510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k161687

B. Purpose for Submission:

Addition of a diagnostic claim to an existing device.

C. Measurand:

Whole Blood Glycated 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-10 Hemoglobin A1c Program

G. Regulatory Information:

Product Code	Regulation Name	Classification	Regulation Section	Panel
PDJ	Hemoglobin A1c Test System	Class II	21 CFR 862.1373	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indication for use below.

2. Indication(s) for use:

The D-10 Hemoglobin A1c Program 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-10 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-10 Hemoglobin A1c Program is intended for professional in vitro diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only.

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.

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.
The HbA1c test 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 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.

4. Special instrument requirements:

   Bio-Rad D-10 Hemoglobin Testing System

I. Device Description:

The D-10 HbA1c assay is run on the D-10 Hemoglobin Testing System instrument.

The Bio-Rad D-10 HbA1c assay includes the following components:

D-10 HbA1c Analytical Cartridge. Cation exchange cartridge (400 tests), 4.0 mm x 30 mm.
D-10 Wash/Diluent Solution. Each bottle contains 1600 mL of deionized water with <0.05% sodium azide as a preservative.
D-10 Elution Buffer 1. Each bottle contains 2000 mL of a Bis-Tris/Phosphate buffer. Contains <0.05% sodium azide as a preservative.
D-10 Elution Buffer 2. Each bottle contains 1000 mL of a Bis-Tris/Phosphate buffer. Contains <0.05% sodium azide as a preservative.
D-10 Calibrator/Diluent Set. One set consisting of 3 vials of Calibrator Level 1, 3 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. The D-10 Calibrator/Diluent Set was previously cleared in k031043.
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.
Sample Vials. 100 polypropylene microvials with pierceable caps, 1.5 mL.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VARIANT II TURBO HbA1c Kit -2.0

2. Predicate 510(k) number(s):

   k142448

3. Comparison with predicate:

Similarities		
Item	D-10 Hemoglobin A1c Program Candidate Device	VARIANT II TURBO HbA1c Kit – 2.0 Predicate Device (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.
Specimen Type	Human Whole blood	Same
Assay Principle	Ion exchange HPLC	Same
Standardization	Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)	Same

Differences		
Item	D-10 Hemoglobin A1c Program Candidate Device	VARIANT II TURBO HbA1c Kit – 2.0 Predicate Device (k142448)
Platform	D-10 Hemoglobin Testing System	VARIANTII TURBO Hemoglobin Testing System and VARIANTII TURBO Link Hemoglobin Testing System
Measuring Range	3.9 to 18.8% (NSGP) 19 – 182 mmol/mol HbA1c (IFCC)	3.4 to 20.6 % (NSGP) 14 – 203 mmol/mol HbA1c (IFCC)

Matrices	K2-EDTA, K3-EDTA	K2-EDTA, K3-EDTA Hemoglobin Capillary Collection Kit

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

CLSI EP05-A2- Evaluation of Precision Performance of Clinical Devices; Approved Guideline

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

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

CLSI EP12-A2- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline

CLSI EP17-A2-Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline

CLSI EP25A-Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI EP28-A3-Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

L. Test Principle:

The D-10 Hemoglobin A1c Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 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-10 software collects raw data from each analysis and calculates HbA1c values based on a bi-level calibration curve. A sample report and a chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the D-10™ Hemoglobin A1c Program was evaluated based on CLSI EP05-A2 guidelines, Evaluation of Precision Performance of Quantitative Measurement Methods using a modified study design. Four K3 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. The samples were run 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. NGSP results are shown in Tables 1-4. IFCC results are shown in Tables 5-8.

