510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K151321

B. Purpose for Submission:

New Device

C. Measurand:

Whole blood Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Ion-Exchange Quantitative High Performance Liquid Chromatography

E. Applicant:

Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:

D-100 HbA1c
D-100 HbA1c Calibrator Pack

G. Regulatory Information:

Regulatory Description	Product Code	Classification	Regulation Section	Panel
Hemoglobin A1c Test System	PDJ, LCP	Class II	21 CFR 862.1373	Chemistry, 75
Calibrator	JIT	Class II	21 CFR 862.1150	Chemistry, 75

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) 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 in identifying patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control 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.

3. Special conditions for use statement(s):

The D-100 HbA1c test is not intended for analysis of samples collected from newborns.

The D-100 HbA1c test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.

HbA1c should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA1c 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 D-100 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.

For prescription use only.

4. Special instrument requirements:

Bio-Rad D-100 Hemoglobin Testing System

I. Device Description:

The D-100 HbA1c assay is run on the D-100 Hemoglobin Testing System instrument.

The Bio-Rad D-100 HbA1c assay includes the following components:

100 TM Prefilters	2000 tests each. Package of 5
100 TM Cleaning Tube.	One microvial containing 1.5 mL of a liquid cleaning solution.
100 TM Sample Diluent.	Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.
100 TM HbA1c Elution Buffer A.	Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.
100 TM HbA1c Elution Buffer B.	Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains<0.1% sodium azide as a preservative.
100 TM HbA1c Wash Solution.	Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.

D-100 TM HbA1c 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.
- 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.

The Calibrators contain lyophilized human whole blood with glycine and trehalose as preservatives.

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.

Controls for the system were previously reviewed and cleared in k070546 and k052838.

J. Substantial Equivalence Information:

1. Predicate Device name(s):

VARIANT II TURBO HbA1c Kit -2.0

VARIANT II Hemoglobin A1c Calibrators

2. Predicate 510(k) number(s)

K142448

K070452

3. Comparison with predicate

Similarities and Differences - Assay		
Item	Candidate Device D-100HbA1c (K151321)	Predicate Device: VARIANT II TURBO HbA1c Kit – 2.0 (K142448)
Intended Use	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.	Intended for the quantitative determination of hemoglobin A1c (IFCC mmol/mol and NGSP %) as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes.
Test principle	Ion exchange HPLC	Same
Sample Types	Human Whole Blood collected with K_2-EDTA, K_3-EDTA Potassium Oxalate/Sodium Fluoride, Sodium Citrate, Sodium Heparin, Lithium Heparin	Human Whole Blood collected with K_2-EDTA, K_3-EDTA Hemoglobin Capillary Collection Kit
Instrument Platform	D-100™ Hemoglobin Testing System	VARIANT™TURBO Hemoglobin Testing System and VARIANT™TURBO Link Hemoglobin Testing System
Measuring Range	3.5 to 20% (NSGP) 15 – 195 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)
Standardization	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and	Same

Similarities and Differences - Assay		
Item	Candidate Device D-100HbA1c (K151321)	Predicate Device: VARIANT II TURBO HbA1c Kit – 2.0 (K142448)
	IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP).	

Similarities and Differences - Calibrators		
Item	Candidate Device D-100 HbA1c Calibrator Pack (K151321)	Predicate Device: VARIANT II Hemoglobin A1c Calibrators (K070452)
Intended Use	Intended for the quantitative determination of hemoglobin A1c in Human Whole Blood	Same
Levels	Levels 1 & 2 Calibration is performed once at the beginning of a new cartridge.	Same
Standardization/Traceability	Each lot of calibrators is value assigned and values are reported in both NGSP and IFCC units.	Same
Matrix	Lyophilized human whole blood with glycine and trehalose as preservatives.	Human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guidelines - Second Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach: Approved Guideline

CLSI EP07-A2: Interference Testing in Clinical Chemistry: Approved Guideline- Second Edition

CLSI EP09-A2-IR: Method Comparison and Bias Estimation Using Patient samples; Approved Guideline- Second Edition

CLSI EP14-A2: Evaluation of Matrix Effect: Approved Guideline-Second Edition

L. Test Principle:

The device is based on chromatographic separation of HbA1c on a cation exchange cartridge. A high pressure pumping system first delivers a buffer solution to a cation exchange cartridge and detector. Whole blood samples undergo automatic hemolysis and dilution before being delivered to the cation exchange cartridge in a buffer gradient of increasing ionic strength. The various forms of hemoglobin exhibit charge differences at the pH of the buffer and are separated on the cation exchange cartridge based on these charge differences. The separated hemoglobin species then pass through a flow cell where changes in absorbance are measured and recorded as a digital chromatogram. Software performs an analysis of the hemoglobin peaks in the chromatogram and identifies peaks as target analytes. 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the D-100 HbA1c test was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guidelines - Second Edition. Four K_3-EDTA whole blood samples at concentrations near 5%, 6.5%, 8% and 12% HbA1c and five whole blood quality control solutions (Control 1, Control 2, QC1, QC2, QC 3)were analyzed in duplicate, twice a day, with three lots of reagents, over 20 days each, on three D-100 Hemoglobin Testing System instruments at two different sites. For each sample there were 720 measurements. Results are shown in the tables below.

