

**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:**

<b>Regulatory Description</b>	<b>Classification</b>	<b>Regulation</b>	<b>Product Code</b>	<b>Panel</b>
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:

<u>Quantity</u>	<u>Description</u>
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:

<b>Similarities</b>		
<b>Item</b>	<b>Candidate Device: VARIANT II TURBO HbA1c Kit – 2.0</b>	<b>Predicate Device: COBAS INTEGRA 800 HbA1c DX Gen. 2</b>
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

<b>Differences</b>		
<b>Item</b>	<b>Candidate Device: VARIANT II TURBO HbA1c Kit-2.0</b>	<b>Predicate Device: COBAS INTEGRA 800 HbA1c DX Gen.2</b>
Sample Types	K <sub>2</sub> -EDTA, K <sub>3</sub> -EDTA, Capillary blood in Hemoglobin Capillary Collection System (HCCS)	K <sub>2</sub> -EDTA, K <sub>3</sub> -EDTA, KF/Na <sub>2</sub> - EDTA, Na-heparin NF/K-oxalate NF/NA <sub>2</sub> - 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
<b>Concentration HbA1c (NGSP%)</b>	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
<b>Concentration HbA1c (NGSP%)</b>	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
<b>Concentration HbA1c (NGSP%)</b>	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
<b>Concentration HbA1c (NGSP%)</b>	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
<b>Concentration HbA1c (IFCC%)</b>	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
<b>Concentration HbA1c (IFCC%)</b>	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
<b>Concentration HbA1c (IFCC%)</b>	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
<b>Concentration HbA1c (IFCC%)</b>	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<sub>2</sub> and K<sub>3</sub>EDTA. Samples with concentration values spanning 3.50% to 20.60% HbA1c were collected in K<sub>2</sub>-EDTA tubes, K<sub>3</sub>-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<sub>2</sub>-EDTA tubes and K<sub>3</sub>-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**

<b>Endogenous Substance</b>	<b>Concentration</b>
	<b>Conventional Units</b>
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  $\pm 7\%$  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
	<b>Conventional (US) units</b>
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  $\pm 7\%$  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**

<b>Hemoglobin Variant</b>	<b>n</b>	<b>Range in % Abnormal Variant</b>	<b>Range in %HbA1c Concentration</b>
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**

<b>Hemoglobin Variant</b>	<b>Relative % Bias from Reference Method at Low and High Concentrations of HbA1c</b>	
	<b>Relative % Bias (StDev) for HbA1c ~6.5%</b>	<b>Relative % Bias (StDev) for HbA1c ~8.0%</b>
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  $\pm 7\%$  from the control. No significant interference was observed for HbC ( $\leq 42.4\%$ ), HbD ( $\leq 41.4\%$ ), HbE ( $\leq 31.4\%$ ), HbS ( $\leq 41.6\%$ ), HbA2 ( $\leq 10.2\%$ ) and HbF ( $\leq 29.4\%$ ) 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.

