

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k070452

B. Purpose for Submission:

Reagent formulation change

C. Measurand:

Hemoglobin A1c

D. Type of Test:

Quantitative High Performance Liquid Chromotography

E. Applicant:

Bio-Rad Laboratories, Inc

F. Proprietary and Established Names:

VARIANT II Hemoglobin A1c Program

G. Regulatory Information:

1. Regulation section:

21CFR Section 864.7470 – Glycosylated Hemoglobin Assay

21CFR Section 864.8165 Calibrator for hemoglobin or hematocrit measurement.

2. Classification:

Class II

3. Product code:

LCP, KRZ

4. Panel:

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The Bio-Rad VARIANT II Hemoglobin A1c Program is intended for the percent determination of hemoglobin A1c in human whole blood using ion-exchange high-performance liquid chromatography (HPLC).

The Bio-Rad VARIANT II Hemoglobin A1c Program is intended for Professional Use Only.

Measurement of percent hemoglobin A1c is effective in monitoring long-term glucose control in individuals with diabetes mellitus.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

VARIANT II Hemoglobin Testing System

I. Device Description:

The VARIANT II Hemoglobin Testing System provides an integrated method for sample preparation, separation, and the percent determination of hemoglobin in EDTA human whole blood. The VARIANT II Hemoglobin Testing System is a fully automated, high-throughput hemoglobin analyzer. It consists of two modules - the VARIANT II Chromatographic Station (VCS) and the VARIANT II Sampling Station (VSS). In addition, a personal computer is used to control the VARIANT II System using Clinical Data Management (CDM) software.

The new VARIANT II Hemoglobin A1c Program contains an analytical cartridge, 2 prefilters, Buffers A and B, Wash/Diluent Solution, Calibrator Diluent Set, and program parameters to run 1000 tests.

J. Substantial Equivalence Information:

1. Predicate device name(s):

VARIANT II Hemoglobin A1c Program

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

k984268

3. Comparison with predicate:

| Similarities | | |
|---------------------|--|--|
| Item | Device | Predicate |
| Intended Use | The Bio-Rad VARIANT II Hemoglobin A1c Program is intended for the percent determination of hemoglobin A1c in human whole blood using ion-exchange high performance liquid chromatography (HPLC). | The Bio-Rad VARIANT II Hemoglobin A1c Program is intended for the percent determination of hemoglobin A1c in human whole blood using ion-exchange high performance liquid chromatography (HPLC). |
| Sample Type | Human anticoagulated whole blood (EDTA) | Human anticoagulated whole blood (EDTA) |
| Standardization | Traceable to the Diabetes Control and Complicaiton Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP). | Traceable to the Diabetes Control and Complicaiton Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP). |
| Assay Priniciple | Cation exchange HPLC | Cation exchange HPLC |

| Differences | | |
|----------------------------------|--|--|
| Item | Device | Predicate |
| Cartridges Included in Kit | 1 cartridge (1000) tests with reduced particle size | 2 cartridges (500 tests each) |
| Calibration frequency | After installation of a new cartridge and every 30 days. | After installation of a new cartridge. |
| hemoglobin variants E and D | Can not report HbA1c in the presence hemoglobin of variants E and D. | Can not report HbA1c in the presence hemoglobin of variants E and D. |
| Calibrator Reconstitution Volume | 7 mL | 5 mL |

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

Medical devices - Application of risk management to medical devices (ISO 14971:2000)

Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied (ISO 15223)

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (CLSI EP5-A2)

Draft Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs (FDA guidance)

Guidance for Off-the-Shelf Software Use in Medical Devices; Final (FDA Guidance for Industry, FDA Reviewers and Compliance)

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (FDA Guidance for Industry and FDA Staff)

Format for Traditional and Abbreviated 510(k)s – (FDA Guidance for Industry and FDA Staff)