Table 1: 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 2: 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 3: 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 4: 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 5: Instrument 1 (%CV by Sample, IFCC)

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 mmol/mol)	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 6: Instrument 2 (%CV by Sample, IFCC)

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 mmol/mol)	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 7: Instrument 3 (%CV by Sample, IFCC)

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 (IFCC mmol/mol)	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 8: 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 mmol/mol)	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/assay reportable range:

A linearity study was performed based on CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures; Linearity across the reportable range was performed using altered patient samples collected using K_3-EDTA. Samples were prepared by first altering two patient whole blood samples to obtain a low HbA1c of 3.5% (15 mmol/mol) and a high HbA1c of 20.0% (195 mmol/mol). These high and low samples were mixed together in varying ratios to obtain 9 additional intermediate

sample levels. The eleven test samples (high, low and 9 intermediates) were run in duplicate on one instrument; measured values were compared to expected values.

The regression parameters (slope, intercept, and R^2) were the following:

Table 9: linearity Regression parameters

Units/Values	Slope	Intercept	R^2
NGPSP	0.9992	0.03961	0.9997
IFCC	0.999	0.455	0/9997

The linearity study was reviewed and found acceptable. Results of the linearity study support the claimed measuring range of the device of 3.5% to 20% HbA1c (15-195 mmol/mol).

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

D-100 HbA1c test standardization is traceable to both the International Federation of Clinical Chemistry (IFCC) reference calibrators and the Diabetes Control and Complications Trial (DCCT). The assay is certified by the National Glycohemoglobin Standardization Program (NGSP).The NGSP certification expires in one year. See the NGSP website for current certification at http://www.ngsp.org.

HbA1c results are provided to users in two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol). Results in % HbA1c from the NGSP correlation are calculated from the individual quantitative results for Hemoglobin A1c. The IFCC units of mmol/mol are calculated using the Master Equation: NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152.

Closed-Vial Stability:

Accelerated and real time stability study protocols and acceptance criteria for reagents including the Analytical Cartridge, Prefilter, Sample Diluent, Elution Buffer A, Elution Buffer B, Wash Solution and Cleaning Tube were reviewed and found to be acceptable. The Analytical Cartridge and Prefilter are stable for 24months when stored at 2–8°C, the Sample Diluent is stable for 24 months when stored unopened at 15–35°C, Elution Buffers and Wash Solution are stable for 24 months when stored unopened at 2–8°C, the Cleaning Tube is stable for 12 months when stored unopened at 15–35°C. Real time stability studies are ongoing.

In-Use Stability:

In-use stability study protocols and acceptance criteria for reagents including the Analytical Cartridge, Prefilter, Sample Diluent, Elution Buffer A, Elution Buffer B, and Wash Solution were reviewed and found to be acceptable. The Analytical

Cartridge and Prefilter are stable for 90 days when stored at 15–35°C when installed on the instrument, the Sample Diluent is stable for 90 days after opening when stored at 15–35°C, the Elution Buffers and Wash Solution are stable for 90 days at 15–35°C when installed on the instrument.

Calibrators and Control Materials

Value assignment:

Value assignment for the D-100 HbA1c Calibrator Pack (Level 1 and Level 2) is conducted by measuring each Level over multiple runs using multiple D-100 Hemoglobin Testing System instruments. The D-100 HbA1c Calibrator Pack Levels are assigned final concentration values based on the mean of multiple runs and comparison to values of IFCC reference calibrators run in parallel.

The commercially available Bio-Rad Lyphocheck Diabetes control materials and the Bio-Rad Liquicheck Diabetes control materials were previously reviewed in k070546 and k052838 respectively.

Open Vial Stability:

The open-vial stability study protocols and acceptance criteria were reviewed and found acceptable. Results support the open vial stability claim for reconstituted D-100 HbA1c Calibrator Pack of 24 hours when stored at 2-8 °C.

