failure or violating a rule.

❻	Comment: Any comments entered regarding the control appear in this field. • Comments can be predefined for Advisor rules or can be entered by the user. To enter a comment or edit an existing comment, touch this field (no limit to number of characters).
❼	**Exclude data point from calculations**: Select this checkbox to exclude the result from the Levey-Jennings calculations. A pop-up message confirms that the QC result will be excluded from the calculations. Touch **OK**. **NOTE:** *Failed QC results are automatically excluded from the calculations.*
❽	**Show Chromatogram** button: Touch this button to view the chromatogram for the control result. Touch ✖ to close the chromatogram view.
❾	**Change View** button: Opens a dialog box, allowing you to select the control data to be viewed. See Figure 4-59.
❿	**Edit QC Lot information** button: Opens a dialog box, allowing you to edit the control lot information. See Figure 4-68.
⓫	**More Details** button: To view additional details regarding the control analysis, touch this button. See Figure 4-46.
⓬	**Print** button: Touch to print the QC Lot Summary report. The report includes the Levey-Jennings chart plus the individual QC results in table format for the control lot. ***Figure 4-62:*** *Print QC Lot Summary Report Dialog Box*  • **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for results printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the QC Summary Report is "QC-System Name-yyyy-mm-dd_hh-mm-ss.pdf". • **Print** button: After selecting options, touch this button to print the report. **NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Levey-Jennings Details screen, then touch **Cancel Printing** in the dialog box.* • **Close** button: Closes the dialog box without printing.
⓭	Touch ▶ to display the next control result or ◀ to display the previous control result. **NOTE:** *To exit the Levey-Jennings Details screen, touch any navigation tab (e.g., touch **Levey-Jennings** to return to the **Levey-Jennings** screen).*

4.4.2 QC/Settings Screen

The settings for QC actions are defined in the **QC/Settings** screen. These settings cannot be changed when the instrument is in Running state.

Figure 4-63: *QC/Settings Screen*



1	Assay: HbA1c.
2	There are 3 actions to define the system behavior when QC rules are violated or QC fails. A Action on QC Warning – Select from the following 2 options to define the system behavior when there is a QC Warning for violating a QC rule (see Section 4.4.3 for QC rules): • **None**: the run will continue and the subsequent patient samples will not be flagged. • **Continue processing; flag results**: the run will continue, but the subsequent patient samples will be flagged, with the Note "QC Warning". B Action on QC Failure – Select from the following 3 options to define the system behavior when there is a QC Failure (i.e., QC result is outside of the fixed control range or fails a QC rule): • **None**: the run will continue and the subsequent patient samples will not be flagged. • **Continue processing; flag results**: the run will continue, but the subsequent patient samples will be flagged, with the Note "QC Failed". • **Stop processing; flag results in process**: the run will stop and any subsequent patient samples that were in process will be flagged, with the Note "QC Failed". C **Repeat control automatically**: To automatically repeat the QC when it fails, select this checkbox; the QC will be repeated one time. **NOTE:** *By default, when a QC warning occurs, the message panel displays the yellow message "QC Warning"; when QC fails, the message panel displays the red message "QC Failed".*

❸	QC Interval: To have the system alert you to run QC at a specific interval, select the **Run QC every** checkbox, select a unit (**Samples** or **Hours**), and enter the desired number. The system indicates when the QC is “Due Next” in the **Home** screen QC Status area. See Section 4.2.4.

4.4.3 QC/Rules Screen

The rules for QC results are defined in the **QC/Rules** screen.

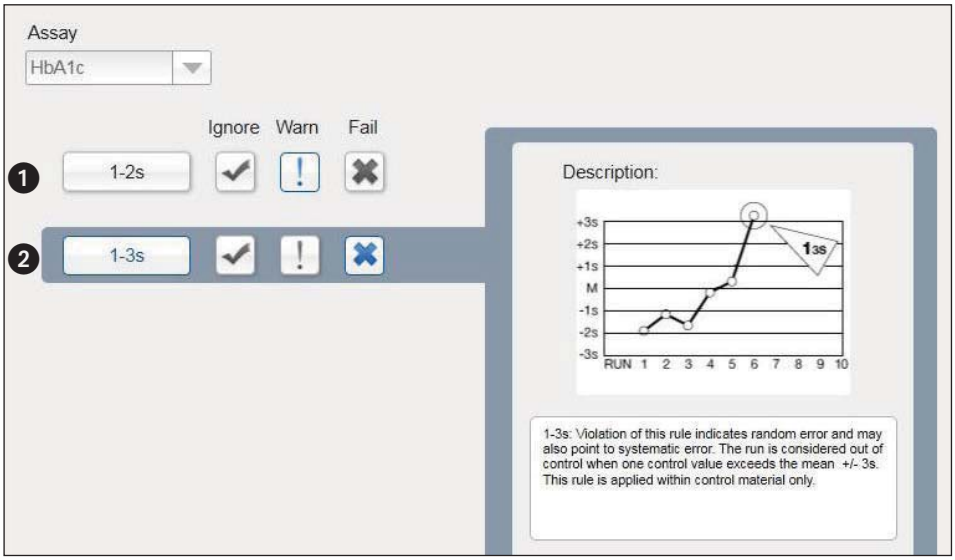
NOTE:

- *These rules apply only when the "**Use QC rules with**" validation method is selected in the Add/Edit Control Lot dialog box (see Section 4.4.4.1, No. 8 B).*
- *The actions taken by the system when these rules are violated are defined in the **QC/Settings** screen (see Section 4.4.2).*

Figure 4-64: *QC/Rules Screen, 1-2s Rule*

1	**1-2s** button: Touch to view or edit the 1-2s rule. See Figure 4-64. This rule is violated when a single control result is outside ±2 SD from the mean. Select an option for handling this rule violation. (The selected option icon appears in blue.) • **Ignore** ✓ : The QC result will not be flagged. • **Warn** ! : The QC result will be flagged, with the Note "QC Warning". • **Fail** ✖ : The QC result will be flagged, with the Note "QC Failure".

❷ ***Figure 4-65:*** *QC/Rules Screen, 1-3s Rule*



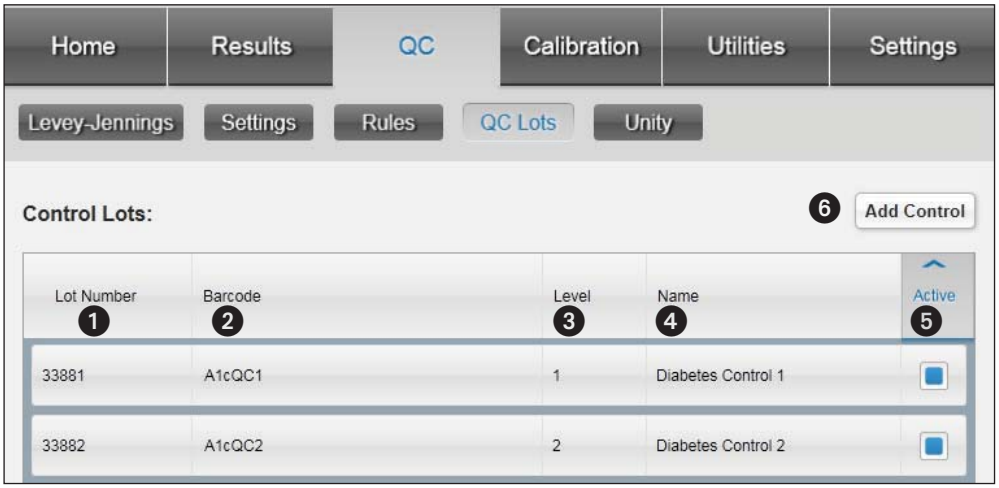
1-3s button: Touch to view or edit the 1-3s rule. See Figure 4-65. This rule is violated when a single control result is outside ±3 SD from the mean. Select an option for handling this rule violation. (The selected option icon appears in blue.)

- **Ignore** : The QC result will not be flagged.
- **Warn** : The QC result will be flagged, with the Note “QC Warning”.
- **Fail** : The QC result will be flagged, with the Note “QC Failure”.

4.4.4 QC/QC Lots Screen

The **QC/QC Lots** screen provides control lot information in table format. QC lots cannot be added or edited when the instrument is in Running state.

Figure 4-66: *QC/QC Lots Screen*



1	Lot Number: control lot number.
2	Barcode: microvial adapter barcode label name.
3	Level: control level (i.e., 1, 2, or 3).
4	Name: control name.
5	Active: The checkbox is selected (blue) if the control lot is currently in use. Clear the checkbox when the lot is no longer in use. The QC Status of the active controls is displayed in the **Home** screen (see Section 4.2.4).

NOTE:

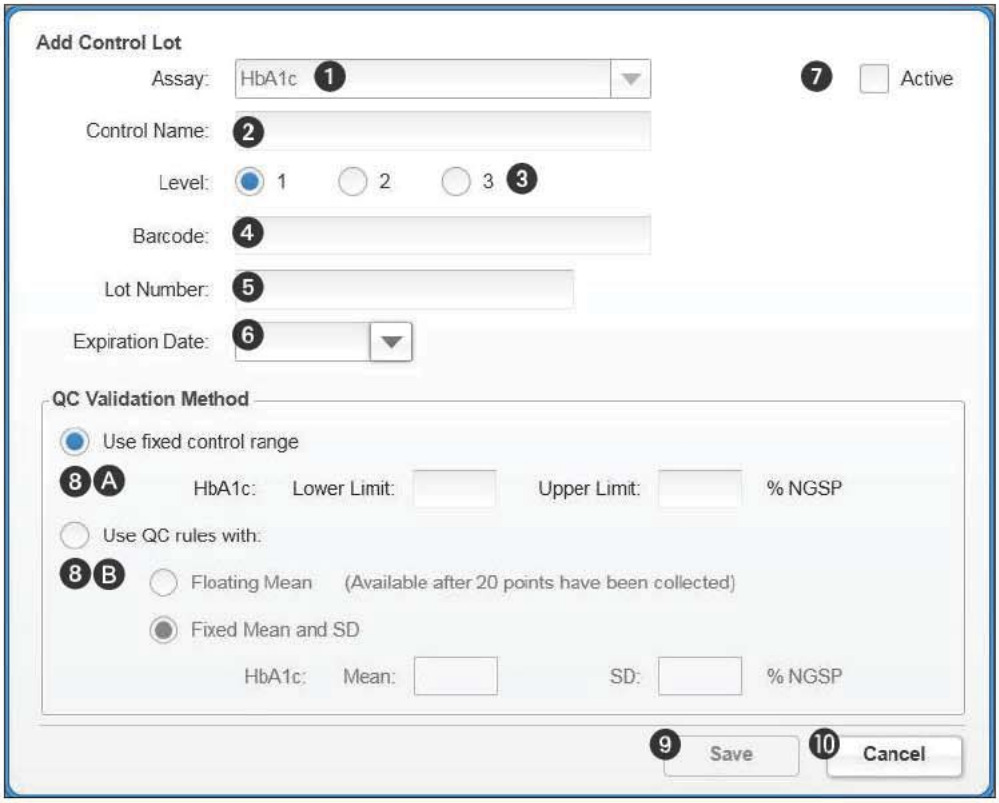
- *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.*
- *To view or edit the control lot information, touch the lot row. See Section 4.4.4.2.*

6	**Add Control** button: To add a new control lot to the system, touch this button. See Section 4.4.4.1.

4.4.4.1 Adding a New QC Lot

From the **QC/QC Lots** screen, touch **Add Control**. The Add Control Lot dialog box appears.

Figure 4-67: *Add Control Lot Dialog Box*



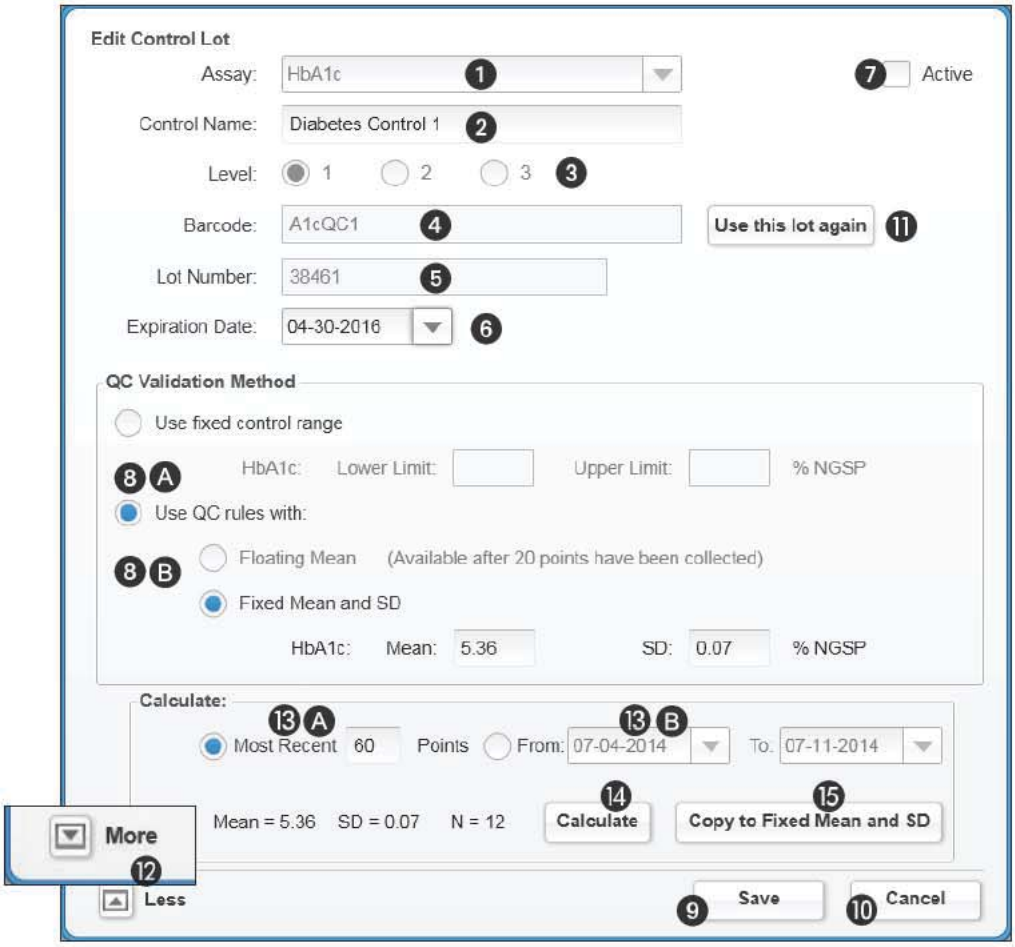
1	Assay: HbA1c.
2	Control Name: Enter the name of the control (maximum: 20 characters).
3	Level: Select control level (1, 2, or 3).
4	Barcode: Enter the microvial adapter barcode label name (maximum: 22 characters; no spaces).
5	Lot Number: Enter the control lot number (maximum: 15 characters).
6	Expiration Date: Enter the control lot expiration date.
7	Active: Select this checkbox to indicate that this is the QC lot currently in use on the system. You will clear the checkbox when the lot is no longer in use.

❽	Select a QC validation method: Ⓐ **Use fixed control range**: Enter the HbA1c **Lower Limit** and **Upper Limit** provided in the QC insert. Ⓑ **Use QC rules with**: This method uses either a **Floating Mean** (which is automatically determined after 20 data points have been collected) or a **Fixed Mean and SD**. If you select the latter option, enter a **Mean** and **SD**. **NOTE:** • *The Floating Mean option cannot be selected until 20 data points have been collected.* • *Ensure all values entered are appropriate for the primary reporting unit shown (i.e., % NGSP or mmol/mol IFCC).*
❾	**Save** button: Touch this button to save the control lot information. A pop-up message confirms that the new lot will now be used for QC rules evaluation. Touch **OK**.
❿	**Cancel** button: Closes the dialog box without saving any information.

4.4.4.2 Viewing or Editing QC Lot Information and Calculation

From the **QC/QC Lots** screen, touch the desired lot row in the table. The Edit Control Lot dialog box appears.

Figure 4-68: *Edit Control Lot Dialog Box (Extended)*



See Section 4.4.4.1 for a description of No. ❶ through ❿ in the dialog box.

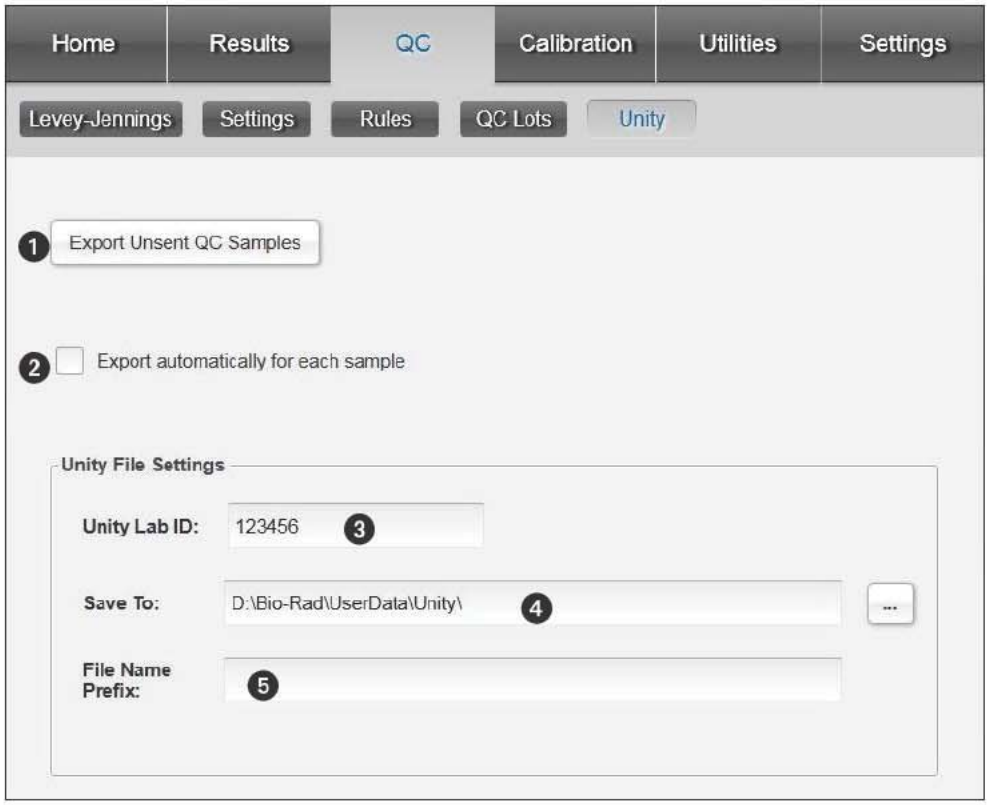
❺	The lot number cannot be edited (appears dimmed) if there is at least one result in the database for that lot.
⓫	**Use this lot again** button: If you want to make a previously used control lot "Active" again, you must touch this button. The same microvial adapter barcode labels are used for all control lots, so this step is necessary to ensure the barcodes are associated with the control lot you are currently using.
⓬	Touch the **More** button to extend the dialog box to view/edit the QC calculation information; touch the **Less** button to condense the dialog box.
⓭	Select from 2 Levey-Jennings calculation options: Ⓐ **Most recent**: Enter the number of most recent data points you want to include in the calculations. Ⓑ **From/To**: Enter a "From" date and a "To" date.

⓮	**Calculate** button: After selecting a calculation option, touch this button to display the calculated Mean, SD, and N.
⓯	**Copy to Fixed mean and SD** button: If you have selected **Use QC rules with Fixed Mean and SD** as your QC validation method (see No. ❽ Ⓑ), touch this button to automatically copy the values to the respective fields.

4.4.5 QC/Unity Screen

The settings for the Bio-Rad Unity Real Time® program are defined in the **QC/Unity** screen.

Figure 4-69: *QC/Unity Screen*



❶	**Export Unsent QC Samples** button: If QC results are not automatically exported to Unity, touch this button to export results.
❷	**Export automatically for each sample**: To automatically export each QC result to Unity immediately after analysis, select this checkbox.
❸	Unity Lab ID: Enter your 6-digit Unity lab ID.
❹	Save To: The default location for exported Unity files is the D:\Bio-Rad\UserData\Unity folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
❺	File Name Prefix: The default file name is "yyyymmddhhmmss.txt". Touch this field to add a prefix to the file name.

4.5 Calibration Screen

The **Calibration** screen provides access to all calibrator results in a table format. This screen can be accessed at any time with the instrument in any state.

Figure 4-70: *Calibration Screen*

1	Date/Time: The date and time the calibration results were generated.
2	Calibrator Lot #: The Calibrator Pack lot number.
3	Cartridge: The lot number and serial number of the cartridge installed for this calibration.
4	Result: The calibration slope, intercept, and reporting unit.
5	Status: The calibration status (i.e., Pass or Fail).
NOTE:	*To view the calibrator results in detail, touch the calibration row. See Section 4.5.1.*

❻ **Calibrate Now** button: Touching this button opens the following dialog box.

NOTE: ***Calibrate Now*** *is disabled when the instrument is in Running state.*

Figure 4-71: *Calibration Dialog Box*

- Ⓐ Assay: HbA1c.
- Ⓑ Lot Number: The Calibrator Pack lot number.
- Ⓒ Expiration Date: The Calibrator Pack expiration date.
- Ⓓ Level 1: Calibrator Level 1 assigned value in the primary reporting unit.
- Ⓔ Level 2: Calibrator Level 2 assigned value in the primary reporting unit.

Ⓕ **Calibrate without reconstituting (calibrate with prediluted material)**: If the Calibrator Pack was previously run/reconstituted on the instrument, this checkbox must be selected.

Ⓖ **Open Stat Area/Load Stat Area** button: This toggle button is used to eject the Stat rack from the Stat Area so that the Calibrator Pack and controls can be inserted, and then load the Stat rack into the Stat Area.

NOTE: *The calibrator information* Ⓑ *through* Ⓔ *is automatically entered from the barcode label when the Calibrator Pack is loaded into the Stat Area.*

Ⓗ **Calibrate Now** button: Touch to start the calibration run.

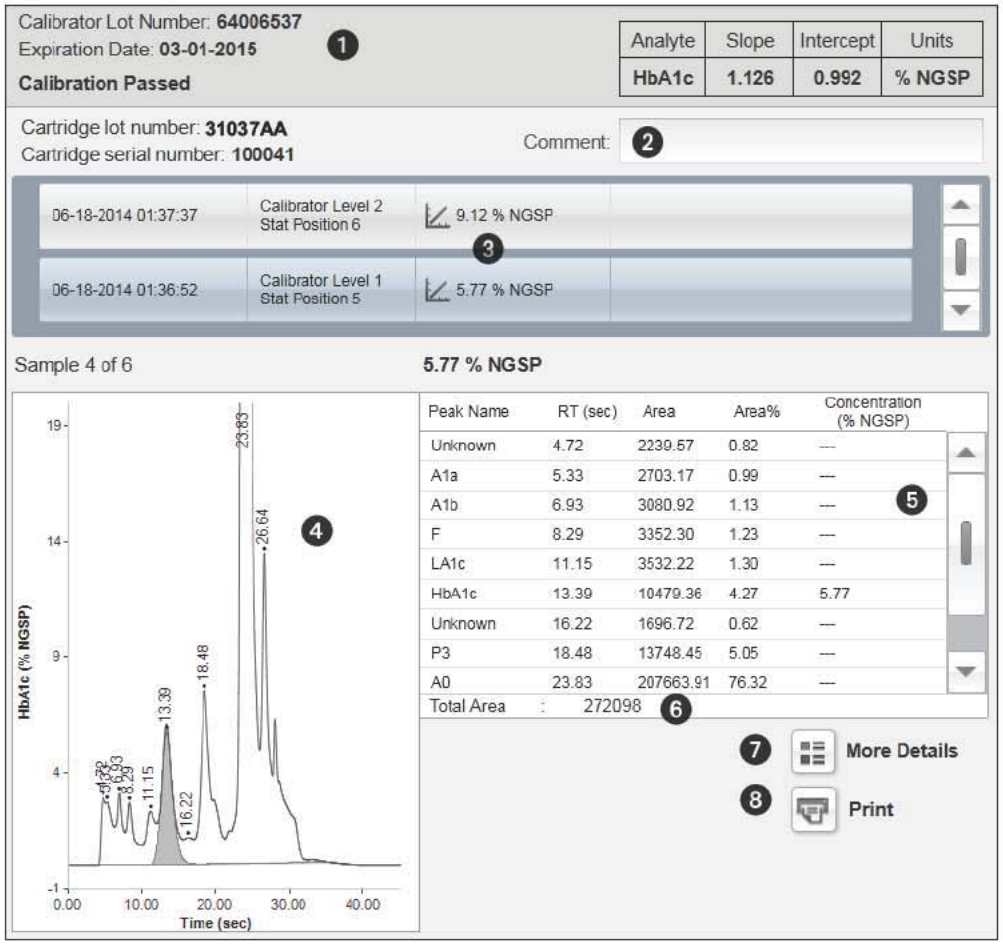
Ⓘ **Cancel** button: Closes the dialog box without running the Calibrator Pack.