L. Test Principle:

The VARIANT II Hemoglobin A1c Program 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 allows molecules to be separated based upon their charge. Separation is optimized to eliminate interferences from hemoglobin variants (HbS, HbC, HbD and HbE), labile A1c, hemoglobin F and carbamylated hemoglobin.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Normal patient, normal control, diabetic patient and diabetic controls were run in the precision study. The precision study was performed on six instruments in three laboratories over 10 days. Each site was provided with the same sample set and directed to perform two replicates of each sample on each of 2 runs/day (morning and evening) for 10 days. Each site conducted the study on two instruments. The position of the precision specimens in each run was

randomized. The results were analyzed by nested Analysis of Variance (ANOVA) with the hierarchical levels of labs, instruments, days, and runs. The estimates of imprecision obtained from the analysis are given in the table below. Patient samples are EDTA human whole blood.

| | Normal Patient (HbA1c) | Diabetic Patient (HbA1c) |
|------------------------|------------------------|--------------------------|
| n= (number of samples) | 240 | 160* |
| Mean (%HbA1c) | 5.5 | 8.8* |
| Within run (%CV) | 0.90 | 0.59 |
| Between day (%CV) | 1.15 | 1.15 |
| Between run (%CV) | 0.64 | 0.46 |
| Within Device (%CV) | 1.60 | 1.38 |
| Total Precision (%CV) | 2.53 | 2.84 |

* One site used a different diabetic sample and data was not included.

| | Normal Control (HbA1c) | Diabetic Control (HbA1c) |
|------------------------|------------------------|--------------------------|
| n= (number of samples) | 240 | 240 |
| Mean (%HbA1c) | 5.6 | 9.5 |
| Within run (%CV) | 1.12 | 0.63 |
| Between day (%CV) | 1.11 | 1.17 |
| Between run (%CV) | 0.43 | 0.56 |
| Within Device (%CV) | 1.64 | 1.44 |
| Total Precision (%CV) | 2.40 | 2.52 |

b. Linearity/assay reportable range:

Linearity across the reportable range was performed using low (3.1%) and high (18.6%) EDTA whole blood patient samples. These were mixed together in varying ratios (i.e. 3:1, 1:1 and 1:3) and the measured values were compared to the theoretical values based upon the dilution factor. The measured values were all within 0.05% of the predicted values. A linear regression was performed and resulted in a slope of 0.998 and a y-intercept of 0.006.

| Sample Pool | Measured %A1c | Predicted %A1c |
|-------------|---------------|----------------|
| 1 | 3.05 | 3.05 |
| 2 | 6.89 | 6.88 |
| 3 | 10.78 | 10.76 |
| 4 | 14.65 | 14.63 |
| 5 | 18.55 | 18.52 |

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

Traceability

Hemoglobin A1c IFCC Reference standards (Kyoto 2002) were obtained from the IFCC Working Group on Standardization on Hemoglobin A1c. These standards and the Manufacturer Reference Calibrators were run on multiple VARIANT II Hemoglobin Testing Systems to assign values to the Manufacturer Reference Calibration materials. The Manufacturer Reference Calibrators are used to transfer IFCC/NGSP traceable values to product calibrators for the VARIANT II Hemoglobin A1c Calibrator/Diluent Set. Both IFCC and NGSP calibrator value assignments are provided in the VARIANT II Hemoglobin A1c Program Calibrator/Diluent Set packaging.

Stability

Real time stability and accelerated stability study protocols and acceptance criteria were reviewed and found to be acceptable.

d. *Detection limit:*

The reportable range is 3.1 to 18.5% HbA1c. See the linearity study above for data on recovery of samples across the measuring range.

e. *Analytical specificity:*

Several interfering substance studies were performed which include Labile A1c, carbamylated A1c, bilirubin, lipemia, hemoglobin F, and hemoglobins S, C, D and E. Whole blood pooled samples with normal and diabetic levels of HbA1c were supplemented with bilirubin, Hemoglobin F, labile A1c. The samples were analyzed in duplicate on the VARIANT II Hemoglobin Testing System. The specification for no interference was less than or equal to 0.3% change in HbA1c value. No interference was seen from bilirubin up to 20 mg/dL, hemoglobin F concentrations up to 10%, and labile A1c at biological levels.