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

Sample ID	HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%C	SD	%CV	SD	%CV	SD	%CV
Patient 1	5.2	0.07	1.3	0.00	0.0	0.04	0.7	0.08	1.6	0.11	2.2
Patient 2	6.7	0.07	1.0	0.00	0.0	0.07	1.1	0.05	0.7	0.11	1.7
Patient 3	8.4	0.05	0.6	0.01	0.2	0.08	1.0	0.02	0.2	0.10	1.2
Patient 4	12.7	0.09	0.7	0.06	0.5	0.17	1.3	0.21	1.7	0.29	2.3
Control 1	5.6	0.07	1.2	0.00	0.0	0.05	0.9	0.05	0.9	0.10	1.8
Control 2	10.4	0.10	0.9	0.00	0.0	0.12	1.1	0.12	1.2	0.20	1.9
QC 1	5.5	0.06	1.0	0.00	0.0	0.05	0.9	0.08	1.5	0.11	2.1
QC 2	10.0	0.13	1.3	0.00	0.0	0.09	0.9	0.06	0.6	0.17	1.7
QC 3	15.6	0.11	0.7	0.15	1.0	0.08	0.5	0.17	1.1	0.27	1.7

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

Sample ID	HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%C	SD	%CV	SD	%CV	SD	%CV
Patient 1	5.2	0.04	0.9	0.02	0.4	0.04	0.8	0.08	1.5	0.10	1.9
Patient 2	6.6	0.03	0.5	0.04	0.6	0.05	0.8	0.02	0.3	0.08	1.2
Patient 3	8.2	0.04	0.5	0.04	0.4	0.07	0.8	0.02	0.2	0.09	1.0
Patient 4	12.5	0.06	0.5	0.08	0.6	0.13	1.1	0.21	1.7	0.27	2.1
Control 1	5.6	0.04	0.8	0.02	0.3	0.06	1.0	0.07	1.2	0.10	1.7
Control 2	10.1	0.04	0.4	0.06	0.6	0.08	0.8	0.09	0.9	0.14	1.4
QC 1	5.5	0.03	0.6	0.02	0.4	0.06	1.0	0.08	1.5	0.11	1.9
QC 2	9.8	0.07	0.7	0.06	0.6	0.09	0.9	0.04	0.4	0.13	1.4
QC 3	15.2	0.07	0.4	0.08	0.5	0.14	0.9	0.24	1.6	0.29	1.9

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

Sample ID	HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Patient 1	5.2	0.06	1.2	0.00	0.0	0.06	1.2	0.05	0.9	0.10	1.9
Patient 2	6.7	0.05	0.7	0.02	0.3	0.08	1.2	0.00	0.0	0.09	1.4
Patient 3	8.3	0.05	0.6	0.03	0.4	0.11	1.3	0.04	0.5	0.13	1.6
Patient 4	12.7	0.07	0.6	0.07	0.6	0.17	1.3	0.21	1.6	0.29	2.2
Control 1	5.6	0.05	0.9	0.00	0.0	0.07	1.2	0.04	0.8	0.10	1.7
Control 2	10.3	0.08	0.8	0.05	0.5	0.14	1.3	0.08	0.8	0.19	1.8
QC 1	5.5	0.04	0.8	0.02	0.3	0.07	1.2	0.07	1.3	0.11	2.0
QC 2	9.9	0.09	0.9	0.00	0.0	0.12	1.2	0.13	1.3	0.20	2.0
QC 3	15.5	0.10	0.6	0.11	0.7	0.18	1.1	0.07	0.4	0.24	1.6

Table 4: Instruments Combined (% CV by Sample (NGSP))

Sample ID	HbA1c %	Repeatability		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Patient 1	5.2	0.06	1.2	0.00	0.0	0.05	0.9	0.00	0.0	0.07	1.4	0.10	2.0
Patient 2	6.7	0.05	0.8	0.01	0.2	0.07	1.0	0.05	0.8	0.03	0.5	0.11	1.6
Patient 3	8.3	0.05	0.6	0.03	0.3	0.09	1.1	0.08	0.9	0.03	0.3	0.13	1.6
Patient 4	12.7	0.08	0.6	0.07	0.6	0.16	1.2	0.00	0.0	0.21	1.7	0.28	2.2
Control 1	5.6	0.06	1.0	0.00	0.0	0.06	1.0	0.00	0.0	0.05	0.9	0.10	1.7
Control 2	10.3	0.08	0.7	0.04	0.4	0.12	1.1	0.14	1.3	0.10	1.0	0.22	2.2
QC 1	5.5	0.05	0.8	0.00	0.1	0.06	1.0	0.00	0.0	0.08	1.4	0.11	2.0
QC 2	9.9	0.10	1.0	0.00	0.0	0.10	1.0	0.05	0.5	0.09	0.9	0.17	1.8
QC 3	15.4	0.09	0.6	0.12	0.8	0.14	0.9	0.20	1.3	0.17	1.1	0.34	2.2