See Section 5.3 for more information regarding running the Calibrator Pack. See Section 5.9 for information regarding running third-party calibrators.

4.5.1 Viewing Calibration Details

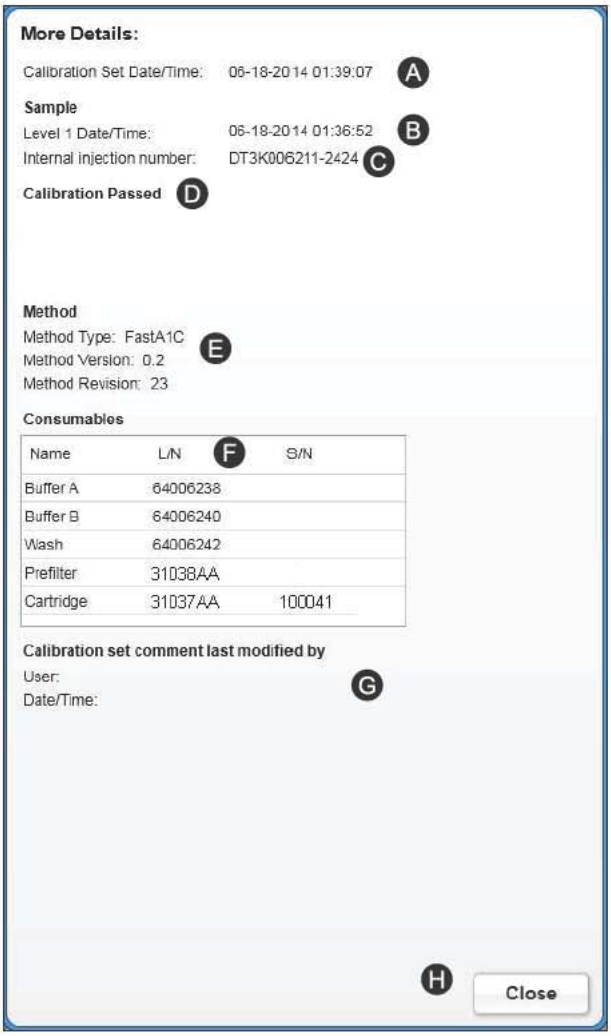
From the **Calibration** screen, touch the desired calibration row in the table. The Calibration Details screen appears.

Figure 4-72: *Calibration Details Screen*



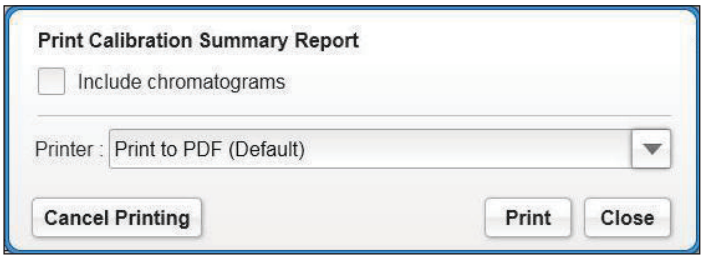
In addition to the calibrator information already provided in the **Calibration** screen (described in Section 4.5), the Calibration Details screen displays the following:

1	Expiration Date: The Calibrator Pack expiration date.
2	Comment: To enter a comment or edit an existing comment for the calibration, touch this field (no limit to number of characters).
3	The results for Calibrator Level 1 and Calibrator Level 2 are displayed in table format, similar to the **Results** screen. For each calibration, there are 3 results for Calibrator Level 1 and 3 results for Calibrator Level 2. The result details displayed are for the result highlighted blue. Touch another result in this table to display the details for that result. **NOTE:** *Use the scroll bar to move up/down the table.*
4	Chromatogram: graph of detector output vs time. For an enlarged view of the chromatogram and peak table, touch the chromatogram (e.g., see Figure 4-45). Touch ✖ to close the enlarged view.

5	Peak Table: This table includes the list of detected peaks, retention time (RT) in seconds, area, area % of non-calibrated peaks, and concentration of HbA1c peak. The HbA_{1c} result appears in the primary reporting unit.
6	Total Area: The sum of all detected peak areas.
7	***Figure 4-73:*** *More Details Pop-Up Window*  **More Details** button: to view additional details regarding the calibrator analysis, touch this button. A pop-up window displays the following: A Calibration Set Date/Time B Sample Date/Time C Internal injection number D Status (i.e., Calibration Passed or Calibration Failed) E Method name, version, and revision F Consumables lot numbers and serial numbers G User name, date, and time of specific user actions H Touch **Close** to close the window.

❽ **Print** button: Touch to print the Calibration Summary Report.

Figure 4-74: *Print Calibration Summary Report Dialog Box*



- **Include chromatograms**: Select this checkbox to include the calibrator chromatograms and peak tables in the printed report.
- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).
  The default location for reports printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Calibration Summary Report is "Calib-System Name-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print the report.

  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the Calibration Details screen, then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

4.6 Utilities Tab

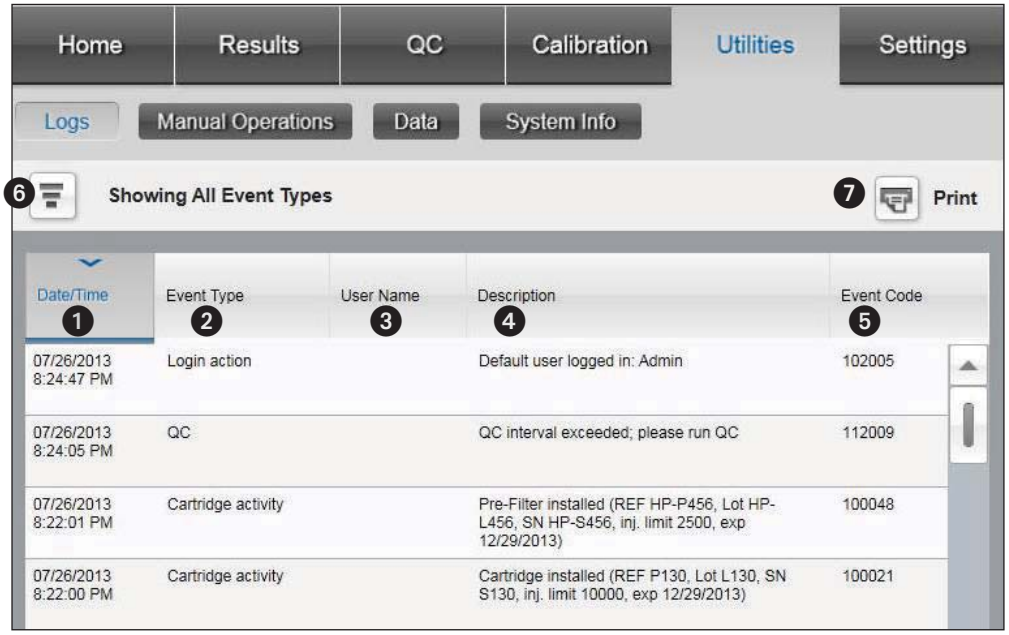
The **Utilities** tab includes the following secondary navigation buttons:

- **Logs** (See Section 4.6.1)
- **Manual Operations** (See Section 4.6.2)
- **Data** (See Section 4.6.3)
- **System Info** (See Section 4.6.4)

4.6.1 Utilities/Logs Screen

Every event that occurs on the system is logged in table format in the **Utilities/Logs** screen.

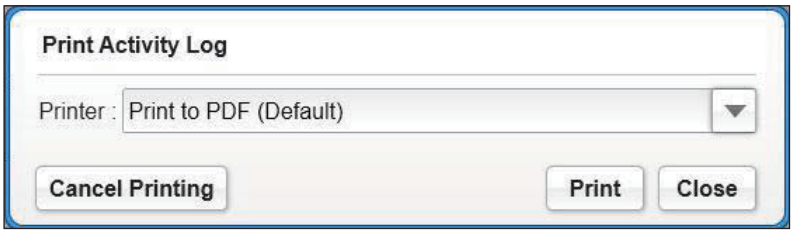
Figure 4-75: *Utilities/Logs Screen*



1	Date/Time: The date and time of the event.
2	Event Type: The general type of event (e.g., Reagent activity, Cartridge activity, Hardware state change, User action, Fault).
3	User Name: The user logged in to the system at the time of the event.
4	Description: The specific description of the event.
5	Event Code: The numerical code for the event.
6	button: Opens the Activity Log Filter dialog box. See Section 4.6.1.1. The filter currently in use is indicated to the right of the button.

❼ **Print** button: Touch to print the Activity Log Report (entire table of filtered log entries). If the log report is lengthy, a pop-up message indicates the number of lines in the log and asks if you want to continue. Touch **Yes** to proceed.

Figure 4-76: *Print Activity Log Dialog Box*



- **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**).

  The default location for logs printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the Activity Log is "Activity Log-System Name-yyyy-mm-dd_hh-mm-ss.pdf".
- **Print** button: After selecting options, touch this button to print the report.

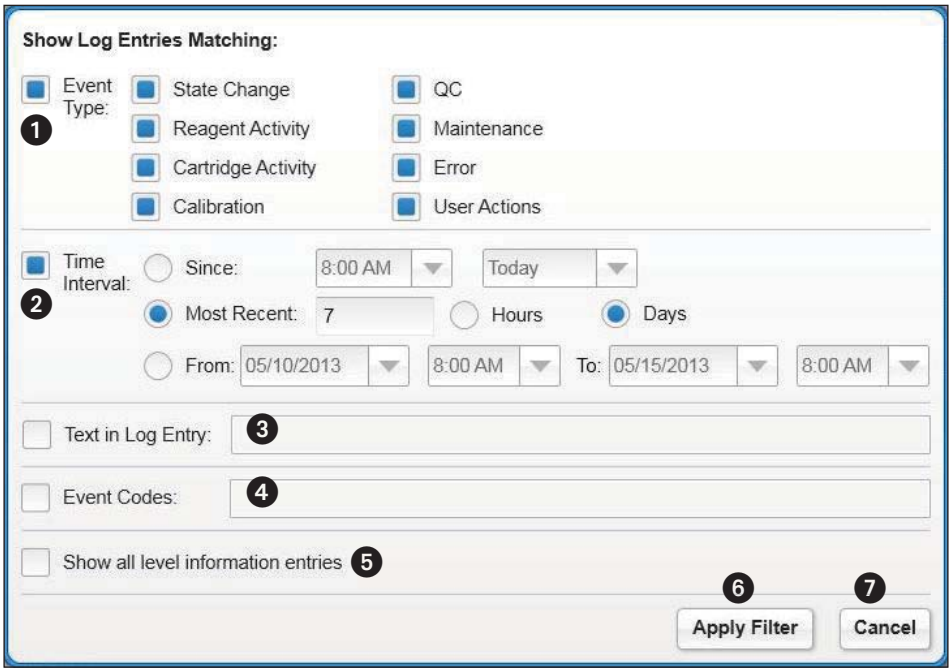
  NOTE: *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Utilities/Logs** screen, touch **Yes** in the pop-up message, and then touch **Cancel Printing** in the dialog box.*
- **Close** button: Closes the dialog box without printing.

NOTE: *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.*

4.6.1.1 Filtering Activity Log Entries

From the **Utilities/Logs** screen, touch  . The Activity Log Filter dialog box appears. The default filter ("Showing All Event Types") criteria are shown in Figure 4-77.

Figure 4-77: *Activity Log Filter Dialog Box, Default Filter*



To create a custom filter for your lab, select or enter the applicable criteria as follows:

1	To filter by the type of event, select **Event Type** and then select one or more of the following options: • **State Change** (i.e., instrument state changes) • **Reagent Activity** (i.e., buffer, wash, and waste events) • **Cartridge Activity** (i.e., cartridge, prefilter, and low-pressure filter events) • **Calibration** (i.e., calibrator events) • **QC** (e.g., QC warning, QC failure, QC lot expired, etc.) • **Maintenance** (e.g., cleaning, change probe, etc.) • **Error** (e.g., SW fault, HW error, etc.) • **User Actions** (e.g., user logged in/out, user requested Run, QC settings changed, etc.)
2	To filter by an interval of time, select **Time interval** and then select one of the following options: • **Since**: enter a time and select a day (**Today** or **Yesterday**) from the drop-down list. • **Most recent**: enter a number and select **Hours** or **Days**. • **From/To**: enter a "From" date and time plus a "To" date and time.
3	To filter by the text in the Description field, select **Text in Log Entry** and then enter the applicable text.
4	To filter by the Event Code, select **Event Codes** and then enter the applicable code.

❺	**Show all level information entries**: Select this checkbox to include all log entries, regardless of level; if not selected, low-level (insignificant) messages will not be shown.
❻	**Apply Filter** button: To apply the filter, touch this button. The Activity Log table will display the filtered results (under the name "Custom Filter") until you log out of the system or change the filter.
❼	**Cancel** button: Closes the dialog box without saving filter selections.

4.6.2 Utilities/Manual Operations Screen

From the **Utilities/Manual Operations** screen, the following functions can be performed:

- **General: Sleep** (See Section 4.6.2.1), **Clean Screen** (See Section 7.4 for instructions), and **Calibrate Touch Screen** (See Section 7.5 for instructions)
- **Clean System** (See Section 7.8 for instructions)
- **Change Probe** (See Section 7.9 for instructions)
- **Prime/Flush** (See Section 7.6 for instructions)
- **Update Method** (See Section 4.6.2.2)

4.6.2.1 Transitioning to Sleeping State

Some functions cannot be performed unless the instrument is in Sleeping state.

Figure 4-78: *Utilities/Manual Operations Screen, General and Sleep Buttons*

1. Go to the **Utilities/Manual Operations** screen.
2. Touch **General**.
3. Touch **Sleep**.

4.6.2.2 Updating Method

Test parameters are automatically updated when the analytical cartridge RFID is read. However, there may be a case where a new method file must be downloaded from a USB flash drive.

Figure 4-79: *Utilities/Manual Operations Screen, Update Method Buttons*

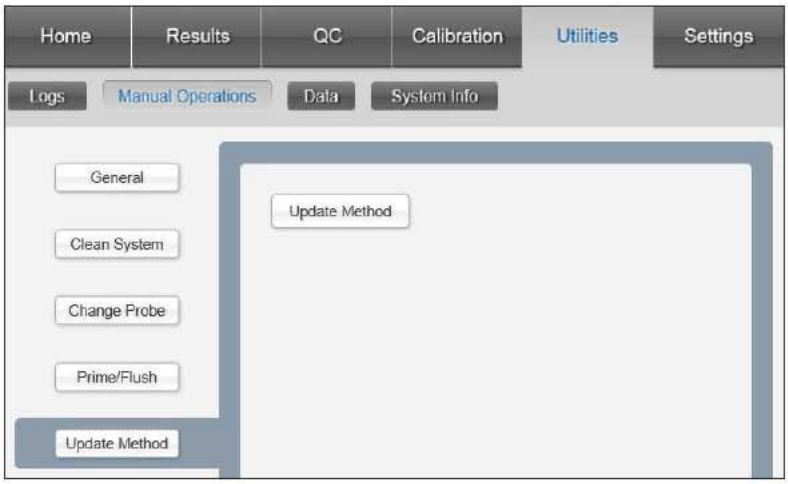
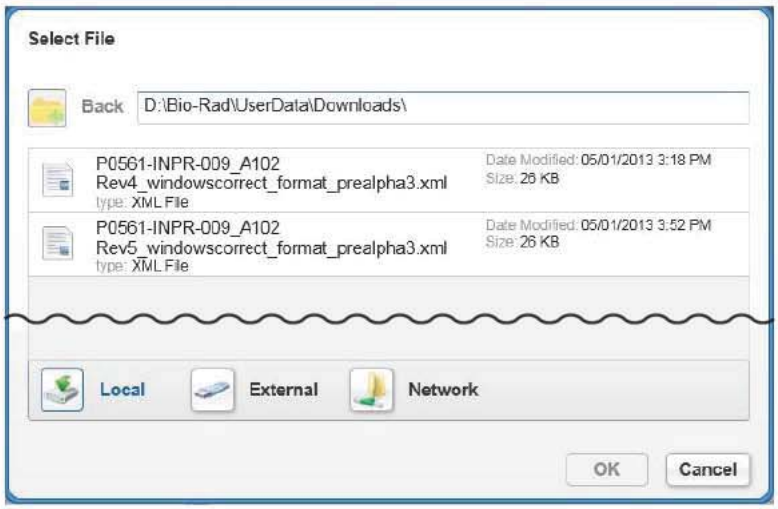


Figure 4-80: *Select File Dialog Box*

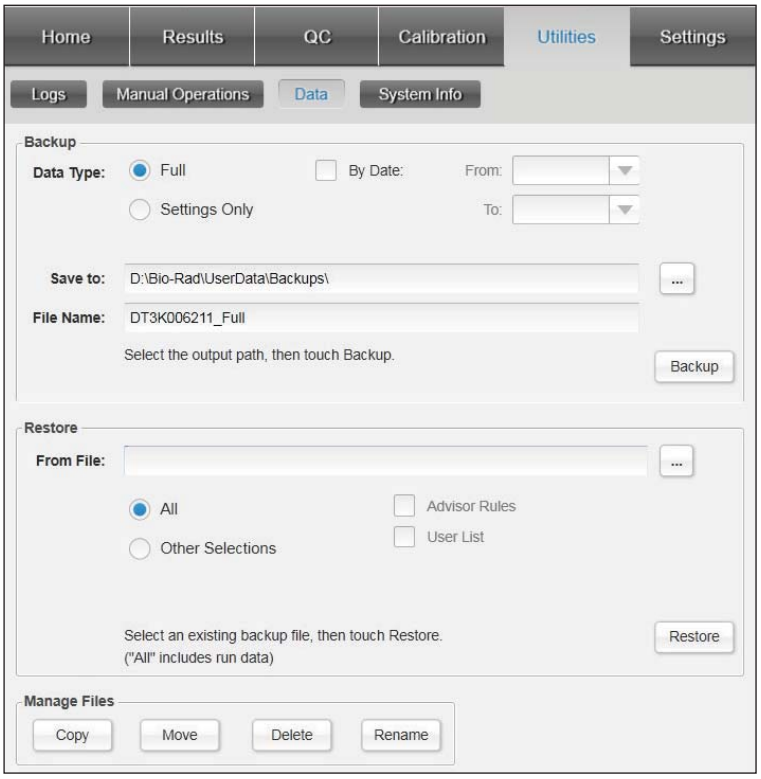


1. The instrument must be in Sleeping or Standby state.
2. Insert the flash drive containing the method file into one of the D-100 USB ports.
3. Go to the **Utilities/Manual Operations** screen.
4. Touch **Update Method**.
5. Touch the second **Update Method** button.
6. In the Select File dialog box, select the corresponding drive and the file name.
7. Touch **OK**.
8. A pop-up message confirms that the new method was imported. Touch **OK**.

4.6.3 Utilities/Data Screen

From the **Utilities/Data** screen, the following functions can be performed:

Figure 4-81: *Utilities/Data Screen*



- Backing up the database (See Section 4.6.3.1)
- Restoring a database (See Section 4.6.3.2)
- Managing files (See Section 4.6.3.3)

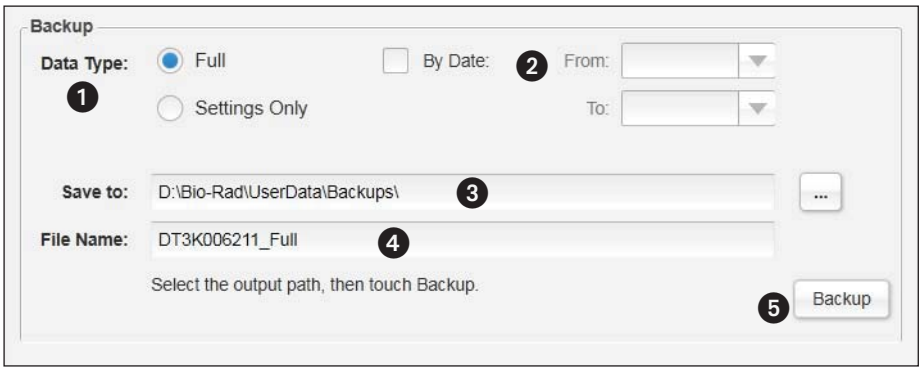
4.6.3.1 Backing Up the Database

IMPORTANT! ***It is highly recommended to back up data on a regular basis.***

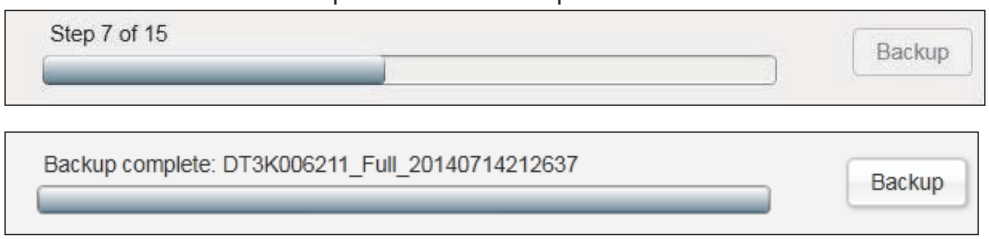
- All data is maintained in a database by the D-100 software.
- Each D-100 database is limited to approximately 100,000 results. When the database reaches its limit, results will be managed on a FIFO (first in, first out) basis as new results are added to the database. There is no warning when the database reaches its limit.
- During the backup process, the **Utilities/Data** screen cannot be exited to perform other system functions. Complete any urgent activities before backing up data.
- To view or print previous data in a backed-up database, use the **View Archive** function (see Section 4.3.3.4). It is not necessary to restore the database.

The instrument must be in Sleeping or Standby state. Go to the **Utilities/Data** screen.

Figure 4-82: *Utilities/Data Screen, Backup Dialog Box*



1	Data Type: Select **Full** to back up the entire database (i.e., configuration, test properties, sample data, and event logs) or **Settings Only** to back up the configuration and user settings only.
2	To back up data from a specific date interval, select **By Date** and then enter a "From" date plus a "To" date.
3	Save to: The default location for database backups is the D:\Bio-Rad\UserData\Backups folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
4	File Name: The default file name is "Instrument Serial Number_Full/Set_yyyymmddhhmmss.back". The File Name field is autopopulated with the instrument serial number and "Full" or "Set", depending on the data type selected. To enter a different prefix for the file name, touch the field and edit the prefix.

❺	**Backup** button: Touch this button to back up the database. A progress bar to the left of the button indicates the status and completion of the backup.  **NOTE:** *The time to back up a database depends on the size of the database and the speed of the drive. For a complete database of 100,000 results, backup may require approximately 45 minutes. More frequent backups of a smaller database using a* ***By Date*** *range will reduce the backup time.*

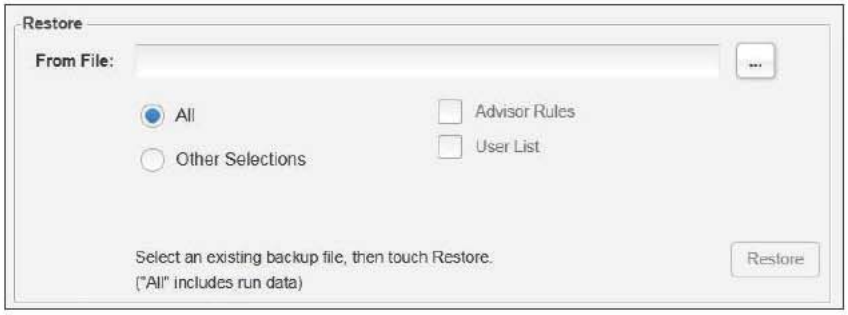
4.6.3.2 Restoring a Database

- The Restore function lets you restore a backed-up database for use on a different D-100 instrument or for troubleshooting purposes.
- During the restore process, the **Utilities/Data** screen cannot be exited to perform other system functions. Complete any urgent activities before restoring data.

NOTE: *When you restore a database, the current database is replaced by the restored database; the current database cannot be restored once it is replaced. To simply view or print previous data, use the* ***View Archive*** *function (see Section 4.3.3.4).*

1. The instrument must be in Sleeping state.
2. Go to the **Utilities/Data** screen.

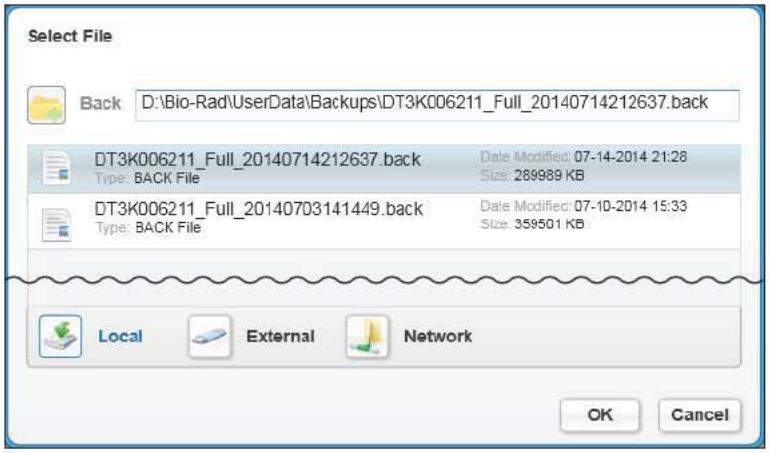
Figure 4-83: *Utilities/Data Screen, Restore Dialog Box*



3. In the Restore dialog box, touch the From File field or the browse button ... to locate the database backup file.
4. In the Select File dialog box, select the file to restore from the applicable drive/folder and touch **OK**.