Closed Vial Stability:

The closed-vial stability study protocols and acceptance criteria were reviewed and found acceptable. Results support a closed vial stability claim for reconstituted D-100 Calibrator Pack of 24 months when stored at 2-8 °C. Real time stability studies are ongoing.

d. Detection limit:

Not applicable.

e. Analytical specificity:

i. Endogenous Interference

An Endogenous Interference studies was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. This study assessed common or known endogenous substances that could interfere with the D-100 HbA1c test. Potentially interfering endogenous substances were analyzed by spiking whole blood samples with HbA1c values of ~6.5% HbA1c and ~8% HbA1c with potentially interfering substances to create test samples. Ten replicates of each test and reference sample

(sample containing no potential interferent) were analyzed and the difference in HbA1c value between the mean of test and reference replicates was determined. Significant interference was defined by the sponsor as ≥7% change in HbA1c value of the mean of the test samples relative to mean of the reference samples.

The following endogenous substances showed no significant interference at the concentrations described below:

Table 10: Endogenous interfering substances tested

Endogenous Substance	Concentration (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 studies was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. This study assessed common or known drugs that could interfere with the D-100 HbA1c Test. Two whole blood sample pools at ~6.5% HbA1c (Low) and ≥8.0% HbA1c (High) were spiked with each potentially interfering drug to create test samples. Ten replicates of each test and reference sample (sample containing no potential interferent) were analyzed and the difference in HbA1c value between the mean of test and reference replicates was determined. Significant interference was defined by the sponsor as ≥7% change in HbA1c value of the mean of the test samples relative to mean of the reference samples.

The following endogenous substances showed no significant interference at the concentrations described below:

Table 11: Highest tested non-interfering drug concentrations

Drug Name	Concentration
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

Hemoglobin Derivatives Interference studies was performed based on CLSI EP07-A2, Interference Testing in Clinical Chemistry. These studies study assessed potential interference from Acetylated hemoglobin (Hb), Carbamylated hemoglobin (Hb) and Labile HbA1c. Two whole blood sample pools at ~6.5% HbA1c (Low) and ≥8.0% HbA1c (High) were spiked with each potentially interfering hemoglobin derivative. Serial dilutions of each high concentration test sample were prepared, resulting in five or six levels of each potentially interfering hemoglobin derivative. Ten replicates of each level and reference sample (sample containing no potential interferent) were analyzed and the difference in HbA1c value between the mean of each level and the reference was determined. Significant interference was defined by the sponsor as ≥7% change in HbA1c value of the mean of the test samples relative to mean of the reference samples. The test result conclusions are as follows:

• Carbamylated Hb - (up to 5% Carbamylated Hb) does not interfere with this assay.

• Labile A1c- (up to 7% Labile A1c, ≈1200mg/dL glucose) does not interfere with

this assay.

• Acetylated Hb- (up to 50 mg/dL acetylsalicylic acid) does not interfere with this assay.

Results showed there was no cross reactivity with these substances at physiological levels and the labeling states that at physiologically occurring concentrations there is no interference from labile A1c, carbamylated hemoglobin or acetylated hemoglobin.

iv. Hemoglobin Variant Interference

A Hemoglobin Variant Interference study was performed using a total of 135 samples known to contain hemoglobin variants S, C, E, D, A2 and F. 78 whole blood patient samples containing an HbA1c ~6.5% and ~9.0% and the appropriate hemoglobin variant concentrations at levels typically found in heterozygous individuals (up to 30% to 40%) were tested using the D-100 HbA1c Test on the D-1000 Hemoglobin Testing System and compared to results obtained by a reference method that has been demonstrated to be free from interference with the hemoglobin variant being tested (Trinity Biotech Ultra2 A1c and Premier Hb9210 analyzers). The following tables show the samples that were measured (Table 12) and the range of biases that were observed (Table 13) for Hemoglobin Variants S, C, D and E, and the mean biases with 2 standard deviations observed for Hemoglobin Variants F and A2 :

Table 12: Hemoglobin variant samples ranges

Hemoglobin Variant	Number of samples	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
HbF	30	4.1-30.2	4.4-14.4
HbA2	25	5.0-13.3	5.0-14.5

Table 13: Relative Bias Summary

Hemoglobin Variant	Relative % Bias (Range of % Bias) observed relative to Reference Method	
	HbA1c ~6.5% A1c	HbA1c ~9% A1c
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)

Significant interference was defined as ≥7% change in HbA1c value in the presence of the hemoglobin variant relative to control. The results show there is no significant interference for HbS (≤40.2%), HbC (≤42.1%), HbD (≤42.6%), HbE (≤32.5%), HbF

(≤30.2%) and HbA2 (≤13.3%).