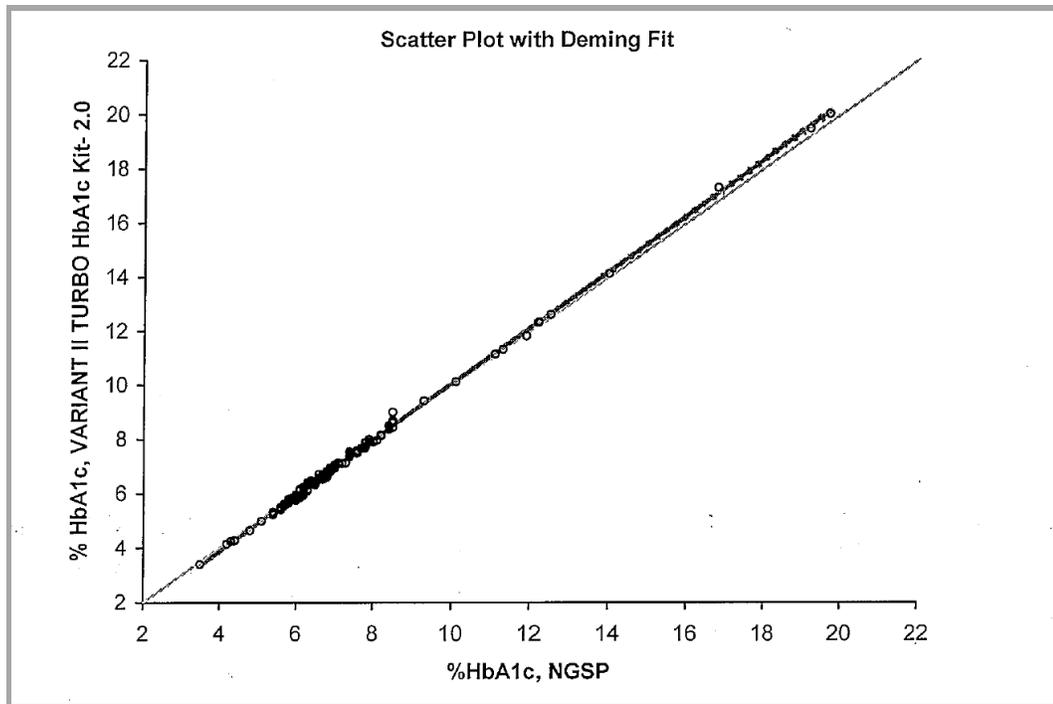
**Distribution of samples**

<b>Hemoglobin A1c level</b>	<b>n</b>	<b>% Samples tested</b>
≤ 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

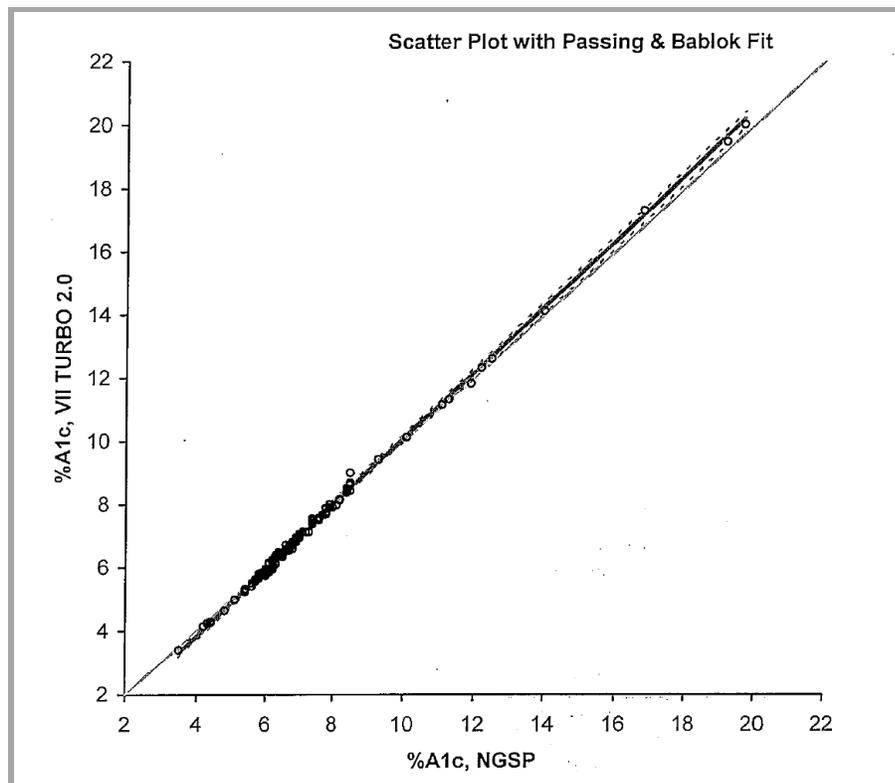
Deming (weighted) and Passing-Bablok regression analyses were performed for the VARIANT™ II TURBO HbA1c Kit – 2.0 versus the NGSP SRL reference method.

**Summary of Method Comparison Results**

	<b>y-Intercept</b>	<b>95% CI</b>	<b>Slope</b>	<b>95% CI</b>
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**

<b>% HbA1c – Decision Level</b>	<b>Bias</b>	<b>% Bias</b>
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**

<b>%A1c</b>	<b>% Bias</b>	<b>% CV</b>	<b>% TE</b>
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<sub>2</sub>-EDTA and K<sub>3</sub>-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<sub>2</sub>-EDTA tubes, K<sub>3</sub>-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<sub>2</sub>-EDTA tubes with fresh whole blood
- K<sub>3</sub>-EDTA tubes with fresh whole blood
- Hemoglobin Capillary Collection System (HCCS) with fresh capillary blood

The regression results are as follows:

<b>Matrix Comparison Results</b>				
<b>Collection Device and Sample Type</b>	<b>Linear Fit</b>			
	<b>Sample Range</b>	<b>Slope (95% CI)</b>	<b>Intercept (95% CI)</b>	<b>r<sup>2</sup> value</b>
K <sub>3</sub> -EDTA versus K <sub>2</sub> -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 <sub>3</sub> -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:

<b>Hemoglobin A1c Expected Values</b>		
<b>Hemoglobin A1c</b>		
<b>NGSP%</b>	<b>IFCC mmol/mol</b>	
$\geq 6.5$	$> 48$	Diabetic <sup>1-3</sup>
5.7 — 6.4	39 — 47	Pre-Diabetic <sup>1</sup>
$< 5.7$	$< 39$	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/](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.