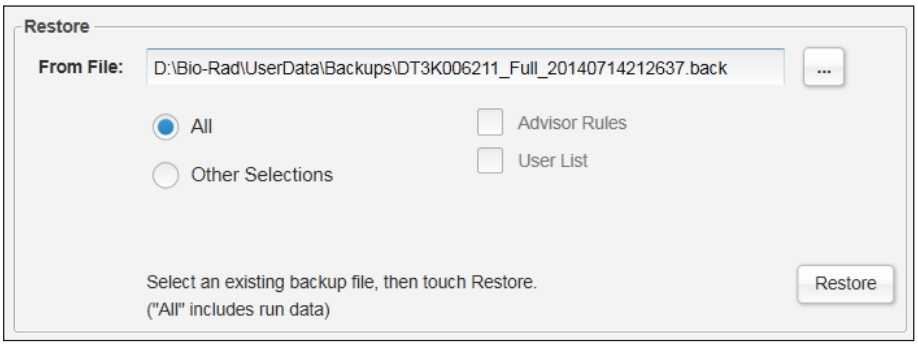
   The default location for database backup files is the D:\Bio-Rad\UserData\Backups folder. Only files with the extension .back are displayed.

Figure 4-84: *Select File Dialog Box*

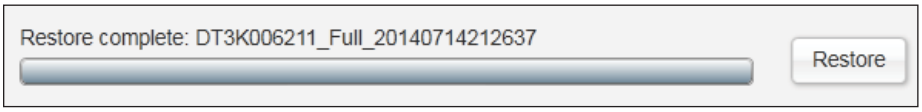


5. The file path appears in the From File field of the Restore dialog box.

Figure 4-85: *Restore Dialog Box after file selected*



6. Select one of the following options for the type of data you want to restore:
   - **All**: The entire database (i.e., configuration, test properties, sample data, and event logs) will be restored.
   - **Other Selections**: Select this option to restore only specific information from the database, and select one or both of the following checkboxes:
     - **Advisor Rules**
     - **User List**
7. Touch **Restore** to restore the file.
8. A pop-up message indicates that your current database will be replaced by the restored database and asks if you want to continue. Touch **Yes** to proceed.
9. Another pop-up message indicates that the system is restoring the database, and a progress bar to the left of the Restore button indicates the status and completion of the restore process.



NOTE: *The time to restore a database depends on the size of the database and the speed of the drive. For a database of 100,000 results, the restore process may require approximately 40 minutes.*

4.6.3.3 Managing Files

The D-100 software lets you copy, move, delete, or rename files.

Figure 4-86: *Utilities/Data Screen, Manage Files Buttons*

4.6.3.3.1 Copying Files

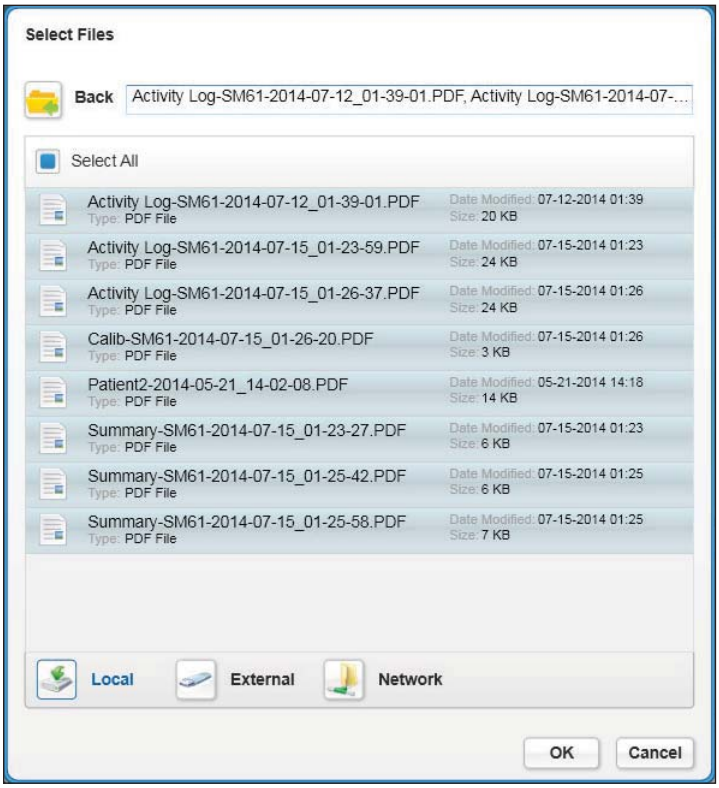
1. Go to the **Utilities/Data** screen.
2. Touch **Copy**.

Figure 4-87: *Utilities/Data Screen, Copy Files Dialog Box*

3. In the Copy Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to copy.
4. In the Select Files dialog box, select the file(s) to copy from the applicable drive/folder and touch **OK**.

   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*

Figure 4-88: *Select Files Dialog Box*



5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Copy Files dialog box.

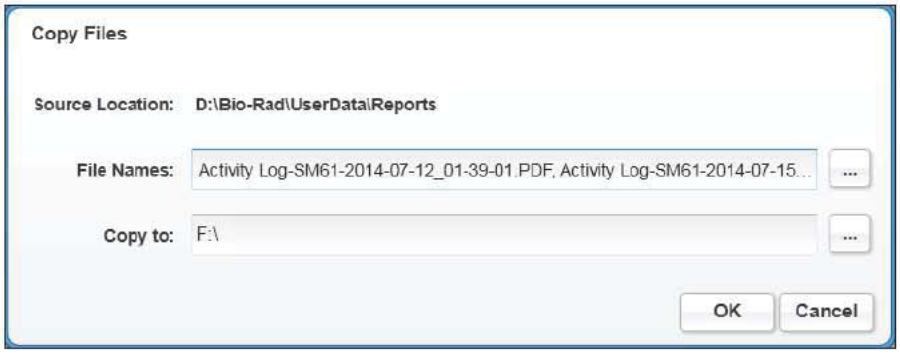
Figure 4-89: *Copy Files Dialog Box after files selected*

6. Touch the Copy to field or the browse button ... to select the destination location.
7. In the Select Folder dialog box, select the destination for the copied files and touch **OK**.

Figure 4-90: *Select Folder Dialog Box*

8. The selected destination location appears in the Copy to field of the Copy Files dialog box.

Figure 4-91: *Copy Files Dialog Box after destination selected*



9. Touch **OK** to copy the file(s).

4.6.3.3.2 Moving Files

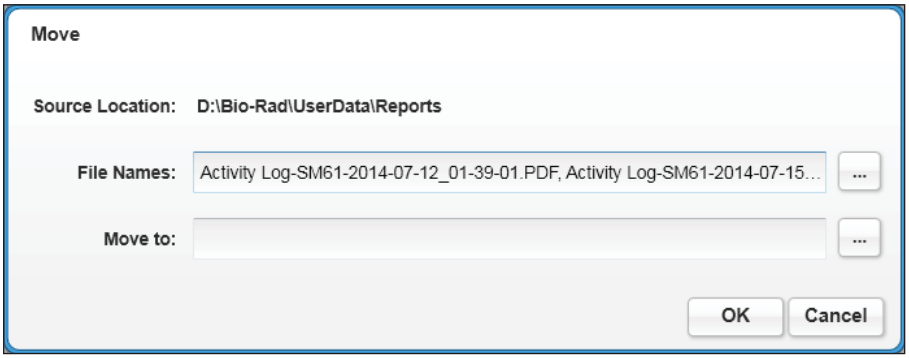
1. Go to the **Utilities/Data** screen.
2. Touch **Move**.

Figure 4-92: *Utilities/Data Screen, Move Files Dialog Box*

3. In the Move Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to move.
4. In the Select Files dialog box (see Figure 4-88), select the file(s) to move from the applicable drive/ folder and touch **OK**.

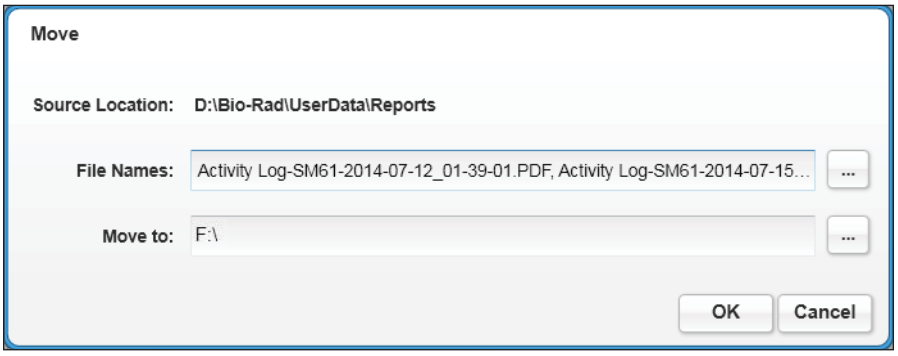
   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*
5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Move Files dialog box.

Figure 4-93: *Move Files Dialog Box after files selected*



6. Touch the Move to field or the browse button ... to select the destination location.
7. In the Select Folder dialog box (see Figure 4-90), select the destination for the moved files and touch **OK**.
8. The selected destination location appears in the Move to field of the Move Files dialog box.

Figure 4-94: *Move Files Dialog Box after destination selected*



9. Touch **OK** to move the file(s).

4.6.3.3.3 Deleting Files

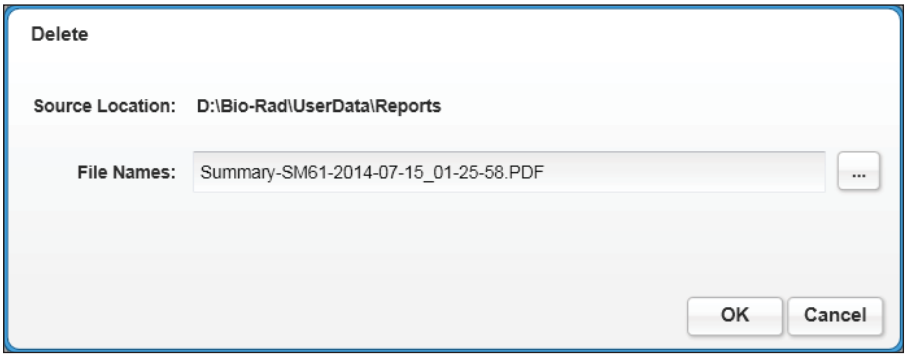
1. Go to the **Utilities/Data** screen.
2. Touch **Delete**.

Figure 4-95: *Utilities/Data Screen, Delete Files Dialog Box*

3. In the Delete Files dialog box, touch the File Names field or the browse button ... to locate the file(s) to delete.
4. In the Select Files dialog box (see Figure 4-88), select the file(s) to delete from the applicable drive/folder and touch **OK**.

   NOTE: *Touch the **Select All** checkbox to select all files within a folder.*
5. The selected file path appears in the Source Location field and the selected files appear in the File Names field of the Delete Files dialog box.

Figure 4-96: *Delete Files Dialog Box after files selected*



6. Touch **OK** to delete the file(s).
7. A pop-up message asks if you are sure you want to delete the files. Touch **Yes** to delete.

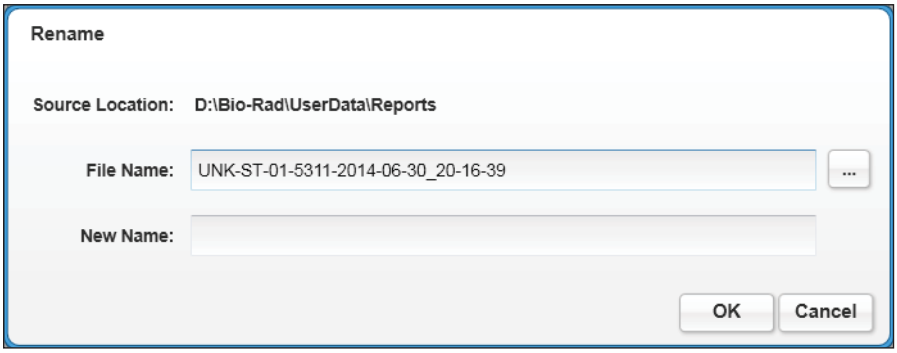
4.6.3.3.4 Renaming Files

1. Go to the **Utilities/Data** screen.
2. Touch **Rename**.

Figure 4-97: *Utilities/Data Screen, Rename File Dialog Box*

3. In the Rename File dialog box, touch the File Name field or the browse button ... to locate the file to rename.
4. In the Select File dialog box (see Figure 4-88), select the file to rename from the applicable drive/ folder and touch **OK**.
5. The selected file path appears in the Source Location field and the selected file appears in the File Name field of the Rename File dialog box.

Figure 4-98: *Rename File Dialog Box after file selected*



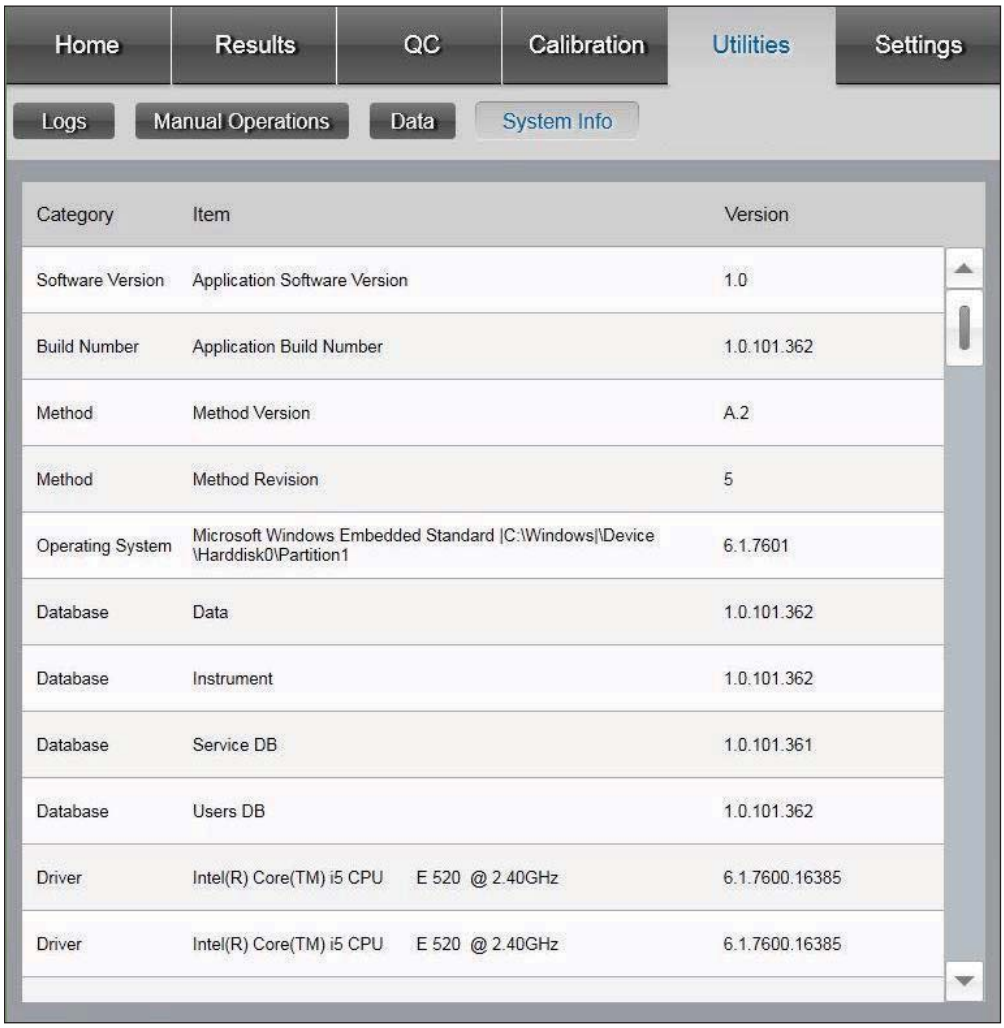
6. Touch the New Name field to enter the new file name.
7. Touch **OK**.

4.6.4 Utilities/System Info Screen

The **Utilities/System Info** screen displays the version numbers for all D-100 software, method, database, and hardware/firmware items currently installed on the instrument.

Use the scroll bar to move up/down the table.

Figure 4-99: *Utilities/System Info Screen*



4.7 Settings Tab

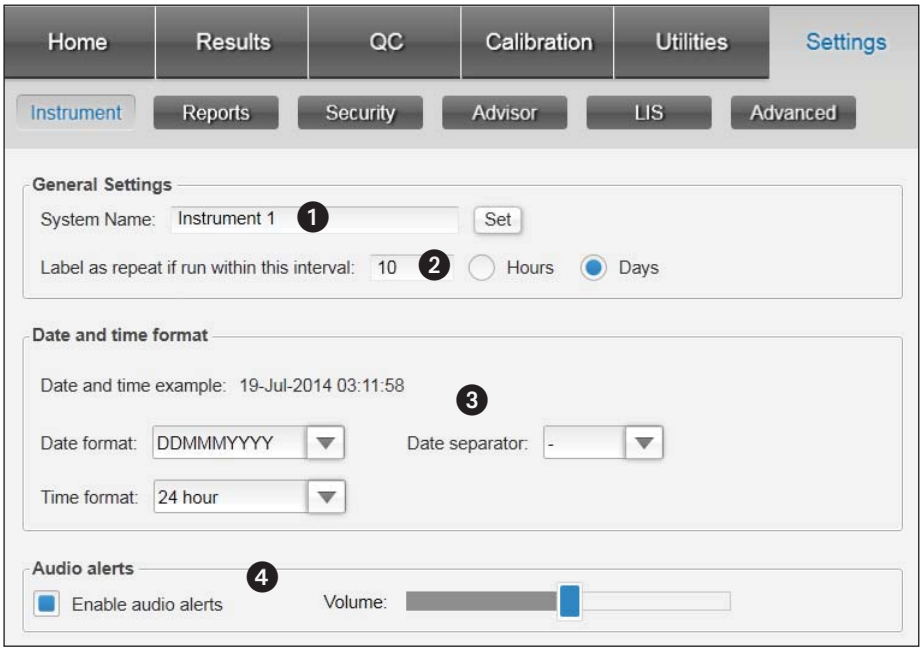
The **Settings** tab includes the following secondary navigation buttons:

- **Instrument** (See Section 4.7.1)
- **Reports** (See Section 4.7.2)
- **Security** (See Section 4.7.3)
- **Advisor** (See Section 4.7.4)
- **LIS** (See Section 4.7.5)
- **Advanced** (See Section 4.7.6)

NOTE: *All settings in the **Settings** tab require Level3 access to modify, except changing one's own password in the **Security** screen.*

4.7.1 Settings/Instrument Screen

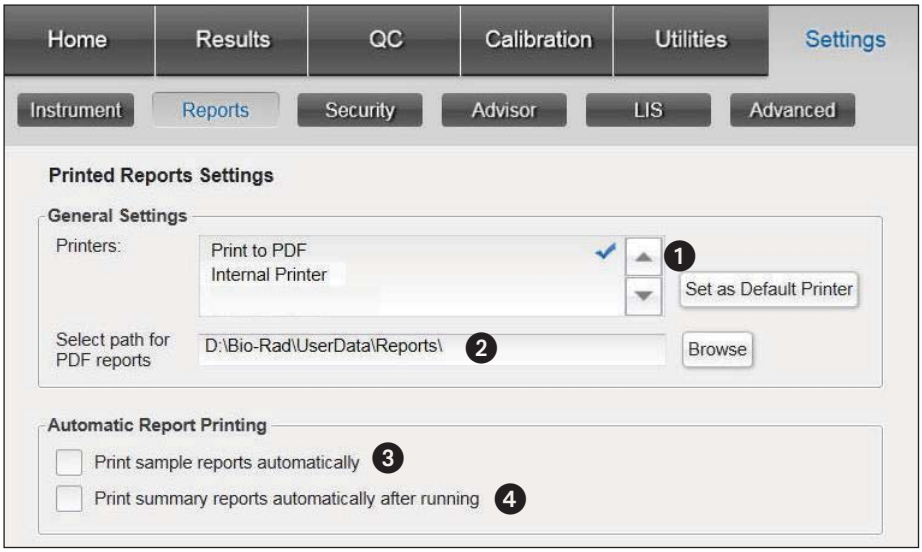
Figure 4-100: *Settings/Instrument Screen*



1	System Name: enter a name for the instrument (maximum: 12 characters). Touch **Set** to save the name.
2	A sample result will be labeled as a repeat (i.e., ⟳) if the sample is processed multiple times within a defined interval. Enter a number and select **Hours** or **Days**.
3	Select the preferred date format, date separator, and time format from the corresponding drop-down lists.
4	**Enable audio alerts**: If this checkbox is selected, audible alarms will accompany important instrument messages or conditions. Select the preferred volume for the alarms; moving the slider to the right increases the volume.

4.7.2 Settings/Reports Screen

Figure 4-101: *Settings/Reports Screen*

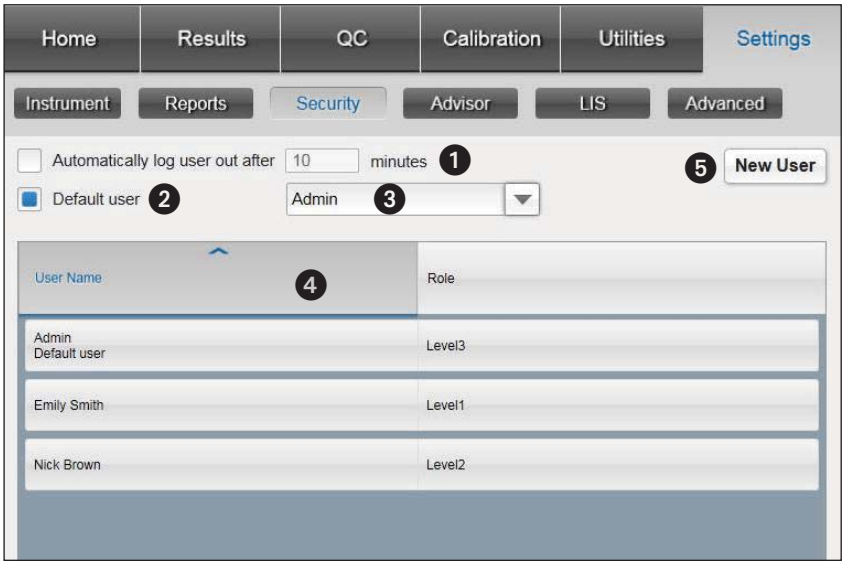


1	Printers: All printers configured for your instrument are listed. Define the default printer by selecting a printer from the drop-down list and then touch **Set as Default Printer**. The default printer is indicated by a blue checkmark and moves to the top of the list.
2	Select path for PDF reports: The default location for all reports (e.g., results, Activity Logs, rules sets, etc.) printed to PDF is the D:\Bio-Rad\UserData\Reports folder. To select a different location, touch **Browse**. In the Select Folder dialog box, select the drive/folder and touch **OK**.
3	**Print sample reports automatically**: Select this checkbox to automatically print the sample report after each sample is processed.
4	**Print summary reports automatically after running**: Select this checkbox to automatically print a summary report after the run is finished.

4.7.3 Settings/Security Screen

The system provides user access through user name and password combinations. This access information is displayed and managed in the **Settings/Security** screen. This screen can be accessed at any time with the instrument in any state.