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. 129 variant-free whole blood K_3-EDTA samples, including 4 spiked samples (2 samples at HbA1c <5% and 2 samples at HbA1c >9%), ranging from 3.4% to 19.2% HbA1c were evaluated using D-100 HbA1c Test on the D-100 HbA1c Testing System. Samples were tested in singlicate over four days. The results were compared to testing performed at a NGSP Secondary Reference Laboratory using a previously cleared HPLC HbA1c assay method (Tosoh G8 HPLC analyzer). To support the diagnostic claim, the distribution of samples spanned around the clinical decision point as follows in the table below.

Table 14: Sample distribution in the method comparison study

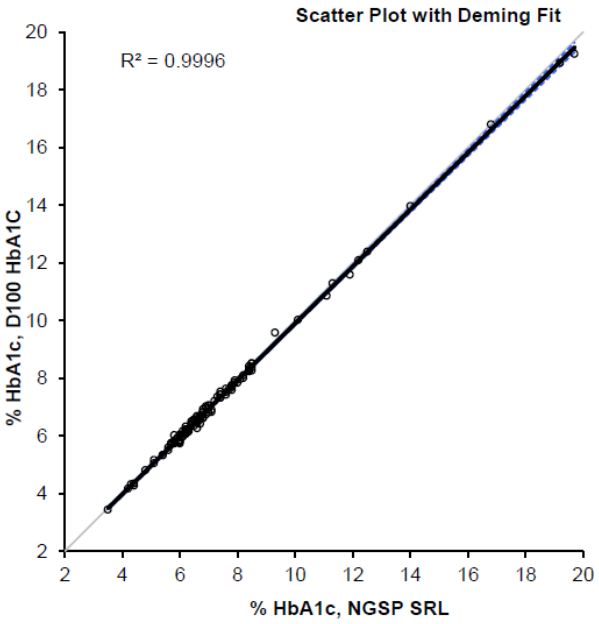
Hemoglobin A1c level	Number of samples	% of Samples
≤ 5%	6	4.7
5 – 6%	17	13.2
6 – 6.5%	31	24.0
6.5 – 7%	33	25.6
7 – 8%	20	15.5
8 – 9%	11	8.5
> 9%	11	8.5
Total samples	129	100

Bias between Candidate and NGSP method

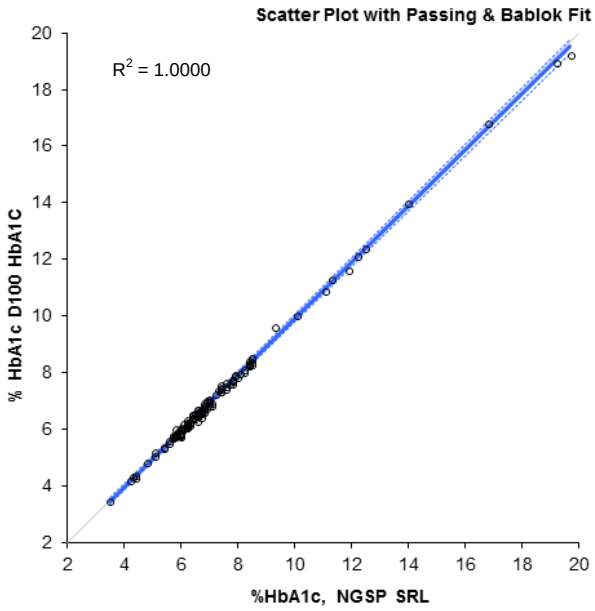
Deming (weighted) and Passing-Bablok regression analyses were performed for the D-100 Hemoglobin Testing System versus the NGSP SRL reference method.

Table 15: 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



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



Scatter Plot using Passing-Bablok Fit, %HbA1c, NGSP SRL vs. D-100 HbA1c

The following biases between the D-100 HbA1c Test run on the D-100 HbA1c Testing System versus the NGSP Reference Method (Tosoh G8 HPLC analyzer) were observed:

Table 16: Deming Regression Analysis (NGSP):

% HbA1c Decision Level	Bias (in % HbA1c)	% Bias
5.0	-0.044	-0.88
6.5	-0.064	-0.98
8.0	-0.084	-1.05
12.0	-0.136	-1.13

Table 17: Passing-Bablok Regression Analysis (NGSP):

% HbA1c Decision Level	Bias (in % HbA1c)	% Bias
5.0	-0.055	-1.09
6.5	-0.068	-1.05
8.0	-0.082	-1.02
12.0	-0.118	-0.99

Total Error Near the Cutoff

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the precision study, Total Error (TE) at four HbA1c concentrations (5.0%, 6.5% , 8.0% and 12.0%) was calculated as follows: %TE =|%Bias| + 1.96 *%CV*(1+%Bias).