Table 5: Instrument 1 (% CV by Sample (IFCC Units- mmol/mol))

Sample ID	HbA1c mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD
Patient 1	33	0.76	2.3	0.00	0.0	0.40	1.2	0.89	2.7	1.24
Patient 2	50	0.76	1.5	0.00	0.0	0.81	1.6	0.54	1.1	1.23
Patient 3	68	0.53	0.8	0.16	0.2	0.92	1.3	0.17	0.3	1.08
Patient 4	116	1.01	0.9	0.65	0.6	1.85	1.6	2.31	2.0	3.19
Control 1	38	0.77	2.0	0.00	0.0	0.56	1.5	0.53	1.4	1.09
Control 2	91	1.08	1.2	0.00	0.0	1.31	1.4	1.34	1.5	2.16
QC 1	37	0.63	1.7	0.00	0.0	0.53	1.4	0.93	2.5	1.25
QC 2	86	1.46	1.7	0.00	0.0	0.96	1.1	0.61	0.7	1.85
QC 3	147	1.25	0.9	1.67	1.1	0.92	0.6	1.90	1.3	2.96

Table 6: Instrument 2 (% CV by Sample (IFCC Units- mmol/mol))

Sample ID	HbA1c mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Patient 1	33	0.49	1.5	0.20	0.6	0.43	1.3	0.84	2.6	1.08	3.3
Patient 2	49	0.36	0.7	0.42	0.9	0.58	1.2	0.24	0.5	0.83	1.7
Patient 3	66	0.40	0.6	0.38	0.6	0.72	1.1	0.19	0.3	0.93	1.4
Patient 4	113	0.63	0.6	0.88	0.8	1.47	1.3	2.27	2.0	2.91	2.6
Control 1	37	0.46	1.2	0.19	0.5	0.61	1.6	0.70	1.9	1.05	2.8
Control 2	87	0.45	0.5	0.66	0.8	0.89	1.0	0.96	1.1	1.54	1.8
QC 1	37	0.38	1.0	0.21	0.6	0.61	1.7	0.91	2.5	1.18	3.2
QC 2	84	0.72	0.9	0.67	0.8	1.00	1.2	0.43	0.5	1.46	1.7
QC 3	142	0.73	0.5	0.83	0.6	1.52	1.1	2.58	1.8	3.19	2.2

Table 7: Instrument 3 (% CV by Sample (IFCC Units- mmol/mol))

Sample ID	HbA1c mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Patient 1	33	0.70	2.1	0.00	0.0	0.66	2.0	0.51	1.6	1.09	3.3
Patient 2	50	0.51	1.0	0.22	0.4	0.85	1.7	0.00	0.0	1.02	2.0
Patient 3	68	0.56	0.8	0.33	0.5	1.20	1.8	0.46	0.7	1.44	2.1
Patient 4	115	0.80	0.7	0.80	0.7	1.81	1.6	2.26	2.0	3.11	2.7
Control	37	0.57	1.5	0.00	0.0	0.73	2.0	0.46	1.2	1.03	2.8
Control	89	0.87	1.0	0.60	0.7	1.51	1.7	0.93	1.0	2.07	2.3
QC 1	36	0.45	1.2	0.18	0.5	0.73	2.0	0.77	2.1	1.16	3.2
QC 2	85	1.00	1.2	0.00	0.0	1.35	1.6	1.44	1.7	2.21	2.6
QC 3	145	1.05	0.7	1.24	0.9	1.93	1.3	0.74	0.5	2.63	1.8

Table 8: Instruments Combined (% CV by Sample (IFCC Units- mmol/mol))