Figure 4-102: *Settings/Security Screen*

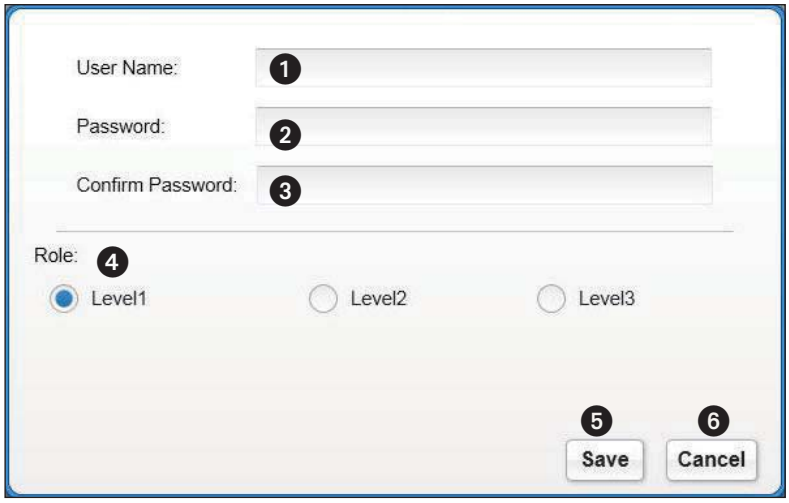


1	**Automatically log user out:** To have the system automatically log out the user after a period of time, select this checkbox and enter the number of minutes. **NOTE:** *If automatic log out occurs during a critical process (i.e., Backup or Restore), the system will delay the log out until the process is completed.*
2	**Default user:** To define a default user profile for the system, select this checkbox and select a user name from the drop-down list. This feature allows system use without manual log-in/password entry. For example, setting a Level 1 user as the default user allows any user to perform daily operation functions without logging in; access to higher level features would require log-in. The default user is automatically logged in when the system is powered on or if a logged-in user logs out.
3	The system is configured with a predefined user profile: Admin. The password is provided to authorized users by Bio-Rad Technical Service. This administrator profile provides full access to all customer-permissible features. This user profile cannot be edited or deleted.
4	Each user name and role (i.e., access level) is displayed in this table. **NOTE:** • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To edit or delete a user profile, touch the row. See Section 4.7.3.2.*
5	**New User** button: To add a new user to the system, touch this button. See Section 4.7.3.1.

4.7.3.1 Adding a New User Profile

From the **Settings/Security** screen, touch **New User**. The User dialog box appears. Only authorized users can add a user profile.

Figure 4-103: *User Dialog Box, New User*



1	User Name: enter the new user name (maximum: 32 characters).
2	Password: enter a password (maximum: 16 characters). **NOTE:** *When logging into the system, user names are not case-sensitive; passwords are case-sensitive.*
3	Confirm Password: re-enter the password to confirm.

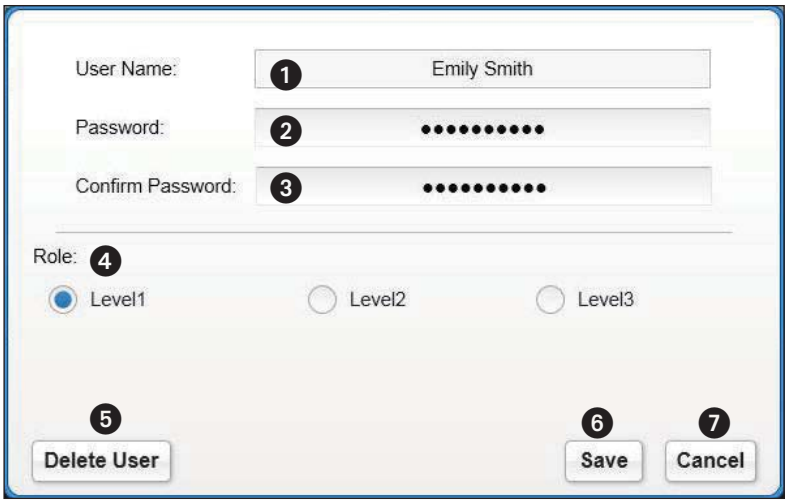
❹	Role: select an access level for the user.	
	Level3 (Supervisor)	Permission to access all customer-permissible features, except cannot restore a database (accessible to Admin user only). Level3 users can release flagged results, modify all instrument settings including Advisor and QC rules and settings, and run with expired consumables.
	Level2 (Lead Technician)	Same permissions as Level3, except the following: • Cannot modify any settings in the **Settings** tab (except own user password) • Cannot modify any settings in the **QC/Settings** or **QC/Rules** screens • Cannot exclude QC data points from calculations • Cannot release or reject flagged results • Cannot run with expired calibrators • Cannot manage files • Cannot calibrate the touchscreen Level2 users can release non-flagged results, enter new QC lot information, back up a database, and export data.
	Level1 (Secondary Technician)	Same permissions as Level2, except the following: • Cannot release or reject any results • Cannot add or edit QC lots • Cannot back up a database • Cannot export data Level1 users can replace consumables, calibrate the D-100, perform a run, and view, print, and add comments to results.
❺	**Save** button: Touch this button to save the user profile.	
❻	**Cancel** button: Closes the dialog box without saving any information.	

4.7.3.2 Editing or Deleting a User Profile

From the **Settings/Security** screen, touch the desired user row in the table. The User dialog box appears. Only authorized users can change or delete a user profile.

NOTE: *Users cannot change their own role or delete themselves; another user with appropriate permission must change the role or delete the user profile.*

Figure 4-104: *User Dialog Box, Existing User*



1	User Name: The user name cannot be edited.
2	Password: To change the password, touch the field and enter a new password (maximum: 16 characters).
3	Confirm Password: If applicable, re-enter the new password to confirm.
4	Role: To change the user's permissions, select the applicable access level.
5	**Delete User** button: To delete the user profile from the system, touch this button.
6	**Save** button: Touch this button to save the changes.
7	**Cancel** button: Closes the dialog box without saving any changes.

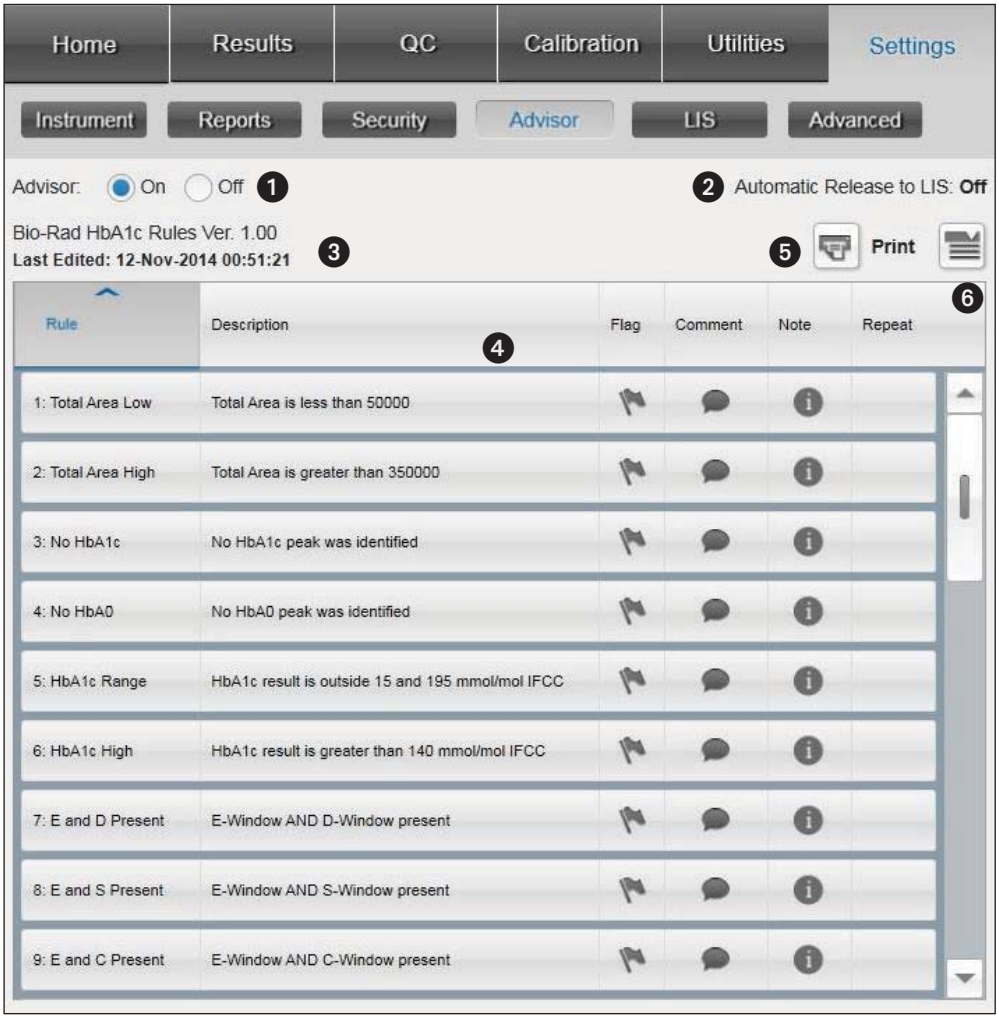
4.7.4 Settings/Advisor Screen

The D-100 System is configured with a set of predefined rules (i.e., "Advisor") that facilitates results reporting by identifying sample results that require further review before they are released to the LIS.

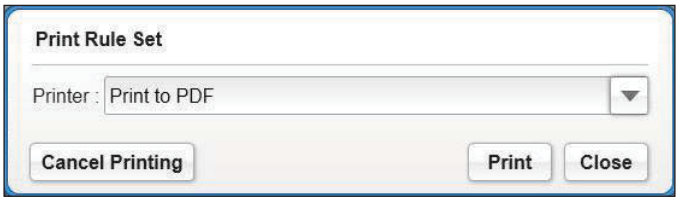
Bio-Rad provides a default rules set based on performance claims in the assay Instructions For Use and additional parameters. See Appendix C for rule details.

The **Settings/Advisor** screen provides access to all rules in table format.

Figure 4-105: *Settings/Advisor Screen*



1	Advisor: You have the option to enable (**On**) or disable (**Off**) rules processing.
2	Indicates whether the Automatic Release to LIS function for patient results is selected (On) or not selected (Off); this function is set in the **Settings/LIS** screen. See Section 4.7.5.
3	Indicates the name of the rules set currently in use and the date/time it was last edited. **NOTE:** *Once a named rule set has been modified, the saved name is no longer displayed. It becomes a modified rule set identified only by the date/time of the last saved edit.*

❹	Each Advisor rule is displayed as a row in this table. The rule name and description are provided. Icons in the last 4 columns indicate the action(s) that will occur when a result triggers the rule: • Flag : The result will be flagged. • Comment : The result will include a comment. • Note : The result will include a note. • Repeat : The sample will automatically be repeated. **NOTE:** • *The table can be sorted by column. Touch the column header; an arrow appears in the column header, indicating ascending or descending order.* • *To view or edit the rule details, touch the row. See Section 4.7.4.1.*
❺	**Print** button: Touch to print the rules set. ***Figure 4-106:*** *Print Rule Set Dialog Box*  • **Printer**: Select an option from the drop-down list (e.g., **Internal Printer** or **Print to PDF**). The default location for rules sets printed to PDF is the D:\Bio-Rad\UserData\Reports folder. The location is set in the **Settings/Reports** screen (see Section 4.7.2). The default file name for the rules set (i.e., Advisor Report) is "Advisor-System Name-yyyy-mm-dd_hh-mm-ss.pdf". • **Print** button: Touch this button to print the rules set. **NOTE:** *After touching **Print**, the dialog box closes. To stop the printing in process, touch **Print** in the **Settings/Advisor** screen, then touch **Cancel Printing** in the dialog box.* • **Close** button: Closes the dialog box without printing.
❻	Advisor Menu button: See Section 4.7.4.2.

4.7.4.1 Viewing/Editing Rule Details

From the **Settings/Advisor** screen, touch the desired rule row in the table. The Rule Details screen appears.

NOTE:

- *Rules can be edited, if necessary, by authorized users only.*
- *Rules cannot be edited while the instrument is in Running state.*
- *Any use of a rule, whether modified or provided as a default rule, is undertaken at the Customer's sole risk. The content and use of each rule depends on the Customer's parameters for processing patient test results. Because the Customer's parameters cannot possibly be known by Bio-Rad Laboratories, Inc., the Customer undertakes sole responsibility for the contents, and resulting actions or uses, of any rule.*

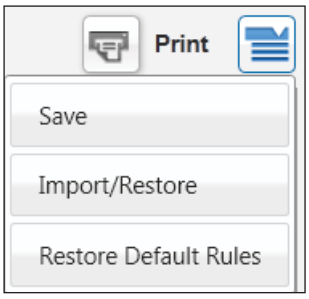
Figure 4-107: *Rule Details Screen, HbA1c Range*

1	Rule Set: The name of the rules set currently in use. **NOTE:** *Once a named rule set has been modified, the saved name is no longer displayed. It becomes a modified rule set identified only by the date/time of the last saved edit.*
2	Rule: The name of the rule.
3	**Enable Rule**: The rule will be included in the rules processing if this checkbox is selected; clear the checkbox to disable the rule.
4	Conditions: The rule parameters are displayed. Parameters vary by rule.

❺	Actions: The rule actions are displayed. The available actions vary by rule. • **Flag Sample**: If this checkbox is selected, the result will be flagged when the rule is triggered. • **Comment**: If this checkbox is selected, the result will include the comment shown when the rule is triggered. Comments can be transmitted to the LIS. • **Note to user**: If this checkbox is selected, the result will include the note shown when the rule is triggered. Notes are not transmitted to the LIS. • **Repeat Sample**: If this checkbox is selected, the sample will automatically be repeated one time (if it is available for processing) when the rule is triggered.
❻	**Save** button: Touch this button to save the changes to the rule. This button is enabled only if the rule has been edited.
❼	**Cancel** button: Closes the Rule Details screen without saving any changes.

4.7.4.2 Advisor Menu

Figure 4-108: *Advisor Menu*



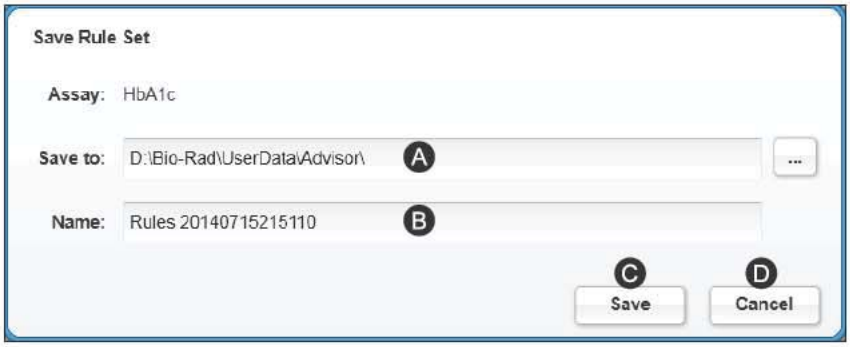
From the **Settings/Advisor** screen, touch . The Advisor Menu appears. There are 3 actions available:

- **Save** (See Section 4.7.4.2.1)
- **Import/Restore** (See Section 4.7.4.2.2)
- **Restore Default Rules** (See Section 4.7.4.2.3)

4.7.4.2.1 Saving a Rules Set

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch .
3. From the Advisor Menu, touch **Save**.

Figure 4-109: *Save Rule Set Dialog Box*



A	Save to: The default location for saving rules sets is the D:\Bio-Rad\UserData\Advisor folder. To select a different location, touch the browse button ... . In the Select Folder dialog box, select the drive/folder and touch **OK**.
B	Name: The default file name is "Rules yyyymmddhhmmss.brs". The date/time is when the rule set was last edited. Touch the field to change the file name.
C	**Save** button: Touch this button to save the rules set.
D	**Cancel** button: Closes the dialog box without saving the rules set.

4.7.4.2.2 Importing/Restoring a Rules Set

The **Import/Restore** function lets you import a saved rules set for use on a different D-100 instrument or import default rules set updates from Bio-Rad.

NOTE: *When you import a rules set, the current rules set is replaced by the imported rules set; the current rules set cannot be restored unless it is saved.*

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch .
3. From the Advisor Menu, touch **Import/Restore**.

Figure 4-110: *Import Rule Set Dialog Box*

A	Rules to Import: Touch this field or the browse button ... to locate the rules set to import. In the Select File dialog box, select the file to restore from the applicable drive/folder and touch **OK**. The default location for rules set files is the D:\Bio-Rad\UserData\Advisor folder. Only files with the extension .brs are displayed.
B	The rules set name and date/time last edited appear in the Import Rule Set dialog box. ***Figure 4-111:*** *Import Rule Set Dialog Box after file selected* 
C	**Import** button: Touch this button to import the rules set. A pop-up message indicates that your current rules will be replaced by the imported rules and asks if you want to continue. Touch **Yes** to proceed.
D	**Cancel** button: Closes the dialog box without saving the rules set.

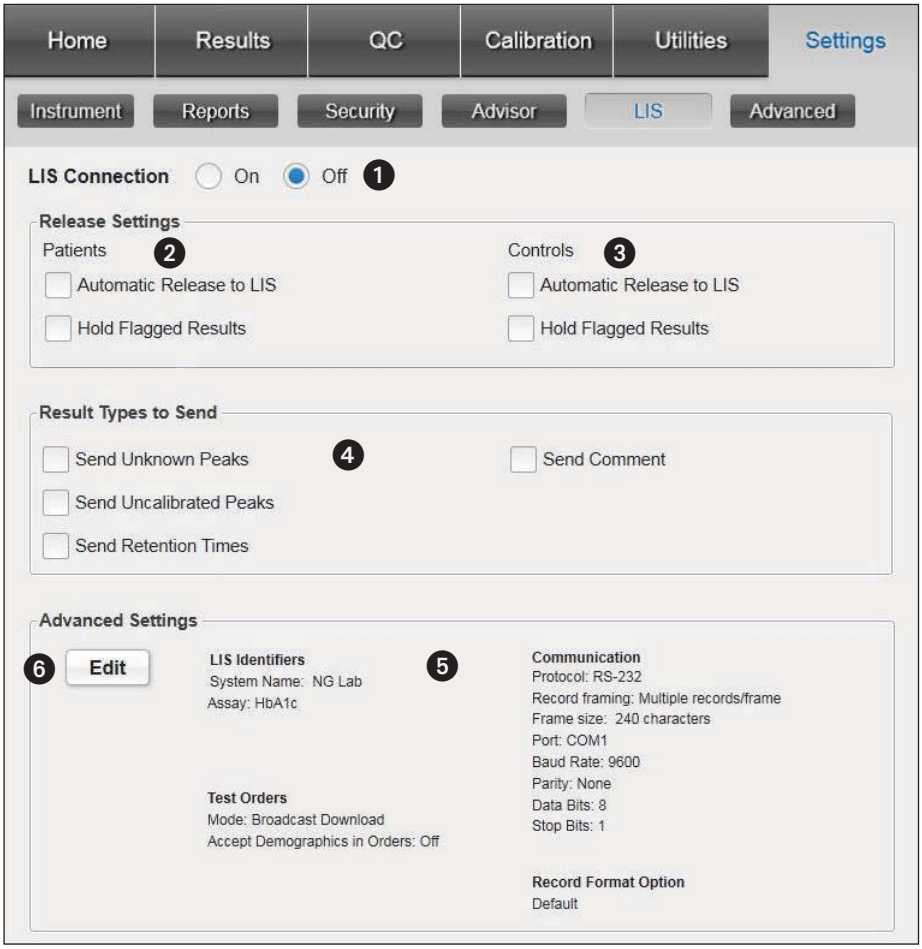
4.7.4.2.3 Restoring the Default Rules Set

The Bio-Rad Default Rules Set can be restored as follows:

1. The instrument must be in Sleeping or Standby state.
2. From the **Settings/Advisor** screen, touch  .
3. From the Advisor Menu, touch **Restore Default Rules**.
4. A pop-up message indicates that your current rules will be replaced by the default rules and asks if you want to continue. Touch **Yes** to proceed.

4.7.5 Settings/LIS Screen

Figure 4-112: *Settings/LIS Screen*

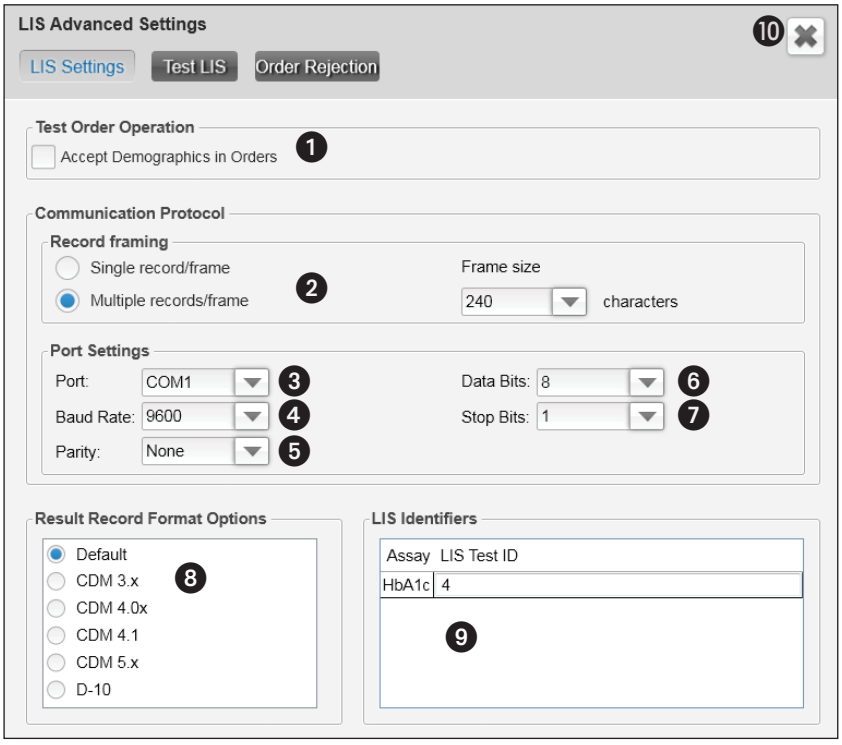


1	LIS Connection: You have the option to enable (**On**) or disable (**Off**) the LIS connection.
2	Release Settings for Patients: • To have the system automatically release patient results to the LIS, select the **Automatic Release to LIS** checkbox. • To have the system hold flagged patient results for manual review, select the **Hold Flagged Results** checkbox.

❸	Release Settings for Controls: • To have the system automatically release QC results to the LIS, select the **Automatic Release to LIS** checkbox. • To have the system hold flagged QC results, select the **Hold Flagged Results** checkbox.
❹	Result Types to Send: You have the option to send specific types of data. • **Send Unknown Peaks**: Peaks that are detected but not identified are labeled "Unknown". If transmission of unknown peaks is required, select this checkbox. **NOTE:** ***Send Uncalibrated Peaks*** *must also be selected for unknown peaks to be transmitted to the LIS.* • **Send Uncalibrated Peaks**: For the HbA1c assay, only the HbA1c peak is calibrated. If transmission of uncalibrated peaks is required, select this checkbox. • **Send Retention Times**: If transmission of peak retention times is required, select this checkbox. • **Send Comment**: If transmission of result comments is required, select this checkbox.
❺	Advanced Settings: Other LIS settings are displayed.
❻	**Edit** button: To edit any of the LIS Advanced Settings, touch this button. The LIS Advanced Settings are organized under 3 tabs: • **LIS Settings** (See Section 4.7.5.1) • **Test LIS** (See Section 4.7.5.2) • **Order Rejection** (See Section 4.7.5.3)

4.7.5.1 LIS Settings Tab

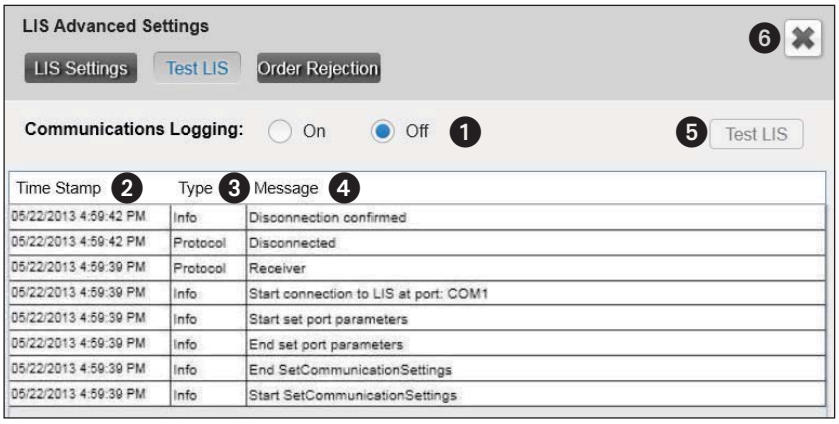
Figure 4-113: *LIS Advanced Settings/LIS Settings Tab*



❶	**Accept Demographics in Orders**: To allow patient demographic data to be included with the test order, this checkbox must be selected.
❷	Record framing: Select one option. • **Single record/frame** • **Multiple records/frame** (default); also select the frame size (**240** or **63993** characters) from the drop-down list. Default is 240.
❸	Port: Select the communication port (**COM1**, **COM2**, etc.) from the drop-down list.
❹	Baud Rate: Select the baud rate from the drop-down list. Default is 9600.
❺	Parity: Select a parity option (**None, Odd, Even, Mark**, or **Space**) from the drop-down list. Default is None.
❻	Data Bits: Select the data bits (**4, 5, 6, 7**, or **8**) from the drop-down list. Default is 8.
❼	Stop Bits: Select the stop bits (**None, 1, 2**, or **1.5**) from the drop-down list. Default is 1.
❽	Result Record Format Options: You can select from a list of existing Bio-Rad result record formats to match the format your lab is currently using.
❾	LIS Test ID: Enter the Test ID for the assay. Default is 4 for the HbA1c assay.
❿	Touch ✖ to close the tab.