To test the level of interference of carbamylated hemoglobin patient EDTA whole blood specimen pools with normal and diabetic levels of A1c were split into two aliquots. The Red Blood Cells (RBCs) of both aliquots were washed with phosphate buffered saline (PBS). One aliquot of each level was incubated with a 0.05 M potassium cyanate solution until the carbamylated hemoglobin level increased by approximately 1, 2, 3, and 4%. Both aliquots from each level were analyzed in duplicate on the VARIANT II Hemoglobin A1c program on two instruments. The specification for interference was that the difference in %HbA1c must be less than or equal to 0.3% for a normal sample up to an increase of 3.0% CHb and less than or equal to 0.5% for a diabetic sample up to an increase of 3.0%. No interference was seen at these levels.

To test for Hemoglobin AD (AD) and Hemoglobin AE (AE) interference 20 AD samples and 23 AE samples were analyzed compared to values obtained

by NGSP SRL Boronate affinity method. Least squares linear regression was performed on the data points collected from this study, comparing VARIANT II HbA1c values with NGSP HbA1c values. The specification for no interference from hemoglobins D and E was that the predicted value of the VARIANT II Hemoglobin A1c result should be within 10% of the NGSP value. All specifications were met.

To test for interference from Hemoglobin AS (AS) and hemoglobin (AC) 20 AS samples and 15 AC samples were analyzed and compared to values obtained by an NGSP SRL Boronate affinity method. Least squares linear regression was performed on the data points collected from this study, comparing VARIANT II Hemoglobin A1c values with NGSP HbA1c values. The specifications for no interference from hemoglobins S and C were that the predicted value of the VARIANT II Hemoglobin A1c result should be within 10% of the NGSP value. All specifications were met.

To determine the effect of lipemia on results generated on the VARIANT II Hemoglobin A1c Program, two pooled EDTA whole blood patients samples representing normal and diabetic HbA1c levels were centrifuged and the plasma fraction was removed from each sample. Serum samples were obtained from a commercial laboratory with normal (86 mg/dL) and abnormal (6060 mg/dL) triglyceride concentrations and used to prepare serum pools. Aliquots of the normal and diabetic patient specimen red blood cells were then combined with an equal volume of the triglyceride serum pools and analyzed in duplicate. The specification for no interference was less than or equal to a 0.3% change in the HbA1c value. No interference was seen.

To study the effects of EDTA on the VARIANT II Hemoglobin A1c Program, samples for the determination of HbA1c were collected from normal and diabetic patients. One milliliter aliquots of patient samples were supplemented with 11 times the EDTA concentration by transferring of the samples to empty potassium EDTA tubes. The controls and supplemented samples were then analyzed in duplicate with the VARIANT II Hemoglobin A1c Program. The results showed a change of less than 0.1% was seen between the controls and samples with up to 11X EDTA.

A study was performed to verify that there is no carryover interference from a variant S, C, D or E sample. The normal quality control material was run in triplicate as patients with variants S, C, D and E samples placed throughout the run in triplicate locations. The specification for carryover interference from hemoglobin variants was that the difference in %HbA1c must be less than or equal to 0.3%. The maximum change in HbA1c was 0.07% in the samples after HbAC, and -0.07% change in the sample after the HbAS samples. The samples after HbAE and HbAD samples showed no change in HbA1c.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

The new VARIANT II Hemoglobin A1c Program was compared to the current VARIANT II Hemoglobin A1c Program. 32 EDTA whole blood patient samples and 18 spiked EDTA whole blood samples were run on the new VARIANT II Hemoglobin A1c Program and the current VARIANT II Hemoglobin A1c Program. The range of samples on the new VARIANT II Hemoglobin A1c Program was 3.5 to 18.55 %A1c. A linear regression was performed resulting in a slope of 0.9673, a y-intercept of 0.0115, and a correlation coefficient of 0.9958.

b. *Matrix comparison:*

Not applicable

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 Value Range was determined from literature (American Diabetes Association. Standards of Medical Care for Patients with Diabetes Mellitus. Diabetes Care 2001, 24 (Suppl. 1), 33-43). Hemoglobin A1c > 8% Action is suggested. Hemoglobin A1c < 7% is the goal. Hemoglobin A1c < 6% is non-diabetic level.

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.