Sample ID	HbA1 mmol/mol	Repeatability		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Patient 1	33	0.66	2.0	0.00	0.0	0.51	1.5	0.00	0.0	0.77	2.3	1.13	3.4
Patient 2	50	0.57	1.1	0.13	0.3	0.76	1.5	0.58	1.2	0.34	0.7	1.17	2.3
Patient 3	67	0.50	0.7	0.31	0.5	0.96	1.4	0.84	1.3	0.30	0.4	1.44	2.1
Patient 4	115	0.83	0.7	0.78	0.7	1.72	1.5	0.00	0.0	2.29	2.0	3.08	2.7
Control 1	37	0.60	1.6	0.00	0.0	0.63	1.7	0.00	0.0	0.57	1.5	1.04	2.8
Control 2	89	0.84	0.9	0.48	0.5	1.26	1.4	1.49	1.7	1.09	1.2	2.44	2.7
QC 1	37	0.50	1.4	0.04	0.1	0.63	1.7	0.00	0.0	0.88	2.4	1.20	3.2
QC 2	85	1.10	1.3	0.00	0.0	1.12	1.3	0.59	0.7	0.93	1.1	1.92	2.3
QC 3	145	1.04	0.7	1.29	0.9	1.52	1.0	2.22	1.5	1.90	1.3	3.69	2.5

b. Linearity/assay reportable range:

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 3.9% HbA1c (19 mmol/mol) and high 18.8% HbA1c (182 mmol/mol) K3 EDTA whole blood patient samples. These samples were mixed together in varying ratios to obtain 9 intermediate sample levels. The measured values were compared to the expected values. The regression parameters (slope, intercept, and r2) follow:

NGSP:

Slope	Intercept	r2	Concentration Range Tested
1.0091	-0.1465	0.9995	3.9 – 18.8 % HbA1c

IFCC:

Slope	Intercept	r2	Concentration Range Tested
1.0091	-1.3867	0.9995	19 – 182 mmol/mol HbA1c

The linearity study supports the device's claimed assay measuring range of 3.9 to 18.8% HbA1c (NGSP) and 19 to 182 mmol/mol HbA1c (IFCC).

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

Traceability:

The D-10 Hemoglobin A1c test standardization is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators.

The D-10 Hemoglobin A1c assay is NGSP certified. The NGSP certification expires in one year. See NGDP 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 available to the customer using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

Controls and calibrators:

D-10 Hemoglobin A1c Calibrators are recommended for use with this device. Value assignment and stability protocols and acceptance criteria were previously reviewed and cleared in k031043.

Bio-Rad recommends commercially available control materials; Lyphochek Diabetes Control was previously cleared in k070546 and Liquichek Diabetes Control was previously cleared in submission k052838.

Sample Stability:

A sample stability study was conducted to show the stability of frozen samples collected in K2 and K3EDTA. Samples with concentration values spanning 3.9% to 17.0% HbA1c were collected in K2-EDTA tubes, samples with concentration values spanning 3.9% to 17.1% HbA1c were collected in K3-EDTA tubes. The aliquots of the whole blood samples were placed at -70°C for twelve months. Study protocols were reviewed and found to be acceptable. The data supports the use of K2-EDTA tubes and K3-EDTA tubes with frozen whole blood aliquoted and stored at -70°C for up to for 12 months when using the D-10™ Hemoglobin A1c Program.

d. *Detection limit:*

The claimed measuring range of 3.9 to 18.8% HbA1c for D-10 Hemoglobin A1c Program is based on linearity, see 1b.

e. Analytical specificity:

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%.

Ten replicates of each pool prepared with the test and control samples were analyzed using the D-10 Hemoglobin A1c on the D-10 Hemoglobin Testing System.

Significant interference was defined as a greater than ±7% change in %HbA1c value of the mean of the test samples relative to the mean of the reference samples.

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

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

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-10 Hemoglobin A1c on the D-10 Hemoglobin Testing System.