4.7.5.2 Test LIS Tab

Figure 4-114: *LIS Advanced Settings/Test LIS Tab*

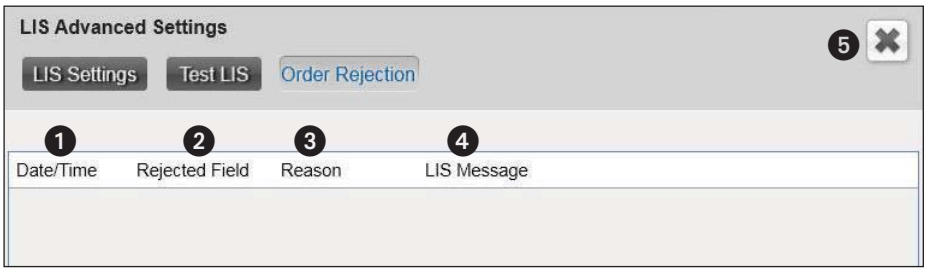


1	**Communications Logging**: You have the option to enable (**On**) or disable (**Off**) the logging of Low Level transmissions between the instrument and the LIS (both directions). **NOTE:** *Disable (turn **Off**) Communications Logging when you are finished checking LIS communications.*
2	Time Stamp: The date and time of the transmission.
3	Type: The type of transmission (e.g., Info, Protocol, Send, Receive).
4	Message: The full contents of the transmission.
5	**Test LIS** button: Touch this button to reset the LIS ports and send a test message to the LIS to verify the connection is working and the LIS is responding.
6	Touch ✖ to close the tab.

4.7.5.3 Order Rejection Tab

Every test order sent by the LIS that is rejected by the D-100 System is logged in table format in the **Order Rejection** tab.

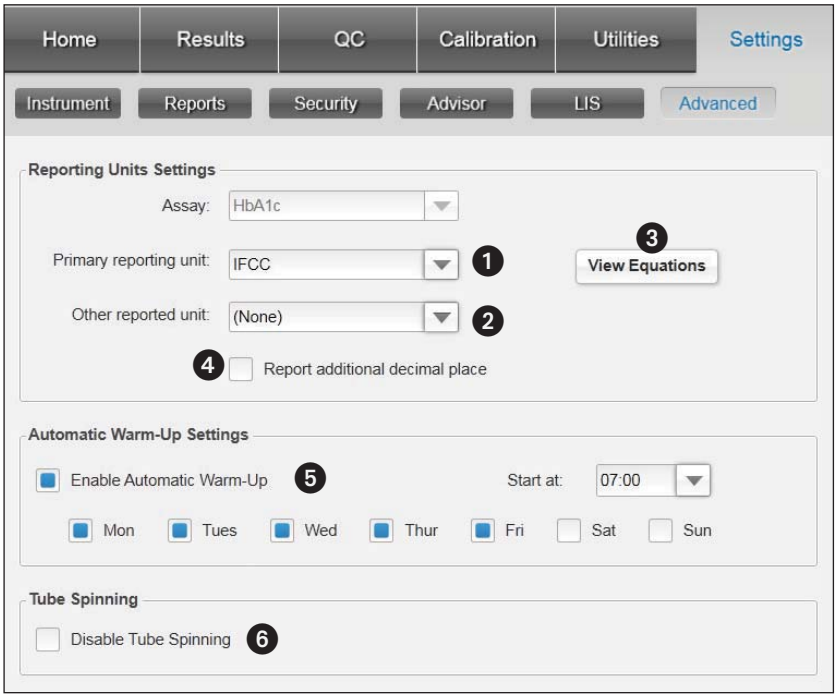
Figure 4-115: *LIS Advanced Settings/Rejection Log Tab*

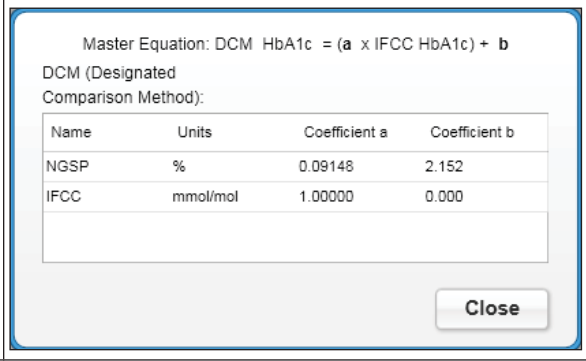


1	Date/Time: The date and time the order was rejected.
2	Rejected Field: “LIS field rejected” or “LIS message rejected”.
3	Reason: A description of why the field or message was invalid.
4	LIS Message: The LIS message that was rejected or that contains the field that was rejected.
5	Touch ✖ to close the tab.

4.7.6 Settings/Advanced Screen

Figure 4-116: *Settings/Advanced Screen*



1	Primary reporting unit: Select your preferred HbA1c primary reporting unit [**IFCC** (mmol/mol) or **NGSP** (%)] from the drop-down list.	
2	Other reported unit: If you want to report using both units, select the secondary reporting unit from this drop-down list.	
3	***Figure 4-117:*** *Master Equation*  	**View Equations**: Touch this button to view the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) standardized HbA1c master equation and DCMs (Designated Comparison Methods) supported by D-100.
4	**Report additional decimal place:** To display and report results with an additional decimal place, select this checkbox. Patient and QC results are reported at the same decimal precision.	
	Default decimal precision • IFCC = xxx (e.g., 43 mmol/mol) • NGSP = xx.x (e.g., 6.1%)	*Additional decimal precision* • IFCC = xxx.x (e.g., 42.7 mmol/mol) • NGSP = xx.xx (e.g., 6.06%)
5	**Enable Automatic Warm-Up:** To have the system automatically perform startup operations, select this checkbox. Also select the checkbox(es) for the applicable day(s) of the week and select the start time.	

❻	**Disable Tube Spinning:** You have the option to disable the tube spinning if you use sample racks from another system that do not allow tube spinning (i.e., tube positions do not have rotary bottoms).

5 Run Operations

5.1 Overview of Sample Analysis

5.1.1 Processing Primary Sample Tubes

a. Barcode information for the sample tube is read by the barcode reader.
b. The sample probe pierces the tube cap, then withdraws sample from the tube.
c. The sample is diluted in the dilution well.
d. The sample is injected into the buffer stream (analytical flow path).
e. The sample probe and line are flushed to prevent cross-contamination between samples.
f. The sample and buffer mixture flows through the cartridge, where the sample is separated into its constituents.
g. The sample constituents and buffer flow through the detector, where the absorbance of each sample constituent is measured.
h. The detector output is plotted as a chromatogram in a result report.
i. A system flush removes any residual sample components.

5.1.2 Processing Prediluted Samples

a. A sensor detects the presence of a microvial adapter in the rack.
b. If present, barcode information on the microvial or adapter is read by the barcode reader.
c. The sample probe pierces the microvial cap, then withdraws sample from the microvial.
d. The sample is injected into the buffer stream (analytical flow path).
e. The sample probe and line are flushed to prevent cross-contamination between samples.
f. The sample and buffer mixture flows through the cartridge, where the sample is separated into its constituents.
g. The sample constituents and buffer flow through the detector, where the absorbance of each sample constituent is measured.
h. The detector output is plotted as a chromatogram in a result report.
i. A system flush removes any residual sample components.

5.2 System Startup

NOTE: *It is not necessary to perform System Startup on a daily basis. During normal operation, it is recommended to keep the D-100 powered on.*

1. Turn on the D-100 power switch (on the rear of the instrument). If the instrument power switch is already turned on, press the soft power button on the front of the instrument to activate the instrument from standby power mode to full operating power. See Figure 2-1, No. 3.
2. The Bio-Rad software startup screen will display while the software is loading.
3. To log into the system, see Section 4.1.1, No. 5.
4. After the software is loaded, touch **Run** in the banner to initiate the system warm-up. The instrument will transition from Sleeping to Warming Up state.

   NOTE: *To enable Automatic Warm-Up, see Section 4.7.6.*
5. After the warm-up is complete, the instrument transitions to Standby state. It is now ready to run.

5.3 Running the Calibrator Pack

See the assay Instructions For Use for information regarding storage of the Calibrator Pack and calibration frequency.

1. The instrument must be in Sleeping or Standby state.
2. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
3. Insert the Calibrator Pack in the dedicated position of the Stat rack with the barcodes facing you.
4. Insert QC samples in positions 1–3 of the Stat rack as instructed in Section 5.4.

   NOTE:

   - *All barcodes are read before processing the first sample in the Stat rack. The Calibrator Pack is processed first, followed by the other Stat rack samples in sequential order.*
   - *Verify that the control lots and barcodes in the **QC/QC Lots** screen match the QC samples being run (see Section 4.4.4).*
5. Touch ⏏ **Load**. The Stat rack is loaded into the Stat Area. The calibrator information is automatically entered from the barcode.
6. The Calibration dialog box appears (see Figure 4-71). Touch **Calibrate Now**.

   NOTE:

   - *If the Calibrator Pack was previously run/reconstituted on the instrument, the **Calibrate without reconstituting** checkbox must be selected.*
   - *Calibration takes approximately 30 minutes. Once the calibration run is started, it cannot be stopped.*
7. After the Stat rack samples are finished being processed, touch ⏏ **Open**.
8. Remove the Calibrator Pack and QC samples from the Stat rack.

5.4 Running Controls (QC)

See the assay Instructions For Use for information regarding preparation of the controls and recommended QC frequency.

1. Check the message panel for red or yellow messages and address as needed.
2. Before running a new lot of QC, go to the **QC/QC Lots** screen (while in Sleeping or Standby state). Touch **Add Control**. Enter the necessary information in the Add Control Lot dialog box (see Section 4.4.4.1).
3. Insert the prediluted QC microvials into the appropriately barcode-labeled microvial adapters.
4. The prediluted QC samples can be run in the Stat Area or in a Sysmex rack.
   a. To run in the Stat Area, touch ⏏ **Open** in the **Home** screen to retrieve the Stat rack. Insert the microvial adapters in positions 1–3 of the Stat rack with the barcodes facing you so they are visibly displayed through the rack slots. Touch ⏏ **Load**. If not in Running state, touch **Run**. After the QC samples are finished being processed, touch ⏏ **Open**. Remove the QC microvials and adapters from the Stat rack.
   b. To run in a Sysmex rack, insert the microvial adapters with the magnet facing the back of the rack. Place the rack in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you. If not in Running state, touch **Run**. Remove the processed Sysmex rack from the output area.

   NOTE: *To avoid processing empty sample positions, ensure empty barcoded microvial adapters are removed from racks before the next run.*

5.5 Running Patient Samples

1. Check the message panel for red or yellow messages and address as needed.
2. Insert the primary sample tubes in the Sysmex racks.

   NOTE:

   - *If the sample is in an abnormal size/type tube, or if the height of the sample in the tube appears to be ≤1 cm, then the sample must be prediluted and placed in a microvial adapter. See the assay Instructions For Use for Specimen Preparation instructions. Insert the microvial adapter with the magnet facing the back of the rack.*
   - *All prediluted sample microvials and primary sample tubes should be capped before loading them onto the system.*
3. Place the racks in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you.
4. If not in Running state, touch **Run**.

5. Remove the processed Sysmex racks from the output area.

   NOTE:

   - *A maximum of 9 racks can be positioned in the rack input area at one time; a 10th rack can be inserted after the run starts and the 1st rack is in the sampling position.*
   - *A maximum of 10 racks are permitted in the rack output area at one time. You may continue adding racks to the input area as you remove completed racks from the output area.*

5.6 Running Urgent (Stat) Samples

Priority samples can be analyzed during a run in progress.

1. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
2. Insert the sample(s) (i.e., primary sample tubes or prediluted samples in microvial adapters) in positions 1–3 of the Stat rack, with the barcode(s) facing you.
3. Touch ⏏ **Load**. The Stat rack is loaded into the Stat Area.
4. The Stat sample(s) will automatically be processed after completion of the last aspirated sample. After the Stat sample(s) are finished being processed, the instrument resumes processing samples in Sysmex racks.
5. After the Stat samples are finished being processed, touch ⏏ **Open**.
6. Remove the samples from the Stat rack.

5.7 Handling Unread Barcodes

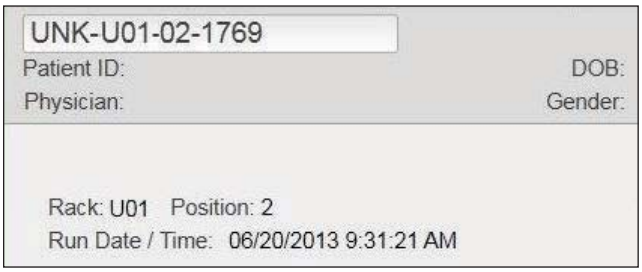
5.7.1 Unread Sample Barcodes

When a patient sample has no barcode or has a damaged barcode that cannot be read, the system automatically generates a “UNK” (Unknown) Sample ID based on the Rack ID (i.e., Sysmex rack number or ST for Stat rack), tube position, and unique injection number (e.g., UNK-001-01-injection number).

The Sample ID can be edited after the sample analysis is complete.

1. From the **Results** screen, touch the desired sample result row in the table. The Result Details screen appears.

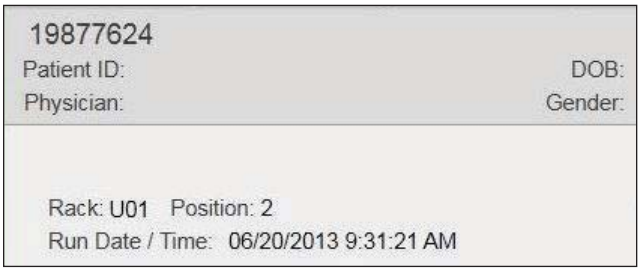
   Figure 5-1: *Result Details Screen, Editable Sample ID Field*



2. Touch the Accession Number/Sample ID field and enter the correct sample ID.
3. The system automatically looks for a corresponding order from the LIS and enters the Patient ID, Physician, DOB, and/or Gender if present.
4. The Confirm New Barcode dialog box appears. Touch **Confirm** to save the corrected Sample ID; once the Sample ID is saved, it cannot be edited.

Figure 5-2: *Confirm New Barcode Dialog Box*

Figure 5-3: *Result Details Screen, Corrected Sample ID*



5.7.2 Unread Rack Barcodes

When a Sysmex rack barcode is missing or cannot be read, the system automatically generates a 3-character Rack ID (e.g., U01, U02, up to U99). The “U” indicates unknown; the number is incremented for each auto-generated ID; the number is reset to 01 on system startup and whenever it reaches 99.

5.8 Repeating Samples

Under certain circumstances, samples will be automatically repeated (if available):

- If a sample's total area exceeds a limit defined in the method, the results for subsequent samples potentially impacted (due to risk of carryover) are not reported. The system is automatically flushed and the subsequent samples are repeated.
- If a sample's HbA_{1c} level exceeds a certain limit defined in the method, the result for the subsequent sample is not reported (due to risk of carryover); the subsequent sample is automatically repeated.
- The system can be configured to automatically repeat a QC sample after a QC rules failure. See Section 4.4.2.
- The system can be configured to automatically repeat a sample when a specific Advisor rule is triggered. See Section 4.7.4.1. The repeat sample will be processed as the next test.

Otherwise, to manually repeat a sample, run it in a Sysmex rack or run it as a Stat sample (see Section 5.6).

5.9 Running Third-Party Calibrators

If there is an occasion when your lab is required to use a third-party liquid calibrator (e.g., IFCC calibrators) to calibrate the D-100, please follow these instructions.

1. Run the D-100 Calibrator Pack to ensure the cartridge is properly conditioned (see Section 5.3).
2. Predilute each third-party liquid calibrator 1:300:
   a. Mix the calibrator by inverting the vial several times.
   b. Pipet 1.5 mL of D-100 Sample Diluent into a labeled 1.5 mL microvial, followed by 5 µL of the calibrator.
   c. Cap the microvial and mix thoroughly.
3. The instrument must be in Sleeping or Standby state.
4. To retrieve the Stat rack, touch ⏏ **Open** in the **Home** screen. The Stat door opens and the Stat rack moves to the loading position.
5. Insert a microvial containing 1.5 mL of Sample Diluent into position 1 to substitute the conditioner.
6. Insert the prediluted calibrator 1 (low level) and calibrator 2 (high level) microvials into unlabeled microvial adapters in positions 2 and 3 of the Stat rack, respectively. Touch ⏏ **Load** or wait until step 8 to load the rack into the Stat Area.
7. Go to the **Calibration** screen and touch **Calibrate Now**.
8. In the Calibration dialog box (see Figure 4-71), touch **Load Stat Area**. The Stat rack is loaded into the Stat Area.
9. Enter the required calibrator information (i.e., assigned values) and select the **Calibrate without reconstituting** checkbox.
10. Touch **Calibrate Now**.
11. After the Stat Area samples are finished being processed, touch ⏏ **Open**.

12. Remove the samples from the Stat rack.

5.10 Stopping a Run

Touch **Stop** in the banner. The instrument state changes from Running to Stopping. The system finishes processing the last aspirated sample and ejects the racks from the shuttle. The instrument performs end-of-run operations and transitions to Standby state.

5.11 Shutting Down and Restarting the D-100 System

NOTE: *It is not necessary to shut down the D-100 on a daily basis. During normal operation, it is recommended to keep the D-100 powered on.*

1. To shut down the system, touch **Log Out**.
2. In the Log Out dialog box, touch **Shut Down**.
3. To restart the system, press the soft power button on the front of the instrument. See Figure 2-1, No. 3.

5.12 Long-Term System Shutdown

The D-100 should remain powered on when idle to keep the reagents pressurized. However, if the instrument is to be shut down for more than 7 days, perform the following procedure to ensure the system remains in optimal operating condition.

1. The instrument must be in Sleeping state. To transition the instrument, go to the **Utilities/Manual Operations** screen and touch **General**, then **Sleep**.
2. Touch **Log Out** in the banner.
3. Touch **Shut Down** in the dialog box.

   NOTE: *If the Stat rack is not loaded in the Stat Area, a pop-up message asks if you are sure you want to exit. It is recommended that you touch **No** and load the Stat rack to ensure it is not removed while the instrument is shut down.*
4. Remove all bottles from the reagent compartment.
5. Remove the analytical cartridge and prefilter from their holders. Cap the ends of the analytical cartridge and prefilter and store at 2–8 °C for reuse.
6. Install the gray utility cartridge and utility prefilter.
7. After the touchscreen has turned off, turn off the power switch on the rear of the instrument.

Restarting the System after a Long-Term Shutdown

1. Turn on the D-100 power switch (on the rear of the instrument).
2. Press the soft power button on the front of the instrument to activate the instrument from standby power mode to full operating power. See Figure 2-1, No. 3.
3. The Bio-Rad software startup screen will display while the software is loading.
4. After the software is loaded, install the reagent bottles.

5. Remove the gray utility cartridge and utility prefilter from their holders.
6. Install the analytical cartridge and prefilter.
7. Touch **Run** in the banner to initiate the system warm-up. The instrument will transition from Sleeping to Warming Up state.
8. After the warm-up is complete, the instrument transitions to Standby state.
9. It is recommended that the system be recalibrated by running the Calibrator Pack and QC samples.

6 Results and Data Management

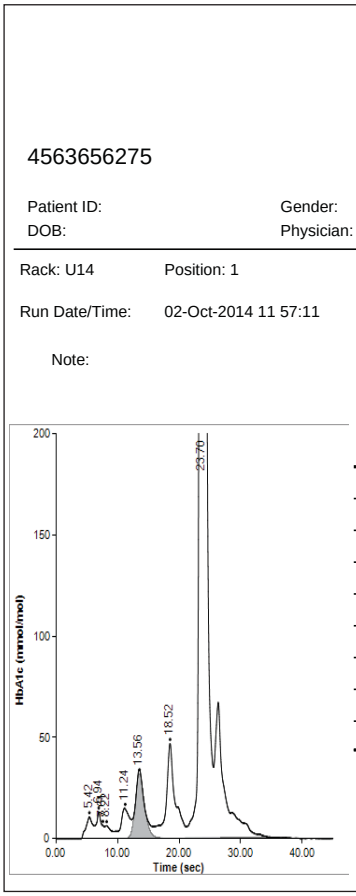
6.1 Reviewing Results

Results are reviewed in the **Results** screen. See Section 4.3 for information.

NOTE: *Bio-Rad recommends that all sample chromatograms be reviewed before releasing results from the D-100.*

Figure 6-1 indicates the items that should be checked on each sample. All items are automatically checked by the D-100 Advisor rules. See Appendix C for rule details.

Figure 6-1: *D-100 HbA1c Example Result Report*



	Item
1	Total Area
2	HbA1c peak
3	A0 peak
4	F peak area
5	P3 peak area
6	Area of Unknown peak
7	HbA1c result

See the assay Instructions For Use and Quick Guide for specific criteria used to interpret the result report.

6.1.1 Viewing Flagged Results

1. In the **Home** screen, touch Flagged to go to the **Results** screen, where results have been filtered to display only flagged samples (i.e., results that meet the **Flagged** filter criteria).
2. In the results table, see the reason why the sample was flagged in the Note/Comment column.
3. To view the sample result in detail, touch the result row.

6.2 Releasing or Rejecting Results

Results can be released or rejected in the results table or in the Result Details screen.

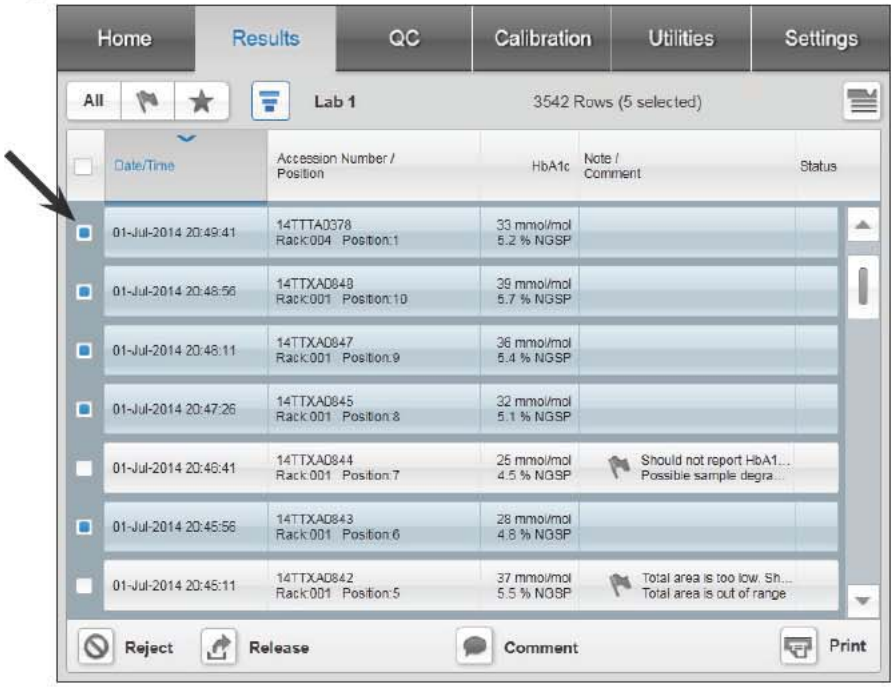
NOTE:

- *If the LIS Connection is **On** (see Section 4.7.5), releasing a result sends it to the LIS. If a questionable result is accidentally released, the result must be manually rejected at the LIS.*
- *Rejected results are not sent to the LIS. If a good result is accidentally rejected, the action can be undone by releasing the result.*

6.2.1 Releasing or Rejecting Results in the Results Table

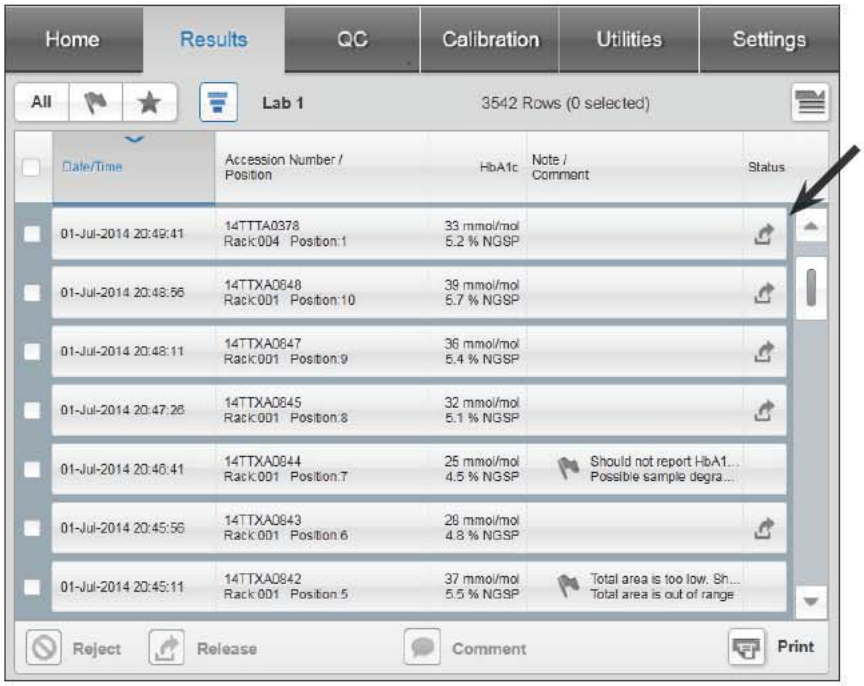
1. In the results table, select the corresponding checkbox for each sample result you want to release.