The results are presented in the tables below.

Table 18: Total Error

Decision Level (% HbA1c)	% Bias	% CV	% TE
5.0	-0.84	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

b. Matrix comparison:

A matrix study was performed to determine the suitability of K_2-EDTA, K_3-EDTA, potassium oxalate/sodium fluoride, sodium citrate, sodium heparin and lithium heparin anticoagulants used with fresh whole blood for use with the D-100 HbA1c Test with the D-100 HbA1c Hemoglobin Testing System. Specimens with concentration values spanning 3.5 to 20.0% HbA1c were collected from a total of 43 different donors. The 43 paired samples included the following sample tube types: (1) K_2 EDTA, (2) K_3 EDTA, (3) Potassium Oxalate/Sodium Fluoride, (4) Sodium Citrate, (5)Sodium Heparin, and (6) Lithium Heparin.

Three samples were altered to cover the extreme ends of the measuring range. All samples were compared to results from whole blood in K_3 EDTA; regression results are as follows.

Table 19: Matrix comparison regression results

Sample Type	N	K_3-EDTA Sample Ranges	Slope	95% CI of Slope		Intercept	95% CI of Intercept		R^2
				Lower	Upper		Lower	Upper	
K_2-EDTA	43	3.53-20.06	0.9929	0.9861	0.9997	0.0270	-0.0204	0.0745	0.9995
K oxalate / NaF	43	3.53-20.06	0.9927	0.9853	1.0002	0.0761	0.0243	0.1279	0.9994
Na citrate	43	3.53-20.06	1.0084	1.0017	1.0152	-0.0429	-0.0899	0.0040	0.9996
Na heparin	43	3.53-20.06	0.9972	0.9907	1.0037	0.0131	-0.0322	0.0584	0.9996
Li heparin	43	3.53-20.06	0.9959	0.9905	1.0013	0.0103	-0.0273	0.0479	0.9997

In this Matrix Comparison study, the following tube types were under evaluation and were found to be acceptable for use with the D-100 HbA1c Test when using the D-100 HbA1c Hemoglobin Testing System :

- K_2-EDTA tubes with fresh whole blood
- K_3-EDTA tubes with fresh whole blood
- potassium oxalate/sodium fluoride with fresh whole blood
- sodium citrate with fresh whole blood
- sodium heparin with fresh whole blood
- lithium heparin with fresh whole 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:

The sponsor provided the following expected values in their labeling:

The following HbA1c 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 HbA1c range for non-diabetic adults is 4–6%.[26]

4. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* **2010,** *33* (Suppl. 1), S62–S69.
5. International Expert Committee. Report on the Role of the A1c Assay in the Diagnosis of Diabetes. *Diabetes Care* **2009,** *32* (7), 1327–1334.
6. World Health Organization. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. http://www.who.int/diabetes/publications/diagnosis_diabetes2011/en/ (accessed May 2015).
26. Clinical Diabetes. Your A1C Results: What Do They Mean? http://clinical.diabetesjournals.org/content/24/1/9. full.pdf+html (accessed November 2015).

N. Instrument Name:

D-100 Hemoglobin Testing System

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes ___X_____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device

using wireless transmission?

Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

3. Specimen Identification:

   Sample identification is via barcoding or manual entry.

4. Specimen Sampling and Handling:

   A study was conducted to show the stability of samples collected in: (1) K_2-EDTA, (2) K_3-EDTA, (3) Potassium Oxalate/Sodium Fluoride, (4) Sodium Citrate, (5) Sodium Heparin, and (6) Lithium Heparin. Study protocols and acceptance criteria were reviewed and found to be acceptable to support storage of samples in all of these matrices at 15-35°C for 1 day, at 2-8°C for 7 days, at -20°C for 7 days and at -70°C for 6 months. Additional real-time studies of sample stability at -70°C are ongoing.

   A study was conducted to show the stability of pre-diluted samples at system operating temperatures. Study protocols and acceptance criteria were reviewed and found to be acceptable to support storage of pre-diluted samples for 3 hours at15-35°C.

5. 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. Calibration is performed using the D-100 HbA1c Calibrator Pack.

6. Quality Control:

   Quality control of the D-100 Hemoglobin Testing System can be conducted using commercially available Bio-Rad Lyphocheck Diabetes control materials and Bio-Rad Liquicheck Diabetes control materials; these were previously cleared in k070546 and k052838 respectively. Labeling for the device states that 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 and that each laboratory should establish its own guidelines for corrective action to be taken if the expected control values are not obtained.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The

"Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.