Significant interference was defined as a greater than ±7% change in %HbA1c value of the mean of the test samples relative to the mean of the reference samples. No significant interference was observed at therapeutic levels up to the stated concentrations as shown below:

Potential Drug Interferent	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

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-10 Hemoglobin Testing System with the D-10 Hemoglobin A1c.

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 49 mg/dL acetylsalicylic acid does not interfere with this assay.
- Carbamylated Hb up to 10 mg/dL potassium cyanate does not interfere with this assay.
- Labile A1c up to 1000 mg/dL glucose does not interfere with this assay.

Hemoglobin Variant Study:

A Hemoglobin Variant study was performed using a total of 87 normal and diabetic whole blood EDTA patent variant samples known to contain hemoglobin variants S, C, E, D, A2 and F. Testing of the samples containing hemoglobin variants S, C, E, and D, A2 and F were performed using the D-10 Hemoglobin A1c on the D-10 Hemoglobin Testing System and compared to results obtained by a NGSP reference method that has been demonstrated to be free from the hemoglobin interferent (Trinity Biotech Ultra2 A1c, Trinity Biotech Premier Hb9210 and Tosoh G8 analyzers). The test result conclusions are as follows:

Variant samples used in Hemoglobin Variant Study

Hemoglobin Variant	n	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbS	22	31 – 43	5.6 – 11.5
HbC	20	31 – 40	5.0 – 10.7
HbD	22	35 – 43	5.8 – 10.0
HbE	23	21 – 32	5.9 – 11.6
HbA2	20	5.0 – 6.2	5.0 - 14.5
HbF	24	3.3 – 32.6	4.7 - 14.4

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	1.1 (-4.7 to 4.9)	0.2 (-6.1 to5.3)
HbC	2.6 (1.4 to 5.9)	-0.9 (-2.8 to 0.9)
HbD	-0.6 (-3.1 to 4.7)	1.7 (-3.6 to 5.1)
HbE	0.5 (-3.3 to 6.2)	2.8 (1.2 to 5.2)
HbA2	0.6 (-1.8 to 1.8)	-1.7 (-2.8 to -0.7)
HbF	3.1 (-1.5 to 8.8)	-0.6 (-2.5 to 4.8)

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 (≤43.0%), HbC (≤40.0%), HbD (≤43.0%), HbE (≤32.0%) and HbA2 (≤6.0%) and HbF (≤ 10%) at the concentrations tested in this study. The labeling states;

> This device has significant positive interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF, including those with known Hereditary Persistence of Fetal Hemoglobin.
>
> Hemoglobin F concentrations up to 10% do not interfere with the test. Any sample with HbF >10% may result in higher than expected HbA 1c values. Any sample with HbF >5% should be suspected of having a hemoglobinopathy.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A Method comparison study was performed per CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 128 variant-free whole blood K3 EDTA samples covering the measuring range were evaluated using the D-10 Hemoglobin A1c Program on the D-10 Hemoglobin 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.