***Figure 6-2:** Results Table, 5 Results Selected to be Released*



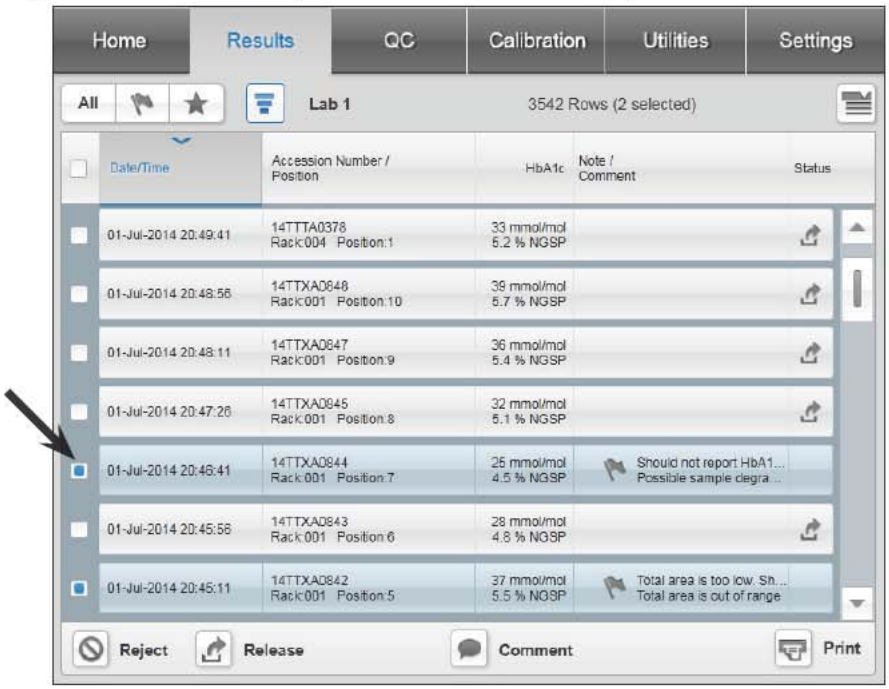
2. Touch **Release** at the bottom of the screen. The result(s) status now indicates .

Figure 6-3: *Results Table, 5 Results Released*



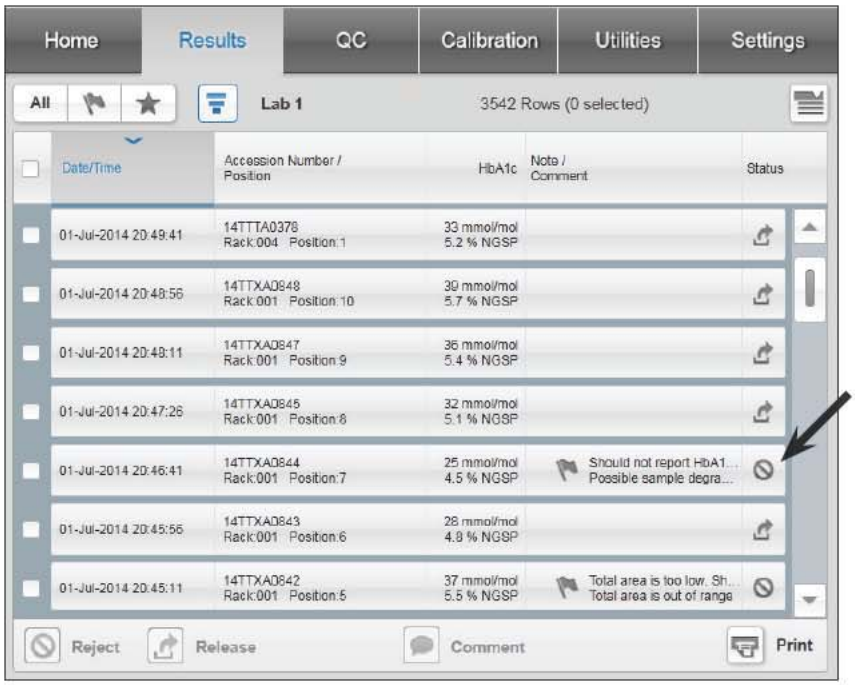
3. Select the corresponding checkbox for each sample result you want to reject.

Figure 6-4: *Results Table, 2 Results Selected to be Rejected*



4. Touch **Reject** at the bottom of the screen. The result(s) status now indicates .

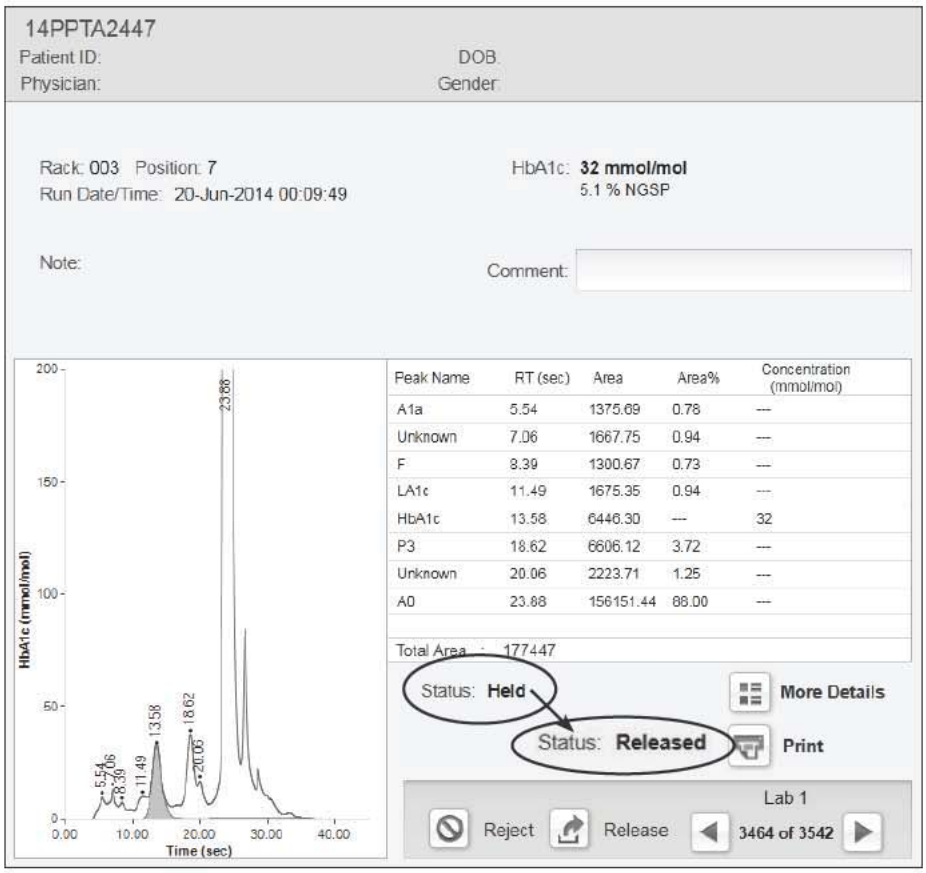
Figure 6-5: *Results Table, 2 Results Rejected*



6.2.2 Releasing or Rejecting Results in the Result Details Screen

1. In the results table, touch the sample result row to open the Result Details screen. The Status indicates "Held".

Figure 6-6: *Result Details Screen, Status*



2. After viewing the details, touch **Release** to release the result or touch **Reject** to reject the result.
3. The system updates the result status ("Released" or "Rejected") accordingly and displays the next sample result.

6.3 Transmitting Results to the LIS

The D-100 can receive orders and send results to a Laboratory Information System (LIS).

When LIS is enabled (i.e., the LIS Connection setting is **On**), results are sent to the LIS according to the settings defined in the **Settings/LIS** screen. See Section 4.7.5 for information.

- Results can be sent to the LIS manually by Releasing the results in the **Results** screen (see Section 6.2).
- Results can be sent to the LIS automatically by selecting the **Automatic Release to LIS** checkbox in the **Settings/LIS** screen.

6.4 Exporting Results

Results can be exported from the **Results** screen. See Section 4.3.3.1 for information.

6.5 Quality Control (QC)

QC results are presented in the **QC** tab. See Section 4.4 for information.

QC results can be exported to the Bio-Rad Unity Real Time® program according to the settings defined in the **QC/Unity** screen. See Section 4.4.5.

- QC results can be sent to Unity manually by selecting **Export Unsent QC Samples** in the **QC/Unity** screen.
- QC results can also be sent to Unity manually by selecting **Export to Unity** from the Results Menu in the **Results** screen. See Section 4.3.3.2.
- QC results can be sent to Unity automatically by selecting the **Export automatically for each sample** checkbox in the **QC/Unity** screen.

6.6 Backing Up Data

To back up the D-100 database, go to the **Utilities/Data** screen. See Section 4.6.3.1 for information.

6.7 Viewing Archived Data

To view or print previous data from a backed-up database, select **View Archive** from the Results Menu in the **Results** screen. See Section 4.3.3.4 for information.

6.8 Restoring Data

To restore a backed-up database for use on a different D-100 instrument or for troubleshooting purposes, go to the **Utilities/Data** screen. See Section 4.6.3.2 for information.

6.9 Managing Files

To copy, move, delete, or rename files, go to the **Utilities/Data** screen. See Section 4.6.3.3 for information.

7 Maintenance

Routine user maintenance for the D-100 is performed at the following intervals:

As Needed:

- Emptying the Waste Bottles (Section 7.1)
- Cleaning Up Spills and Decontaminating Surface Area (Section 7.2)
- Cleaning the Stat Rack and Sysmex Racks (Section 7.3)
- Cleaning the Touchscreen (Section 7.4)
- Calibrating the Touchscreen (Section 7.5)
- Priming/Flushing (Section 7.6)
- Manually Priming the Pumps (Section 7.7)

Every 3 cartridges or 30,000 tests:

- Cleaning the System (Section 7.8)

Maintenance is necessary to maintain optimum system performance.

WARNING: *All maintenance procedures described in this manual can be safely performed by qualified personnel. Maintenance not covered in this manual should be performed only by a Bio-Rad representative.*

BIOHAZARD: *Performing maintenance procedures may expose you to biohazardous conditions. Wear appropriate personal protective equipment.*

NOTE: *The user has responsibility for appropriate decontamination in case of spillage of hazardous material on or inside the equipment.*

Before the use of any decontamination or cleaning methods other than those recommended, users should check with Bio-Rad that the proposed method will not damage the equipment.

7.1 Emptying the Waste Bottles

The Waste indicator becomes red in the consumables panel when a waste bottle is full. A red warning message appears in the message panel when both waste bottles are full.

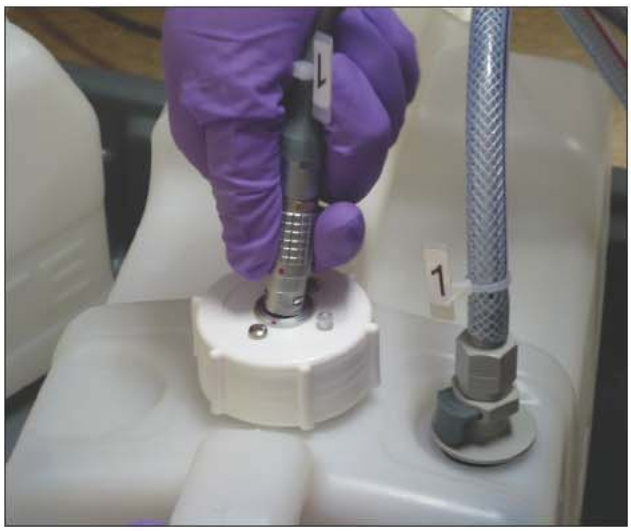
A waste bottle can be emptied at any time, even when the instrument is in Running state, as long as a second waste bottle is installed and not full. If the bottle being disconnected is active, the D-100 automatically switches to the second bottle.

NOTE: *The waste tubings and level sensor cables are labeled as 1 or 2 to correspond with the Waste indicators in the consumables panel.*

WARNING: *Some reagents used with the D-100 contain sodium azide as a preservative (see labels). Azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of reagents containing sodium azide, always flush with large volumes of water to prevent metal azide buildup. For further information, consult the manual Safety Management, No. CDC-22, "Decontamination of Laboratory Sink Drains to Remove Azide Salts" (Centers for Disease Control and Prevention, Atlanta, GA, April 30, 1976).*

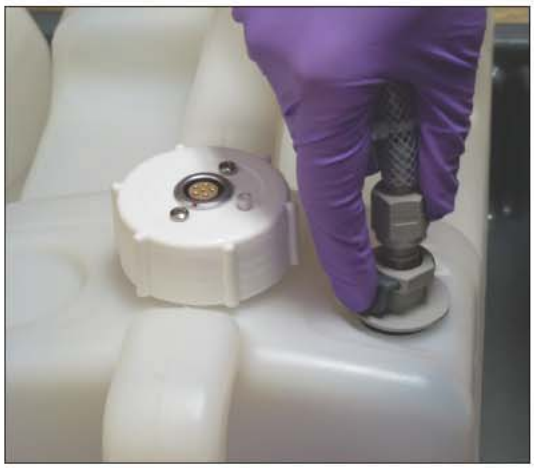
1. Disconnect the gray electrical cable from the level sensor cap by pulling the connector.

Figure 7-1: *Disconnecting Level Sensor Cable from Waste Bottle*



2. Disconnect the braided waste tubing from the bottle by pressing the thumb latch on the quick-disconnect valve. Place the tubing on an absorbent towel.

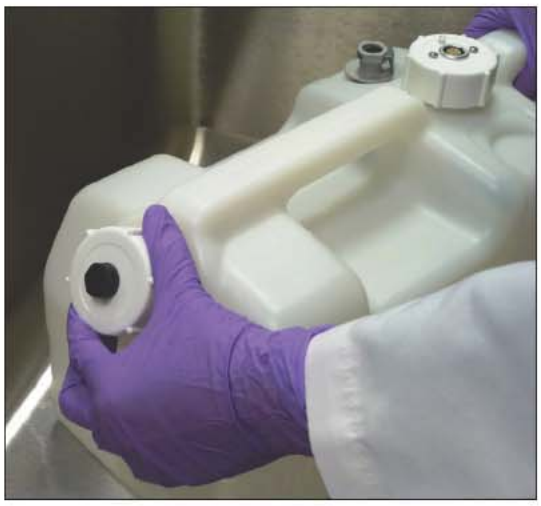
Figure 7-2: *Disconnecting Waste Tubing from Waste Bottle*



3. Transfer the waste bottle to the appropriate disposal area.

4. Unscrew the waste cap on the rear of the bottle. Place the cap on an absorbent towel.

   Figure 7-3: *Unscrewing Waste Cap*



5. Dispose of the waste properly, as directed by laboratory safety procedures.

   NOTE: *Liquid waste should be decontaminated on site if possible. It is recommended to mix liquid waste with household bleach (5% sodium hypochlorite) at a ratio of 1 part bleach to 10 parts waste. For example, add 500 mL of household bleach to a full 5 L waste bottle. Let the mixture stand for at least 30 minutes before emptying.*

6. Reinstall and secure the waste cap.
7. Reconnect the braided waste tubing to the valve. Ensure that the waste tubing is not looped or crimped and is sloped downward.
8. Reconnect the gray electrical cable to the level sensor cap. The red dots on the level sensor cap and the connector must be aligned to reconnect (see Figure 7-1).

7.2 Cleaning Up Spills and Decontaminating Surface Area

Clean up any spills when they occur. Sample spills are potentially biohazardous; treat appropriately. If any sample spills occur in the rack handler area, decontaminate the area using 70% isopropyl alcohol.

1. Ensure the instrument is not in Running state.
2. Remove all racks from the rack handler.
3. Prepare a 70% isopropyl alcohol solution. Do not use corrosive liquids (e.g., bleach).
4. Dampen a disposable towel with the decontamination solution.
5. Wipe the rack handler area and conveyor belts with the damp towel.
6. After decontamination, let the belts air-dry before use.

7.3 Cleaning the Stat Rack and Sysmex Racks

Clean the racks as needed.

1. Remove all samples from the racks.
2. Remove the Stat rack from the instrument: press and hold the button on the front of the rack while lifting up the rack.

Figure 7-4: *Removing Stat Rack*

3. Clean the Stat rack and Sysmex racks using 70% isopropyl alcohol and a disposable towel.

   If a rack is contaminated with dried blood, first wet the area with a detergent disinfectant. Carefully remove with a disposable towel to prevent scattering potentially infectious material. After removal, disinfect the cleaned surface with 70% isopropyl alcohol.
4. Inspect the sample racks to ensure they are in good working condition.
5. Reinstall the Stat rack: while pressing and holding the button on the front of the rack, set the rack in its loading position on the instrument. Release the button to secure the rack.

   NOTE: *The 3 slots beneath the rack align with the 3 posts on the instrument to ensure proper installation.*

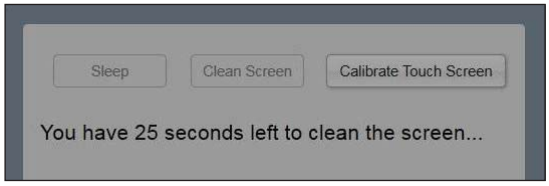
7.4 Cleaning the Touchscreen

Clean the touchscreen as needed.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Clean Screen**.

Figure 7-5: *Utilities/Manual Operations Screen, Clean Screen Button*

4. The touchscreen is disabled for a 30-second period to allow cleaning. The countdown is displayed on the screen.
   During this period, clean the touchscreen using 70% isopropyl alcohol and a soft towel.



7.5 Calibrating the Touchscreen

If the touchscreen is not responding accurately (i.e., the touch targets are misaligned), it may require recalibration. Calibrate the touchscreen as needed.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Calibrate Touch Screen**.

Figure 7-6: *Utilities/Manual Operations Screen, Calibrate Touch Screen Button*

4. A series of 4 red calibration points are displayed one at a time on each corner of the screen. Touch each calibration point within the 15-second countdown period indicated, until the point turns blue and the countdown timer changes to “OK!”.
5. After acknowledging the 4th calibration point, a pop-up message indicates the parameters are being read. The touchscreen calibration is now completed.

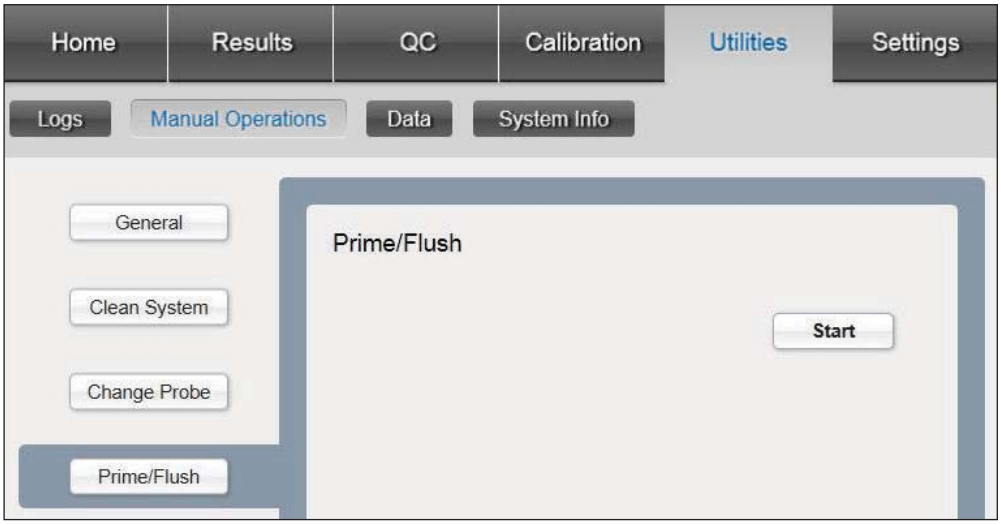
7.6 Priming/Flushing

The **Prime/Flush** function flushes the fluidics with buffer.

Pressure variations >±5% may indicate the presence of air in the buffer lines. Perform the **Prime/Flush** to remove air bubbles.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Prime/Flush**.

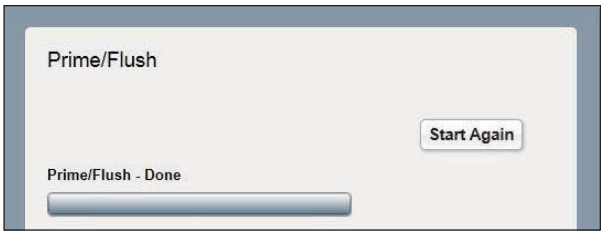
Figure 7-7: *Utilities/Manual Operations Screen, Prime/Flush Button*



4. Touch **Start**. The screen indicates that the Prime/Flush is in progress and when it is complete. The process takes approximately 8 minutes.

5. To repeat the Prime/Flush, touch **Start Again**.

NOTE: *If the pressure variations continue, perform the manual pump prime procedure. See Section 7.7.*



7.7 Manually Priming the Pumps

This manual pump prime procedure can be performed as a supplement or alternative to the automated **Prime/Flush** (Section 7.6). Perform this procedure as needed to remove air bubbles from the buffer lines.

1. The instrument must be in Sleeping state with the reagents pressurized.
2. Open the cartridge/prefilter compartment door to access the high-pressure pumps.
3. Insert a 10–20 mL syringe into the pump A purge valve to collect the leaking buffer. Open the pump A purge valve approximately ½-turn counterclockwise or until buffer begins flowing.

Figure 7-8: *Syringe in Pump B Purge Valve*

4. Leave the valve open until there is a uniform buffer flow without any bubbles, then close the valve by turning it clockwise until secured.
5. Remove the syringe and dispose of the liquid. Use a disposable towel to wipe any buffer from the outside of the purge valve.
6. Repeat steps 3–5 with the pump B purge valve.
7. Close the cartridge/prefilter compartment door.

7.8 Cleaning the System

Clean the system every 3 cartridges or 30,000 tests. Depending on usage conditions, a message may appear prompting you to clean the system sooner. If that occurs, clean the system as soon as possible in addition to the typical cleaning frequency.

NOTE: *Failure to clean the system every 30,000 tests may result in carryover.*

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Clean System**. The software provides step-by-step instructions for cleaning the system.

Figure 7-9: *Utilities/Manual Operations Screen, Clean System Button*

4. Touch **Open** to retrieve the Stat rack from the Stat Area.

5. Insert the Cleaning Tube in a microvial adapter in the Stat rack. Touch **Clean Now**.

6. The screen indicates that the cleaning is in progress and when it is complete. The process takes approximately 2 minutes.

7. The Stat Area opens automatically when the cleaning is complete. Remove the Cleaning Tube from the Stat rack and discard. Touch **Load**.

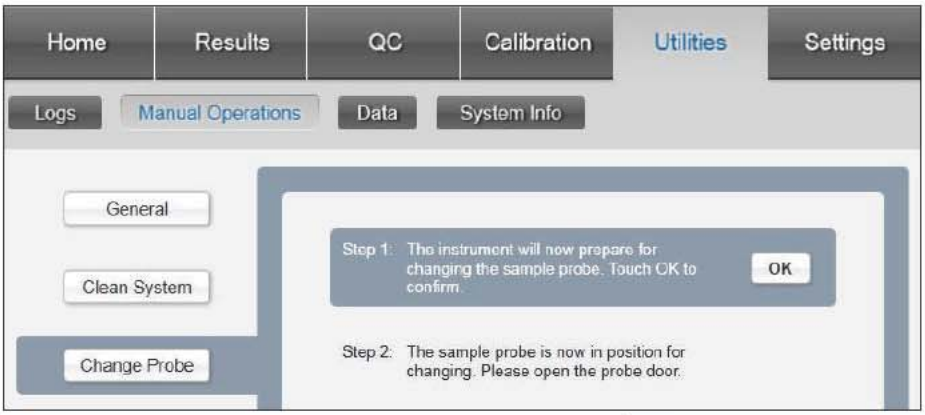
7.9 Replacing the Sample Probe

WARNING: *The sample probe is very sharp. Use caution when handling to avoid injury. Do not attempt to replace a broken probe that has detached from its base. Contact your local Bio-Rad office for technical assistance.*

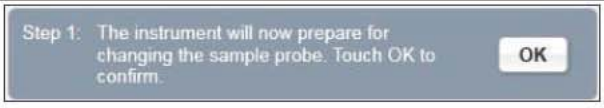
The sample probe will be replaced by Bio-Rad Service during scheduled maintenance; however, you may need to replace the probe if instructed by Technical Service. As an extra biohazard precaution, Bio-Rad recommends cleaning the system (see Section 7.8) before replacing the sample probe. If the probe is damaged, and it is not possible to perform that procedure, carefully wipe down the probe using a towel moistened with 5% sodium hypochlorite solution prior to removing.

1. Go to the **Utilities/Manual Operations** screen.
2. The instrument must be in Sleeping state. To transition the instrument, touch **General**, then **Sleep**.
3. Touch **Change Probe**. The software provides step-by-step instructions for changing the sample probe.

Figure 7-10: *Utilities/Manual Operations Screen, Change Probe Button*



4. Touch **OK** to move the sample probe to the change position.



5. Open the probe door.

   NOTE:

   - *The probe door is interlocked. When open, the interlock switch shuts off power to the probe assembly.*
   - *The Change Probe screen indicates when the probe door is open.*

6. Unscrew the tube fitting from the top of the sample probe. See Figure 7-11.
7. Grasp the probe and slide it up and out through the side of the probe carrier. See Figure 7-12.

Figure 7-11: *Unscrewing the Tube Fitting from the Sample Probe**

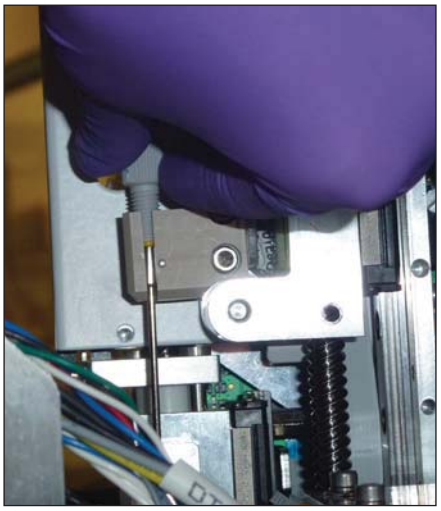
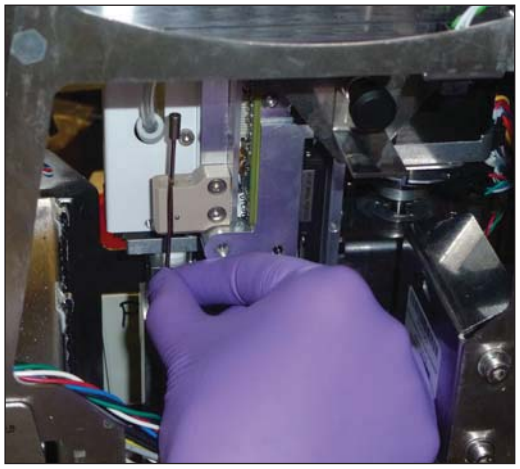


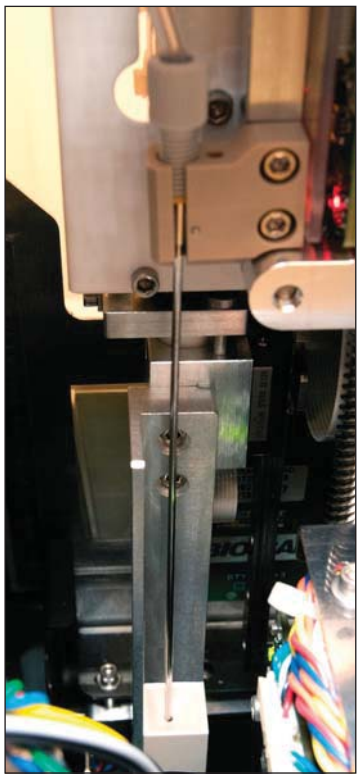
Figure 7-12: *Removing the Sample Probe from the Probe Carrier**



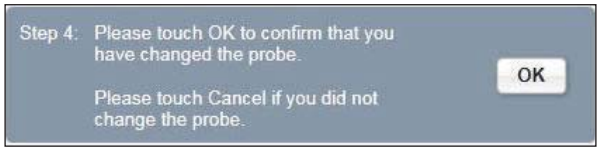
8. Discard the old probe according to your laboratory's standard operating procedures for sharps.
9. Remove a new sample probe from its shipping tube; remove the tip cover.
10. Insert the new probe into the probe carrier, being careful to lower the probe tip into the center of the tube holder, which has a conical base (see Figure 7-13). There is a flat surface on the probe base to ensure a proper fit; rotate the probe until it drops into place.
11. Reconnect the tube fitting.
12. Close the probe door.

** The instrument cover was removed for visibility in these photos.*

Figure 7-13: *Probe Tip in Center of Tube Holder Base**



13. Touch **OK** to confirm that you changed the sample probe. (If you did not change the probe, touch **Cancel**.)



8 Troubleshooting

8.1 Important Troubleshooting Information

Troubleshooting advice for problems you may encounter while using the D-100 is organized as follows:

- General Error Messages (Section 8.2)
- Hardware Error Messages (Section 8.3)
- Chromatography Problems (Section 8.4)
- Other Problems (Section 8.5)

The recommended solutions include abbreviated procedures; see appropriate sections for detailed procedures.

If the problem persists after completing the recommended solution(s), or if the problem is not addressed in this operation manual, contact Bio-Rad Technical Service.

In the USA and Puerto Rico: Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week.

Outside the USA: Contact your regional Bio-Rad office. Go to www.bio-rad.com for contact information.

NOTE: *When performing the recommended solution to a problem requires turning the main power switch off, always attempt to shut down the instrument first by touching* ***Log Out*** *in the banner and touching* ***Shut Down*** *in the dialog box. Wait for the touchscreen to turn off, then turn off the power switch on the rear of the instrument.*

After the troubleshooting is completed, turn the power switch back on. Press the soft power button on the front of the instrument to activate the power-on sequence.

8.2 General Error Messages

The following error messages appear in the message panel when the errors occur. Check the **Utilities/Logs** screen for the description and event code.

Error Message	Probable Cause	Event Code	Recommended Solution
X exceeded recommended test limit	The stated cartridge or prefilter is now past the recommended number of tests.	113033, 113042	Replace the cartridge or prefilter at your earliest convenience.
X is empty	Both bottles of the stated reagent are empty.	113006, 113009, 113012	You must replace the reagent before you can start a run.
X level is low	The stated reagent level is low.	113005, 113008, 113011, 113017	Replace the reagent at your earliest convenience to prevent the run from stopping.

Error Message	Probable Cause	Event Code	Recommended Solution
X lot expired	The stated consumable has reached its expiration date.	113031, 113034, 113036, 113038, 113040, 113043, 113078, 113081, 113084	Replace the consumable at your earliest convenience.
X onboard stability expired	The open/installed stability of the stated consumable has expired.	113032, 113035, 113037, 113039, 113041, 113044, 113079, 113082, 113085	Replace the consumable at your earliest convenience.
X unavailable	There are no bottles of the stated reagent installed.	113062, 113064, 113066, 113072	Install reagent.
Calibration failed for X	Calibrator failed for the stated assay.	123003, 123005	See Section 8.4 for “Calibrator Pack failure” solutions.
Calibration required – new cartridge installed	A new cartridge was installed on the instrument.	113057	Perform calibration.
Calibrator skipped; please load in Stat rack	Calibrator pack inserted in Sysmex rack	123009	Remove calibrator pack from Sysmex rack and insert in Stat rack.
Cannot print PDF file	Selected folder for PDF reports is full.	NA	Move or delete files from the applicable drive to provide space (Section 4.6.3.3).
Cartridge reloaded – calibration recommended	A previously installed cartridge was reinstalled on the instrument.	113058	Recalibration is recommended, but not required.
Cartridge reloaded – calibration required	There is no passed calibration for the cartridge.	113013	Perform calibration.
Cartridge unavailable	No cartridge installed	113060, 113070	Install cartridge.
Cartridge unusable – Data tag error	RFID tag missing	100040, 100044	Discard and replace the cartridge. Contact Bio-Rad Technical Service for a replacement.
	RFID tag failed		1. Remove and reinstall the cartridge. 2. If problem persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
	Electromagnetic interference		Identify and remove the interfering device.
	Failed RFID reader		Contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
Could not close stat area; reopening	Obstruction to movement of Stat Area door	101009	Check for and remove any obstacles from the Stat Area.
Error (see activity log)	Varies	100006, 100014, 100015, 100016, 100017, 100018	Go to the **Utilities/Logs** screen for a description of the error. Contact Bio-Rad Technical Service.
Error in Stat Area	Obstruction to movement of Stat Area door	101013, 101014	Check for and remove any obstacles from the Stat Area.
Export failed	Connectivity failure	100026	• Verify the "Save to" location is correct. • If the "Save to" location is an "External" USB drive, ensure that it is properly connected to a USB port on the D-100. • If the "Save to" location is on the "Network": 1. Ensure the D-100 is connected to the network (i.e., the cable is connected to the correct network port and the cable is connected to the LAN port on the rear of the D-100). 2. Verify the network location is active (i.e., navigate to that folder from another connected instrument to confirm it is online and available). 3. Verify the network location is not full.
Fluidics error; Clean system or contact service	System cleaning required	101051	You must clean the system (Section 7.8) before you can start a run.
Fluidics warning; please clean system	System cleaning recommended	101050	Warning will be cleared after cleaning the system (Section 7.8) or if the subsequent system check passes.
Incompatible bottle in X position	The batch number of reagent installed in the stated position is not compatible.	113022, 113024, 113026, 113028, 113030	Replace the reagent with one that has a compatible batch number.
Invalid calibrator pack loaded	Incompatible lot of calibrator pack installed	123010	Replace with a compatible lot of calibrator pack.

Error Message	Probable Cause	Event Code	Recommended Solution
LIS is not responding	Incorrect LIS settings	107001	Check LIS settings (Section 4.7.5) and correct as needed.
	LIS is not connected to D-100		1. Ensure LIS is connected to the D-100 serial port via RS-232 cable. 2. Touch the **Test** button in the LIS Advanced Settings/**Test LIS** tab to verify the connection.
One or more active control lots has expired	The QC currently in use has expired.	112001	Use a new lot of QC.
Paused – Insufficient level resource	The system has transitioned from Running to Paused state because it is out of reagents or waste space.	101045	1. Replenish the resource. 2. Touch **Resume** to continue the run.
Prefilter unavailable	No prefilter installed	113068	Install prefilter.
Printer error	External printer is not available	100024	Verify the external printer is connected, powered on, and has no errors indicated.
	Check the internal printer error LED. A red light indicates a problem: 1 flash = Printer memory is full and cannot receive additional data		Turn the D-100 main power switch off and then on again as instructed in the NOTE in Section 8.1. If error persists, contact Bio-Rad Technical Service.
	2 flashes = Printer head temperature too high		1. Remove the thermal paper from the printer and leave the paper door open until the printer head has cooled down. 2. Reinstall the paper (Section 3.11).
	Permanent blink = No paper or paper door is open		• If needed, install new roll of paper (Section 3.11). • Close the printer paper door.
	1 long flash and 1 short flash = Printer head failure		Contact Bio-Rad Technical Service.
Printer error: internal sw error	NA	NA	Shut down and restart the D-100 (Section 5.11).
Printer: PDF Error: folder is not available	Selected folder for PDF reports is not available	100057	Verify the selected path for PDF reports (Section 4.7.2) in the **Settings/Reports** screen.

Error Message	Probable Cause	Event Code	Recommended Solution
Probe door is not locked	Probe compartment door is open	101020	Close the door.
QC Failed	QC result is outside of the fixed control range or failed a QC rule	100030, 100031	Rerun QC.
	The QC values entered do not correspond to the primary reporting unit (i.e., % NGSP or mmol/mol IFCC).		Enter the correct values for the primary reporting unit in the Edit Control Lot dialog box (Section 4.4.4.2) in the **QC/QC Lots** screen.
QC interval exceeded; please run QC	QC is now due to be run	112009	Run QC.
QC Warning	QC has violated the 1-2s or 1-3s QC rule	100032	Rerun QC.
Rack handler error	Obstruction to movement of rack handler	101015, 101016	Check for and remove any obstacles from the rack input, output, and sampling areas.
Rack loaded backwards; please reload rack	A Sysmex rack was positioned incorrectly in the rack input area.	101006	To prevent the run from stopping, immediately remove and reposition the rack so that the rack barcode faces the instrument and the Sysmex logo faces you.
Remove racks: Output full.	The rack output area contains the maximum number of racks.	101047	Remove the completed Sysmex racks from the rack output area.
Run stopped due to full disk	D drive is full	110001	Move or delete files from the D drive to provide space (Section 4.6.3.3).
Sample path maintenance due; contact service	Low-pressure filter is approaching its test limit	114001	Contact Bio-Rad Technical Service to replace the low-pressure filter at your earliest convenience. **NOTE:** *You may continue to run the system until Service arrives.*
Sample path maintenance warning; contact service	Low-pressure filter is now past its test limit	113045	Contact Bio-Rad Technical Service to replace the low-pressure filter as soon as possible.

Error Message	Probable Cause	Event Code	Recommended Solution
Stat area is jammed; please clear	Stat rack is incorrectly installed	101004	Remove and reinstall the Stat rack (Section 7.3).
	Sample or Calibrator Pack is incorrectly inserted in the Stat rack		Remove and reinsert the sample or Calibrator Pack (Section 3.6).
	There is an obstacle inside the Stat Area.		Contact Bio-Rad Technical Service.
Stopped due to backwards rack	The run has stopped and the system has entered Standby state because the next Sysmex rack to be processed is positioned incorrectly in the rack input area.	101007	1. Remove and reposition the rack so that the rack barcode faces the instrument and the Sysmex logo faces you. 2. Restart the run.
Stopped due to full output area	The Sysmex rack just finished being processed cannot be ejected from the shuttle because the rack output area contains the maximum number of racks. The system has transitioned from Running to Paused state.	101001	1. Remove the completed Sysmex racks from the rack output area. 2. Touch **Resume** to continue the run.
Units changed – Calibration required.	The primary reporting unit (i.e., NGSP or IFCC) has been changed.	104003	Perform calibration.
Unity folder error (see activity log)	Network folder is unavailable	100037, 100055	• Ensure the D-100 is connected to the network. • Ensure the computer that hosts the network folder is connected and running.
Waste level is almost full	The external waste level is almost full.	113049	Empty the waste bottles at your earliest convenience to prevent the run from stopping.
Waste level is full	Both external waste bottles are full.	113050	You must empty the waste bottles before you can start a run.

8.3 Hardware Error Messages

The following hardware error messages appear in the message panel when the errors occur. Check the **Utilities/Logs** screen for the description and event code.

Error Message	Probable Cause	Event Code	Recommended Solution
Buffer error; check bottles, touch Run or Reset	A reagent bottle is not correctly installed.	131206	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Touch **Run** or **Reset**.
	A faulty reagent bottle is leaking air.	133209	1. Replace the applicable buffer bottle. 2. Touch **Run** or **Reset**.
Cannot read tag; please replace consumable	Checksum verification failed.	130504	1. Remove and reinstall the consumable. 2. If error persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
Cartridge error; check cartridge, touch Reset/ Run	The Cartridge holder was opened while in use.	131704	1. Ensure the cartridge is still installed. 2. Close the cartridge holder door and the cartridge/prefilter compartment door. 3. Touch **Reset** or **Run**.
	The pumps were running with no cartridge installed.	133074	1. Wipe up any leaks. Leaks from the cartridge holder are potentially biohazardous; handle and treat appropriately. 2. Install a cartridge. 3. Close the cartridge holder door and the cartridge/prefilter compartment door. 4. Touch **Reset** or **Run**.
Close all doors to continue	Failed to lock a door because it is not closed.	132203	Close the open instrument door(s).
Data tag error; replace bottle A/B/Wash position 1/2	Failed to write data to the RFID tag for the stated reagent bottle.	132766-132771	• Replace the stated reagent. • If error persists, contact Bio-Rad Technical Service.
Data tag error; replace Cartridge	Failed to write data to the RFID tag for the Cartridge.	131228, 132772	• Replace the cartridge. • If error persists, contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
Data tag error; replace consumable bottle	Failed to write data to the RFID tag of a consumable bottle.	131230	• Replace the applicable bottle. • If error persists, contact Bio-Rad Technical Service.
Data tag error; replace Low Pressure Filter	Failed to write data to the RFID tag for the Low Pressure Filter.	132773	Contact Bio-Rad Technical Service.
Data tag error; replace Prefilter	Failed to write data to the RFID tag for the Prefilter.	131229, 132774	• Replace the prefilter. • If error persists, contact Bio-Rad Technical Service.
Detector warning; contact Technical Service	LED could be at the end of its life.	132507, 132513, 132514	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Door error; check doors and touch Reset or Run	Door open (and not allowed) or interlock error.	133012	1. Close the open instrument door(s). 2. Touch **Reset** or **Run**.
Door open; close doors and touch Reset or Run	The operation failed because a door is open.	131203	1. Close the open instrument door(s). 2. Touch **Reset** or **Run**.
Error; check output area and touch Reset or Run	Moving the rack failed (Pushers motors error).	133058	1. Check for and remove any obstacles from the rack output area. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Eject sequence failed (Output motor error).	133060	
In-use waste bottle removed; check for spills	A waste bottle was removed while being filled.	132699	1. Wipe up any spills. Waste spills are biohazardous; handle and treat appropriately. 2. Ensure at least one empty waste bottle is available and correctly installed (Section 7.1).
Low Buffer A/B; replace bottle, touch Run or Reset	The process failed because there is not enough of the stated buffer to complete.	131208, 131209	1. Replace the applicable buffer bottle(s). 2. Touch **Run** or **Reset**.
Low Pressure filter error; please check	The Low Pressure Filter holder was opened while in use.	131705	Contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
No cap; check tube or contact Technical Service	No cap was detected while moving into the sample tube.	131231	1. Locate and correct sample tube with no cap. 2. Touch **Run** or **Reset**. 3. If error persists, contact Bio-Rad Technical Service.
Please replace cartridge; cannot read tag	The calculated checksum of the Cartridge extended data does not match the stored value.	131406	1. Remove and reinstall the cartridge. 2. If error persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
	The Cartridge extended data could not be read.	131407	
Please replace consumable; cannot read tag	Decoding of the Data Tag failed (i.e., the RFID tag could not be read).	130500	1. Remove and reinstall the consumable. 2. If error persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
	Reading of a data block failed.	130501	
	Reading of a (signed) value block failed.	130502	
	Reading of a (unsigned) value block failed.	130503	
Power supply warning; contact Technical Service	The power supply is beginning to fail.	132698	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Prefilter error; check prefilter, touch Reset/ Run	The Prefilter holder was opened while in use.	131706	1. Ensure the prefilter is still installed. 2. Close the prefilter holder door and the cartridge/prefilter compartment door. 3. Touch **Reset** or **Run**.
	The pumps were running with no prefilter installed.	133076	1. Wipe up any leaks. Leaks from the prefilter holder are potentially biohazardous; handle and treat appropriately. 2. Install a prefilter. 3. Close the prefilter holder door and the cartridge/prefilter compartment door. 4. Touch **Reset** or **Run**.

Error Message	Probable Cause	Event Code	Recommended Solution
Pressure fault; Replace Prefilter	Pressure higher than upper limit.	133068, 133208	1. Replace prefilter. 2. If error persists, replace cartridge. Contact Bio Rad Technical Service for a replacement.
Pressure warning: Replace Prefilter	Pressure higher than warning limit.	132546	1. Replace prefilter. 2. If error persists, replace cartridge. Contact Bio-Rad Technical Service for a replacement.
Rack jam; clear shuttle and touch Reset or Run	Unexpected motors error while testing the shuttle.	133061	1. Check for and remove any obstacles from the shuttle. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Unexpected motors error while clearing the shuttle.	133062	
Rack jam; clear shuttle to avoid stopping the run	A Sysmex rack is jammed in the shuttle.	132710	Remove Sysmex rack(s) from the shuttle and correctly position racks in the rack input area to prevent the run from stopping.

Error Message	Probable Cause	Event Code	Recommended Solution
System error; contact Technical Service	Dilution module pressure low. Possible clog in sample path.	133047	Contact Bio-Rad Technical Service.
	Dilution module pressure high. Possible clog in sample path.	133048	Contact Bio-Rad Technical Service.
	Leak has been detected. Leak in the reagent compartment.	133085	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Wipe up any reagent in the leak tray. 3. If error persists, contact Bio-Rad Technical Service.
	The pressure is too low when running gradients (Separation).	133207	1. Ensure the reagent bottles are properly installed (Section 3.10). 2. Prime/flush system (Section 7.6). 3. If error persists, contact Bio-Rad Technical Service.
	The pressure is too high when running gradients (Separation).	133208	1. Replace prefilter. 2. If error persists, replace cartridge. 3. If error persists, contact Bio-Rad Technical Service.
	Varies	Varies	Contact Bio-Rad Technical Service.
System error; touch Reset or Run and try again	A Sysmex rack in process was removed by the user before it was moved to the rack output area.	133057	1. Remove rack from the shuttle. 2. Touch **Reset** or **Run**. 3. If error persists, contact Bio-Rad Technical Service.
	Transient sensor malfunction		
	Varies	Varies	1. Touch **Reset** or **Run**. 2. If error persists, contact Bio-Rad Technical Service.
Tube spin failure; contact Technical Service	Failed to spin a tube in the rack.	132781	Contact Bio-Rad Technical Service.
Warming up failed; please try again	Normal operating conditions were not achieved within the expected warm-up time.	131700, 131701, 131703	1. Touch **Reset** or **Run**. 2. If error persists, contact Bio-Rad Technical Service.

Error Message	Probable Cause	Event Code	Recommended Solution
Warning: a sample was skipped	Unable to access the sample tube. Either something prevents the robot module from moving or it needs to be recalibrated.	131222	1. Check Activity Log for sample location. 2. Rerun sample. 3. If error persists, contact Bio-Rad Technical Service.
	Bottom of sample tube was not found.	131404	
Warning: Pressure low; call Technical Service	Pressure lower than lower limit.	132547	Contact Bio-Rad Technical Service for guidance to check for leaks. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Warning: Rack handling fault; stat samples only	The Rack Handling driver failed to initialize.	132776	1. Contact Bio-Rad Technical Service. 2. You may continue to run samples in the Stat Area until Service resolves the rack handler fault.
Warning: Stat area fault; rack handling only	The Stat Area driver failed to initialize.	132777	1. Contact Bio-Rad Technical Service. 2. You may continue to run samples in the Sysmex racks until Service resolves the Stat Area fault.
Warning: Temperature high; call Technical Service	Cartridge temperature higher than upper limit.	132544	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Warning: Temperature low; call Technical Service	Temperature lower than lower limit.	132545	Contact Bio-Rad Technical Service. **NOTE:** *Although a warning level has been reached, the system is still within operating range. You may continue to run the system until Service arrives.*
Wash empty; replace bottle and touch Run or Reset	The process failed because there is not enough Diluent to complete.	131210	1. Replace the Wash Solution bottle(s). 2. Touch **Run** or **Reset**.

Error Message	Probable Cause	Event Code	Recommended Solution
Waste error; check waste and touch Run or Reset	The process failed because there is no waste bottle or lab drain ready to collect waste from the instrument.	131207	1. Ensure at least one empty waste bottle is available and correctly installed (Section 7.1). 2. Touch **Run** or **Reset**. 3. If error persists, contact Bio-Rad Technical Service.
	Failed to drain to waste port 1.	132778	
	Failed to drain to waste port 2.	132779	
	Failed to drain the waste combiner.	132780	
Waste error; contact Technical Service	The waste valve is stuck.	133083	Contact Bio-Rad Technical Service.
Waste full; empty bottle and touch Run or Reset	The process failed because there is not enough space in the waste bottles to collect waste from the instrument.	131211	1. Empty the waste bottle(s). 2. Touch **Run** or **Reset**.

8.4 Chromatography Problems

Problem	Probable Cause	Recommended Solution
Calibrator Pack failure	Barcode error	Verify assigned calibrator values entered correctly in Calibration dialog box (Section 4.5).
	Calibrator Pack placed in wrong position in Stat rack	Ensure Calibrator Pack is in dedicated position in Stat rack.
	Inadequate calibrator volume	Verify microvials contain sufficient volume of calibrator.
	Calibrator Pack reconstituted more than once	Use new Calibrator Pack.
	Air in detector or high-pressure pump(s)	Prime/flush system (Section 7.6).
	Dirty detector	Contact Bio-Rad Technical Service.
	Suspect reagent or cartridge	Replace suspect component.