Distribution of samples

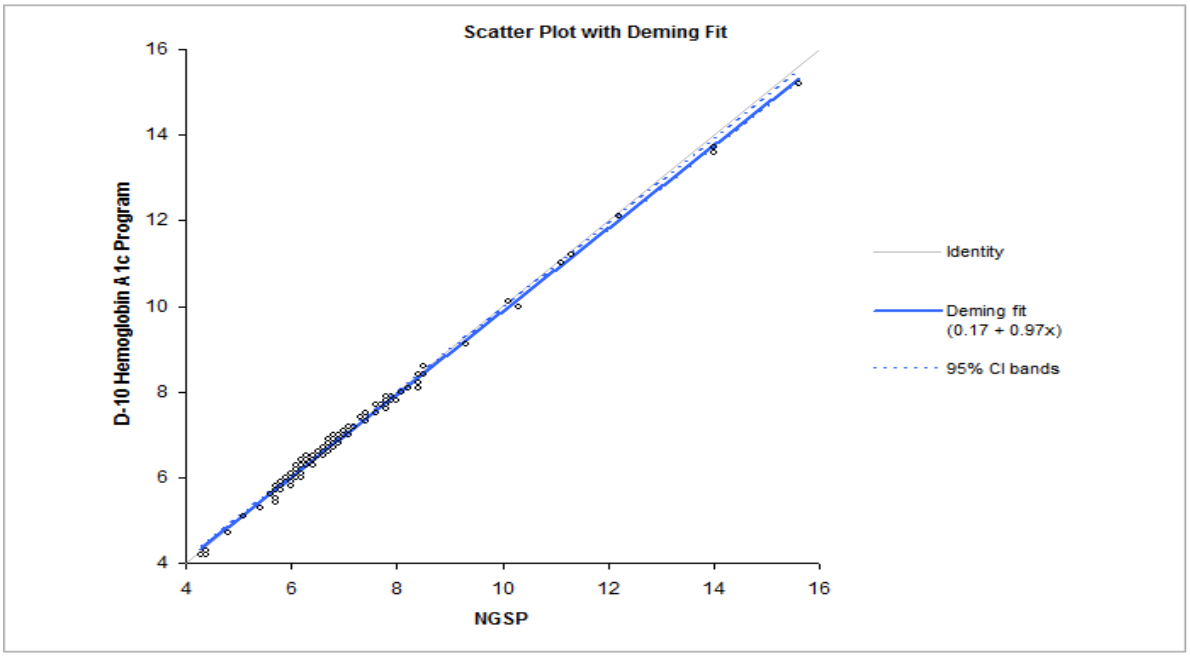
Hemoglobin A1c level	n	% of Samples tested
≤ 5%	4	3.1
5 – 6%	19	14.8
6 – 6.5%	33	25.8
6.5 – 7%	33	25.8
7 – 8%	20	15.6
8 – 9%	10	7.8
> 9%	9	7.0
Total samples	128	100.0

Linear, Deming (weighted), and Passing-Bablok regression analyses were performed for the D-10 Hemoglobin A1c Program on the D-10 Hemoglobin Testing System versus the comparative G8 HPLC method (NGSP) and are summarized below:

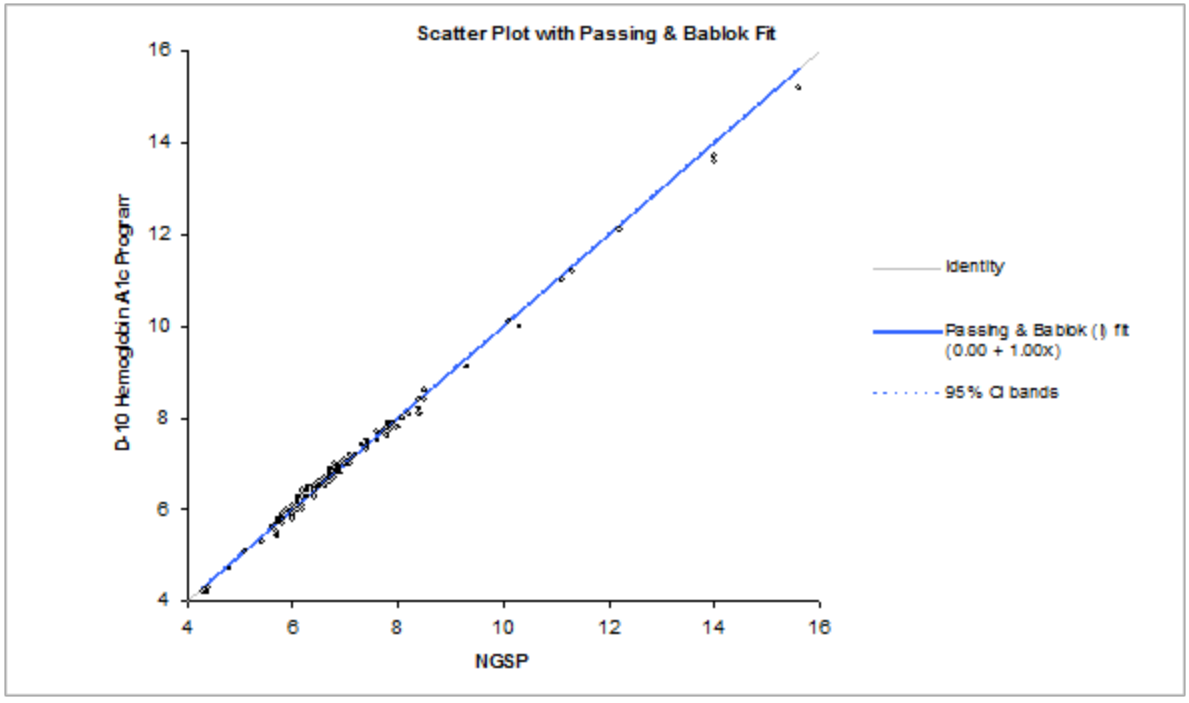
Summary of Method Comparison Results

	Slope	95% CI	y-Intercept	95% CI	R^2
Linear	0.9701	0.9587 – 0.9816	0.1801	0.0980 – 0.2622	0.9955
Deming	0.9722	0.9579 – 0.9866	0.1654	0.0637 – 0.2670	1.0000
Passing-Bablok	1.0000	1.0000 – 1.0000	0.0000	0.0000 – 0.0000	1.0000

Scatter Plot using Deming Fit, %HbA1c, NGSP vs. D-10 HbA1c



Scatter Plot using Passing-Bablok Fit, %HbA1c, NGSP vs. D-10 HbA1c



The following biases between D-10 Hemoglobin A1c run on the D-10HbA1c Testing System versus the NGSP Reference Method (Tosoh G8 HPLC analyzer) were observed:

Bias Estimation

% HbA1c – Decision Level	Bias	% Bias
5.0±0.5	-0.05	-0.96
6.5±0.5	0.00	0.03
8.0±0.5	-0.08	-0.98
12.0±1.0	-0.10	-0.87

Total Error Calculations:
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 as follows:

Total Error Estimation

% A1c – Decision Level	% Bias	% CV	% TE
5.0	0.96	2.0	4.9
6.5	0.03	1.6	3.2
8.0	0.98	1.6	4.1
12.0	0.87	2.2	5.2

b. Matrix comparison:

The matrix comparison study was performed, based on CLSI guidance documents EP14-A2, Vol 25, No 4 "Evaluation of Matrix Effect: Approved Guideline – Second Edition, EP09-A3, Vol 33, No. 11 "Measurement Procedure Comparison and Bias Estimation Using Patient samples; Approved Guideline – Third Edition. Whole blood samples from 44 patients were collected in K3-EDTA sample tubes and K2-EDTA sample tubes. Once the blood samples were collected, they were kept at 2-8°C and shipped to Bio-Rad Laboratories, CSD, within 1 day of draw. Analyses were performed once the samples were received. To cover both extreme ends of the measuring range one high sample was prepared by adding purified A1c and one low sample by adding purified Ao to non-variant whole blood samples. The two (2) spiked samples were less than 15% of the total forty four (44) samples analyzed. Linear regression analysis was performed using K3-EDTA results as the reference and the results are summarized below:

Sample Type	N	Sample Ranges (% A1c)	Slope (95% Cl)	Intercept (95% Cl)	R2
K2-EDTA	44	4.1-17.5	1.0077 (0.9980-1.0174)	-0.0383 (-0.1154-0.0388)	0.9990

The data support the use of K3-EDTA blood collection tubes with fresh whole blood with the D-10 Hemoglobin A1c System.

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		Suggested Diagnosis
NGSP %	**IFCC mmol/mol**	
>6.5	>48	Diabetic
5.7 – 6.4	39-47	Pre-Diabetic
<5.7	<39	Non-Diabetic

   The expected HbA1c range for non-diabetic adults is 4-6%.

   The following references were cited:

- American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2014, 37 (Suppl. 1), S81–S90

- World Health Organization. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. http://www.who.int/diabetes/publications/diagnosisdiabetes2011/en /

- Clinical Diabetes. Your A1CResults: What Do They Mean? http://clinical.diabetesjournals.org/content/24/1/9.full.pdf+html

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.