Problem	Probable Cause	Recommended Solution
Carryover	Sample not capped	1. Rerun all samples with suspected carryover. 2. All primary sample tubes should be capped before loading them onto the system. 3. If cap is missing, predilute sample in microvial prior to analysis.
	System not cleaned as directed	Clean the system as directed in Section 7.8.
	Inadequate wash of sample probe or inadequate drain of dilution well	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
Early retention times	Elevated sample concentration; high total areas.	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
	Cartridge temperature too high	Contact Bio-Rad Technical Service.
	Expired or suspect buffer(s)	Replace buffer(s).
	Suspect cartridge	Replace cartridge.
High Total Area	High hematocrit sample	Manually dilute sample at higher dilution ratio (1:400) and rerun.
	Problem venting sample tubes	Manually dilute sample and rerun.
	Inadequate wash of sample probe or inadequate drain of dilution well	Check dilution well and wash well for waste backup. Contact Bio-Rad Technical Service.
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
Late retention times	Low sample concentration; clotted sample.	Manually dilute sample and rerun.
	Low total areas; sample probe is bent or blocked.	Replace probe (Section 7.9).
	Air in high-pressure pump(s)	Prime/flush system (Section 7.6).
	Leak in flow path	Check for leaks. Contact Bio-Rad Technical Service.
	Cartridge temperature too low	Contact Bio-Rad Technical Service.
	Expired or suspect buffer(s)	Replace buffer(s).
	Suspect cartridge	Replace cartridge.

Problem	Probable Cause	Recommended Solution
Low Total Area	Short sample or clotted sample	Manually dilute sample and rerun.
	Unsupported tube type	
	Low hematocrit sample	Manually dilute sample at lower dilution ratio (1:100) and rerun.
	Prediluted sample not mixed	Remake prediluted sample, mix, and rerun.
	Prediluted sample treated as whole blood	1. Ensure the microvial adapter is properly seated in the rack. 2. If problem persists, discard and replace the adapter.
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
	Leak at dilution well	Contact Bio-Rad Technical Service.
	Sample tubing or probe clogged	Clean system (Section 7.8).
	Sample probe is bent or blocked	Replace probe (Section 7.9).
No HbA_{1c} result	Error occurred during result calculation (Note: Peak integration error)	Rerun sample.
	Very high total area carryover (Note: Automatic repeat - carryover risk)	
	Very high HbA_{1c} carryover (Note: Repeat sample - possible HbA1c carryover)	
Noise spikes appear on chromatogram/drifting baseline	Air in detector or high-pressure pump(s)	Prime/flush system (Section 7.6).
	Dirty detector	Contact Bio-Rad Technical Service.
	Detector board fault	Contact Bio-Rad Technical Service.
No peaks appear on the chromatogram; report shows no data.	Insufficient sample in the tube	Manually dilute sample and rerun.
	Clotted sample	
	Unsupported tube type	
	Air in dilution pump syringe	Prime/flush system (Section 7.6).
	Damaged cartridge	Replace cartridge.
	Sample tubing or probe clogged	Clean system (Section 7.8).
	Sample probe is bent or blocked	Replace probe (Section 7.9).
Poor peak shape (broad peaks, poor EMG fit, tailing)	Expired or suspect prefilter	Replace prefilter.
	Expired or suspect cartridge	Replace cartridge.
	Expired or suspect buffer(s)	Replace buffer(s).
	Dirty detector	Contact Bio-Rad Technical Service.
	Coeluting variant peak	Confirm by alternative methods.

Problem	Probable Cause	Recommended Solution
QC out of range	Low and high QC switched or barcoded incorrectly	Verify correct barcode and position in rack.
	Improper reconstitution of QC	Reconstitute new vials of QC.
	Improper predilution of QC	Prepare new manual dilutions of QC.
Third-party calibrator failure	Data entry error	Verify assigned calibrator values entered correctly in Calibration dialog box (Section 4.5).
	Calibrators placed in wrong position in Stat rack	Ensure Sample Diluent, calibrator level 1, and calibrator level 2 are in positions 1–3 respectively in Stat rack.
	Improper predilution of calibrators	Ensure third-party calibrators are manually diluted 1:300 with D-100 Sample Diluent before running (Section 5.9).

8.5 Other Problems

Problem	Probable Cause	Recommended Solution
Advisor flags applied incorrectly	Misconfigured/incorrectly edited rule(s)	Restore the default rules set (Section 4.7.4.2.3). Otherwise, contact Bio-Rad Technical Service for assistance.
Barcode: Sample barcode misread	Instrument damages barcode	Replace damaged barcode or enter barcode manually (Section 5.7.1). If problem persists, contact Bio-Rad Technical Service.
	Incompatible barcode symbology	See Section B.7 for supported barcode symbologies.
	Poor barcode quality	Replace barcode or enter barcode manually (Section 5.7.1).
	Multiple barcodes on tube	Remove barcodes and replace with single barcode or enter barcode manually (Section 5.7.1).
Barcode: Sample barcode not read	Incompatible barcode symbology	See Section B.7 for supported barcode symbologies.
	Incorrect placement of barcode on tube	Replace barcode (see Section B.7 for placement zone) or enter barcode manually (Section 5.7.1).
	Poor barcode quality	Replace barcode or enter barcode manually (Section 5.7.1).

Problem	Probable Cause	Recommended Solution
Calibrator Pack barcode information not read	Barcode label missing	Discard and replace the Calibrator Pack. Contact Bio-Rad Technical Service for a replacement.
	Barcode damaged	If the last barcode on the label is read and the system recognizes it as a Calibrator Pack, enter the human-readable lot number, expiration date, and assigned values manually in the Calibration dialog box (Section 4.5). Otherwise, discard and replace the Calibrator Pack. Contact Bio-Rad Technical Service for a replacement.
	Barcode reader fault	Contact Bio-Rad Technical Service.
Cartridge leaking	Installed incorrectly	Reinstall the cartridge correctly (Section 3.9).
Consumable installed on system is not detected	RFID tag missing	Discard and replace the consumable. Contact Bio-Rad Technical Service for a replacement.
	RFID tag failed	1. Remove and reinstall the consumable. 2. If problem persists, replace consumable. Contact Bio-Rad Technical Service for a replacement.
	Electromagnetic interference	Identify and remove the interfering device.
	Consumable incorrectly installed	Remove and reinstall the consumable. If problem persists, contact Bio-Rad Technical Service.

Problem	Probable Cause	Recommended Solution
Export: System fails to export results or information	Connectivity failure	• Verify the "Save to" location is correct. • If the "Save to" location is an "External" USB drive, ensure that it is properly connected to a USB port on the D-100. • If the "Save to" location is on the "Network": 1. Ensure the D-100 is connected to the network (i.e., the cable is connected to the correct network port and the cable is connected to the LAN port on the rear of the D-100). 2. Verify the network location is active (i.e., navigate to that folder from another connected instrument to confirm it is online and available). 3. Verify the network location is not full.
Internal printer paper jam	Paper installed incorrectly	Install the paper as instructed in Section 3.11.
	Incorrect paper used	Use only D-100 Thermal Printer Paper (REF 290-1013).
LIS: System not communicating with LIS	Incorrect LIS settings	Check LIS settings (Section 4.7.5) and correct as needed.
	LIS is not connected to D-100	Ensure LIS is connected to the D-100 serial port via RS-232 cable.
Power: Instrument does not power on when power switch is turned on, or loses power	Soft power button was not pressed	Press the soft power button on the front of the instrument to activate the power-on sequence.
	Power outage	Check main incoming circuit breaker.
	Fuse failure	Replace fuses (Section 8.6).
	Power switch failure	Contact Bio-Rad Technical Service.
Prefilter leaking	Installed incorrectly	Reinstall the prefilter correctly (Section 3.8).
Pressure variations	Air in high-pressure pump(s)	Prime/flush system (Section 7.6).

Problem	Probable Cause	Recommended Solution
Probe breaks	Unsupported tube type	1. Replace probe (Section 7.9). 2. Predilute sample in microvial prior to analysis.
	Probe is misaligned	Replace probe as instructed in Section 7.9, ensuring the probe is properly centered in the tube holder.
	Hardware failure	Contact Bio-Rad Technical Service.
Probe cannot pierce seal	Unsupported tube type	Predilute sample in microvial prior to analysis.
QC: System indicates QC Passed when actually failed or QC Failed when actually passed	Wrong values entered by user	Ensure the correct QC values are entered in the Edit Control Lot dialog box in the **QC/QC Lots** screen.
	Wrong sample used as QC	Ensure the QC samples are correctly labeled when prediluting.
	Low and high QC switched or barcoded incorrectly	Verify correct barcode and position in rack.
	Rules configured incorrectly by user	Verify the settings in the **QC/Rules** screen.
QC: System not importing/ exporting QC status/results from/to Unity	Connectivity/external media failure	See “Export: System fails to export results or information” solutions.
QC: The wrong QC result is excluded from calculations	User error	Clear the **Exclude data point from calculations** checkbox for that QC result in the Levey-Jennings Details screen.
Reagents: Excessive time for pressurization of newly installed reagent	Leaking connector	Replace the newly installed reagent bottle.
Sample Result: Bad result accidentally released	Operator error	If the LIS Connection is **Off** or no LIS is used, the action can be undone by rejecting the result. However, if the LIS Connection is **On**, releasing a result sends it to the LIS; the bad result must be manually rejected at the LIS.
Sample Result: Good result accidentally rejected	Operator error	The action can be undone by releasing the result.
Sample tube cannot spin	Multiple barcodes on tube	Remove barcodes and replace with single barcode or enter barcode manually (Section 5.7.1).
Sample tube cap sticks to tube spinner	Tube spinning incompatible with tubes	Disable tube spinning (Section 4.7.6).

Problem	Probable Cause	Recommended Solution
Sample tube does not fit in rack	Large-diameter or abnormal tube type	Predilute sample in microvial prior to analysis.
Sample tube is broken by tube spinner	Using non-D-100 sample racks that do not have rotary bottoms	Disable tube spinning (Section 4.7.6).
	Tube spinning incompatible with tubes	
Sample tube is loose in rack	Small-diameter tube	Use appropriate size rack insert. If tube is <12 mm in diameter, predilute sample in microvial prior to analysis.
Sample type misidentified	Wrong tube adapter used	Ensure you are using the correct tube adapters for prediluted samples: • Use QC1, QC2, and QC3 barcoded adapters for QC levels 1, 2, and 3, respectively. • Use non-barcoded adapter for prediluted patient samples.
Sysmex Racks: Input area incorrectly loaded	Too many racks in input area	A maximum of 9 racks can be positioned in the rack input area between the stopper pins and the front of the instrument; a 10th rack can be inserted after the run starts and the 1st rack is in the sampling position.
	Incorrect orientation	Remove and reposition the rack(s) so that the rack barcode faces the instrument and the Sysmex logo faces you.
Sysmex Racks: Rack jam	Non-D-100 rack used	1. Remove jammed rack. 2. Transfer sample tubes to a D-100 Sysmex rack, placing it in the rack input area. 3. Restart run.
	User tried to access rack that was being processed	1. Remove jammed rack. 2. Place the rack in the rack input area. 3. If necessary, restart run.

Problem	Probable Cause	Recommended Solution
Sysmex Racks: Racks not transported from one area to other	Rack jam; rack loaded incorrectly.	Remove and reposition the rack(s) in the input area, between the stopper pins and the front of the instrument, with the rack barcode facing the instrument and the Sysmex logo facing you.
	Rack not detected in rack input area	Contact Bio-Rad Technical Service.
	Failure of transport mechanism	Contact Bio-Rad Technical Service.
Sysmex Racks: System moves unprocessed rack to rack output area	User stopped the run and rack was ejected from the shuttle	Move rack to input area and start a new run.
	Power outage	After power is restored, move rack(s) to input area and start a new run.
Third Party Calibrator: System cannot process third party calibrators	User incorrectly loads calibrators	Follow the instructions in Section 5.9.
	User does not identify sample properly	
User Access: Unauthorized user access allowed	Improper user management	Edit the user's role (Section 4.7.3.2).
Waste container overflows without warning	Sensor failure	Contact Bio-Rad Technical Service.
Waste: System not discharging waste; waste backup	Kink in waste tubing backs up the waste combiner	Ensure that the waste tubing is not looped or crimped and is sloped downward at all times.
	Blockage	Contact Bio-Rad Technical Service.
	Pump failure	
	Sensor failure	

8.6 Fuse Replacement

Figure 8-1: *Power Switch Turned Off (O)*

1. Turn off the power switch on the rear of the instrument.
2. Remove the power cord.

Figure 8-2: *Removing Fuse Holder*

3. With an appropriate tool (e.g., standard screwdriver), remove the fuse holder.

Figure 8-3: *Fuse in Fuse Holder*

4. Remove both fuses (one on each side of the holder) and install 2 new 10 A/250 V fuses.

Figure 8-4: *Fuse Inserted for 220-240 V* 	5. Reinsert the fuse holder in the correct direction, based on the voltage used. The white arrow to the right of the voltage in use should be pointing to the white rectangle on the bottom.
6. Reinstall the power cord and turn on the power switch.	
7. Press the soft power button on the front of the instrument to activate the power-on sequence.	

Appendix A Replacement Parts

When ordering replacement parts, please refer to the list below for the catalog number, description, and quantity required. Quantities listed below indicate the minimum units available.

REF	Description	Quantity
740	Lyphochek® Diabetes Control Bilevel, 6 x 0.5 mL	1 pkg
740X	Lyphochek® Diabetes Control Bilevel MiniPak, 2 x 0.5 mL	1 pkg
171	Liquichek™ Diabetes Control, Level 1, 6 x 1.0 mL	1 pkg
172	Liquichek™ Diabetes Control, Level 2, 6 x 1.0 mL	1 pkg
173	Liquichek™ Diabetes Control, Level 3, 6 x 1.0 mL	1 pkg
172X	Liquichek™ Diabetes Control, Trilevel MiniPak, 3 x 1.0 mL	1 pkg
12000070	Lyphochek® Hemoglobin A1C Linearity Set (1 each of 6 levels), 6 x 0.5 mL	1 pkg
290-1004	D-100 HbA_{1c} Analytical Cartridge/Calibrator Pack	1 pkg
290-1006	D-100 HbA_{1c} Calibrator Pack	1 ea
290-1007	D-100 HbA_{1c} Prefilters (5 per package)	1 pkg
290-1008	D-100 Cleaning Tube	1 ea
290-1009	D-100 Sample Diluent	1 ea
290-1010	D-100 HbA_{1c} Elution Buffer A	1 ea
290-1011	D-100 HbA_{1c} Elution Buffer B	1 ea
290-1012	D-100 Wash Solution	1 ea
290-1013	D-100 Thermal Printer Paper (10 rolls per package)	1 pkg
12000063	D-100 Operation Manual with Multi-Language CD	1 ea
12000175	Fuses (10 A/250 V)	2 ea
12000182	D-100 Sample Probe	1 ea
12000230	External Waste Bottle	1 ea
12000231	External Waste Tubing	1 ea
12000232	Sysmex Rack	1 ea
12000233	Rack Inserts, 12 mm (10 per package)	1 pkg
12000234	Rack Inserts, 13 mm (10 per package)	1 pkg
12000235	Rack Inserts, 14 mm (10 per package)	1 pkg
12000236	Microvial Adapters (10 per package)	1 pkg
12000237	Sysmex Rack Barcode Labels (1–100)	1 pkg
12000238	Microvial Adapter QC Barcode Labels (3 sheets of 3 levels)	1 pkg
12000243	Sample Vials (polypropylene microvials with pierceable caps), 100 x 1.5 mL	1 pkg

REF	Description	Quantity
12000244	D-100 USB Flash Drive (8 GB)	1 ea
12000296	D-100 Utility Cartridge	1 ea
12000297	D-100 Utility Prefilter	1 ea

Appendix B Specifications

B.1 D-100 General Specifications

- Dimensions: 660 mm (W) x 650 mm (D) x 725 mm (H)
  25.98 in. (W) x 25.59 in. (D) x 28.54 in. (H)
- Weight uncrated: 103 kg (227 lb) dry; 121 kg (266 lb) wet
- Operating Environment

  Temperature: 15–35 °C

  Humidity: 20–80%, non-condensing

  Altitude: 3048 m (10,000 ft) max

  Heat Generation: 4092 BTU/h
- Storage Conditions

  Temperature: –10 °C to 50 °C

  Humidity: 20–80%

  NOTE: *Failure to store the D-100 under these conditions may result in damage to the system.*
- Power Input Requirements: 100–240 V~, 50–60 Hz
- Power Consumption: 1100 VA max
- Fuses: 10 A/250 V TLAG (2 fuses)
- Sound Level: <70 dBA
- Sample Requirements: Refer to assay IFU
- Sample Throughput: Refer to assay IFU
- User Interface: Integrated LCD touchscreen
- Adapter/Ports: Ethernet on RJ45 port; RS232 on DB9 port; External DVI-I connectors (with VGA); 6 External USB ports
- External Waste Bottle Volume: 5 L x 2 bottles

B.2 Pump Module Specifications

- Type: Pair of dual-piston HPLC pumps
- Flow Rate Range: 0.05–5.00 mL/min
- Maximum Pressure: 350 bar

B.3 Pressure Transducer Specifications

- Construction: Corrosive resistant titanium (6AL4V)
- Pressure Rating: 344.7 bar
- Over Pressure Range: 689.4 bar

B.4 Injection Valve Specifications

- Type: 3-position, 4-port Titan HT
- Loop Size: 5 µL x 2 internal injection loops

B.5 Sample Handling Specifications

- Sample Rack: Sysmex, 10 positions
- Sample Capacity: 10 racks in input area, 10 racks in output area
- Primary Tubes: width 12–16 mm, height 75–100 mm
- Sample Vials: 1.5-mL microvials
- Rack Inserts: 12 mm, 13 mm, 14 mm
- Microvial Adapters for 1.5-mL microvials
- Sample Probe: Stainless steel 316L, 143 mm
- Sample Dilution

  Dilution capability: 1:300 in 2 steps

  Dilution well volume: 405 µL

  Sample pickup volume: 10 µL

  Syringe volume: 978 µL

  Diluted sample pickup volume: 220 µL

B.6 Stat Area Specifications

- Sample Rack: Customized
- Sample Capacity: 3 positions for primary tubes or microvial adapters, 3 positions for Calibrator Pack

B.7 Sample Identification (Barcode) Specifications

- Barcode Symbologies supported:

Code 39
Code 128
Codabar
Interleaved 2 of 5
EAN
UPC

NOTE: *It is recommended to use a barcode symbology that includes a check digit (checksum character) to reduce the risk of sample misidentification.*

- Number of Characters: 1–22
- Barcode Symbol Dimensions: 10 mm min height, 67 mm max width
- Barcode Symbol Placement Zone: 20 mm above the bottom of the tube to 14 mm below the top of the tube, excluding the cap

B.8 Reagent Compartment Specifications

- Capacity: Accepts 2 bottles of Elution Buffer A, 2 bottles of Elution Buffer B, and 2 bottles of Wash Solution

B.9 External Waste Bottle Specifications

- Capacity: 5 L x 2 bottles
- Material: Polyethylene
- Level Sensor: 3 float switches

B.10 System Controller Specifications

- Central Processing Unit: Onboard PC
- Operating System: Microsoft® Windows® Embedded Standard 7
- Memory: 4 GB DDR3

B.11 Touchscreen Specifications

- Type: Integrated LCD touchscreen
- Dimensions: 282 mm (W) x 109 mm (D) x 377 mm (H)
- Angular Adjustment: 0 to10°
- Pixel format: 1024 horizontal by 768 vertical

B.12 Cartridge Holder Specifications

- Heating Device: Peltier HPE-128-10-05 Multicomp
- Temperature Sensor: PT100 4 wires measurement
- Temperature Accuracy: ±0.3 °C
- Temperature Stability: ±0.5 °C
- Overheating protection: Thermal fuse, 76 °C to 80 °C opening T

B.13 Internal Printer Specifications

- Dimensions: 178 mm (W) x 178 mm (D) x 325 mm (H)
- Paper Width: 76 mm (3 in.)
- Paper Roll: 50 mm
- Power Consumption: 1.5 A max (24 V); 200 mA (5 V)
- Printer Resolution: 8 dots/mm (203 dpi)
- Printer Technology: Thermal Printer
- Signal Connection: USB
- Printer Speed: 200 mm/s max

B.14 External Network Printer Specifications

- Compatible operating system: Microsoft® Windows® 7
- Connectivity: Hi-Speed USB 2.0, Ethernet
- Printing color: Monochrome (black & white)

Appendix C Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
1	Total Area Low*	The Total Area is less than the cutoff.	50000		Low total area	Should not report HbA1c	No	Enabled
2	Total Area High*	Total Area is greater than the cutoff.	350000		High total area	Should not report HbA1c; predilute/rerun	No	Enabled
3	No HbA1c	No HbA1c peak was identified.	NA		No HbA1c peak	No HbA1c result	No	Enabled
4	No HbA0	No HbA0 peak was identified.	NA		No HbA0 peak	No HbA1c result	No	Enabled
5	HbA1c Range*	The HbA1c result is outside the reportable range.	3.5–20.0 (%) **or** 15–195 (mmol/mol)		HbA1c result is out of range	Should not report HbA1c	No	Enabled
6	HbA1c High*	The HbA1c result is greater than the cutoff.	15 (%) **or** 140 (mmol/mol)		High HbA1c	Possible variant interference	No	Enabled
7	E and D Present	Peaks are present in the E-Window and D-Window.	NA		E-Window and D-Window present	Possible variant interference	No	Enabled
8	E and S Present	Peaks are present in the E-Window and S-Window.	NA		E-Window and S-Window present	Possible variant interference	No	Enabled
9	E and C Present	Peaks are present in the E-Window and C-Window.	NA		E-Window and C-Window present	Possible variant interference	No	Enabled
10	D and S Present	Peaks are present in the D-Window and S-Window.	NA		D-Window and S-Window present	Possible variant interference	No	Enabled
11	D and C Present	Peaks are present in the D-Window and C-Window.	NA		D-Window and C-Window present	Possible variant interference	No	Enabled
12	S and C Present	Peaks are present in the S-Window and C-Window.	NA		S-Window and C-Window present	Possible variant interference	No	Enabled

Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
13	Minor Peak(s) > 10%*	The Unknown 1, Unknown 2, Unknown 3, Unknown 4, A1a, A1b, or P3 Area% is greater than the cutoff.	10 (%)		Minor peak(s) > 10%	Possible variant interference	No	Enabled
14	HbS Cutoff	The S-Window Area% is greater than the cutoff.	60 (%)		Elevated peak in S-Window	Possible variant interference	No	Enabled
15	HbC Cutoff	The C-Window Area% is greater than the cutoff.	60 (%)		Elevated peak in C-Window	Possible variant interference	No	Enabled
16	HbD Cutoff	The D-Window Area% is greater than the cutoff.	43 (%)		Elevated peak in D-Window	Possible variant interference	No	Enabled
17	HbE Cutoff	The E-Window Area% is greater than the cutoff.	39.1 (%)		Elevated peak in E-Window	Possible variant interference	No	Enabled
18	HbF Cutoff*	The F Area% is greater than the cutoff.	30 (%)		Elevated HbF	Should not report HbA1c	No	Enabled
19	S-Window Present	A peak is present in the S-Window.	NA		Peak in S-Window	NA	No	Disabled
20	C-Window Present	A peak is present in the C-Window.	NA		Peak in C-Window	NA	No	Disabled
21	D-Window Present	A peak is present in the D-Window.	NA		Peak in D-Window	NA	No	Disabled
22	E-Window Present	A peak is present in the E-Window.	NA		Peak in E-Window	NA	No	Disabled
23	Unread Barcode	A sample tube or microvial barcode was not read.	NA		NA	Unread barcode	No	Disabled
24	LA1c Cutoff	The LA1c Area% is greater than the cutoff.	7 (%)		NA	High LA1c (info only)	No	Disabled
25	Baseline Slope	The A1c Slope-To-Area Ratio is outside the acceptable range.	0.00–0.90	No	NA	Baseline slope outside range (info only)	No	Disabled

Advisor Default HbA1c Rules Set, Version 1.00

No.	Rule Name	Explanation	Cutoff Value(s)	Flag Sample	Comment	Note to User	Auto Repeat	Rule Enabled or Disabled
26	A1c Sigma	The A1c Sigma is outside the acceptable range.	0.30–0.90	No	NA	A1c sigma outside range (info only)	No	Disabled
27	A1c Tau	The A1c Tau is outside the acceptable range.	0.168–2.200	No	NA	A1c tau outside range (info only)	No	Disabled
28	A1c Tau/Sigma	The A1c Tau/Sigma Ratio is outside the acceptable range.	0.00–3.90	No	NA	A1c tau/sigma outside range (info only)	No	Disabled
29	A1c Fit Crest Time	The A1c Fit Crest Time Diff is outside the acceptable range.	1.40–3.20	No	NA	A1c fit crest time outside range (info only)	No	Disabled

***NOTE:** *The cutoff values for these rules correspond to the performance claims in the assay Instructions For Use.*

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