

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

**A. 510(k) Number:**

k040872

**B. Purpose of the Submission:**

Improvements over the predicate chromatographic device known as the VARIANT II Hemoglobin A<sub>1c</sub> Program [k984268]

**C. Analyte:**

Glycosylated hemoglobin (Hemoglobin A<sub>1c</sub> or HbA<sub>1c</sub>)

**D. Type of Test:**

Quantitative

**E. Applicant:**

Bio-Rad Laboratories, Inc.

**F. Proprietary and Established Names:**

VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program

**G. Regulatory Information:**

1. Regulatory Section: 21 CFR 864.7470 [Assay, glycosylated hemoglobin]
2. Classification II
3. Product Code: LCP
4. Panel 81 - Hematology

**H. Intended Use:**

1. Intended Use(s): The Bio-Rad VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is intended for the percent determination of hemoglobin A<sub>1c</sub> in human whole blood using ion-exchange high performance liquid chromatography (HPLC). For In Vitro Diagnostic Use.
2. Indication(s) for Use: The Bio-Rad VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is intended for the percent determination of hemoglobin A<sub>1c</sub> in human whole blood using ion-exchange high performance liquid chromatography (HPLC). The Bio-Rad VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is intended for Professional Use Only. For In Vitro Diagnostic Use. Measurement of percent hemoglobin A<sub>1c</sub> is effective in monitoring long-term glucose control in individuals with diabetes mellitus.
3. Special condition for use statement(s): NA
4. Special Instrument Requirements:  
VARIANT II TURBO Hemoglobin Testing System

## I. Device Description :

This VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is a testing system that uses the principles of HPLC for the chromatographic separation of Hemoglobin A<sub>1c</sub> present in human blood. The kit contains supplies for 2000 tests and consists of an analytical cartridge, guard cartridge, elution buffers, wash/diluent Solution, calibrators, calibrator diluent and whole blood primer.

## J. Substantial Equivalence Information:

1. Predicate Device Name:  
VARIANT II Hemoglobin A<sub>1c</sub> Program
2. Predicate K number(s)  
k984268
3. Comparison with predicate:

### Similarities

| Characteristic Item or Parameter                        | VARIANT II TURBO Hemoglobin A <sub>1c</sub>  | VARIANT II Hemoglobin A <sub>1c</sub>  |
|---|--|--|
| Analyte Measured: Reported                              | % Hemoglobin A <sub>1c</sub>   | % Hemoglobin A <sub>1c</sub>   |
| Intended Use  | The Bio-Rad VARIANT II TURBO Hemoglobin A <sub>1c</sub> Program is intended for percent determination of HbA <sub>1c</sub> in whole blood using ion-exchange high performance liquid chromatography (HPLC). The Bio-Rad VARIANT II TURBO HbA <sub>1c</sub> Program is intended for Professional Use only. For In Vitro Diagnostic Use. | The Bio-Rad VARIANT II Hemoglobin A <sub>1c</sub> (HbA <sub>1c</sub> ) Program is intended for percent determination of HbA <sub>1c</sub> in human whole blood using ion-exchange high performance liquid chromatography (HPLC). |
| Assay Principle with Visible Light Absorbance Detection | Cation exchange high performance liquid chromatography (HPLC) with detection by Absorbance at 415 nm wavelength  | Cation exchange high performance liquid chromatography (HPLC) with detection by Absorbance at 415 nm wavelength  |
| Sample Type   | Human anticoagulated whole blood (EDTA)  | Human anticoagulated whole blood (EDTA)  |
| Sample Volume   | 23 µL  | 23 µL  |
| Use of Controls   | Bilevel Control for A <sub>1c</sub> per run.   | Bilevel Control for A <sub>1c</sub> per run.   |
| Standardization   | Traceable to the Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP).   | Traceable to Diabetes Control and Complications Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP).   |
| Measurement Type  | Quantitative area percent  | Quantitative area percent  |
| Design  | Modular system that is designed for a medium size laboratory.  | Modular system that is designed for a medium size laboratory.  |
| Instrumentation   | VARIANT II TURBO is one system with 3 discrete units; VARIANT Sampling Station (VSS), VARIANT Chromatographic Station (VCS) and computer.  | VARIANT II is one system with 3 discrete units; VARIANT Sampling Station (VSS), VARIANT Chromatographic Station (VCS) and computer.  |

## Differences

| Characteristic Item/ Parameter                    | VARIANT II TURBO Hemoglobin A <sub>1c</sub>  | VARIANT II Hemoglobin A <sub>1c</sub>  |
|---|--|--|
| Analytes Reported                                 | Hemoglobin A1a, A1b, F, A <sub>0</sub> , S, C, CHb LA <sub>1c</sub> , P3 and VARIANT window.   | Hemoglobin A1a, A1b, F, A <sub>0</sub> , C, LA <sub>1c</sub> , P3 and E, D, S window.  |
| Calibration                                       | 2 point calibration, once every new analytical or guard cartridge  | 2 point calibration, once every new analytical cartridge.  |
| Reagents  | Analytical cartridge, Guard cartridge, Elution buffers, Wash/Diluent Solution, calibrators, calibrator diluent and whole blood primer.   | Analytical cartridge, Elution buffers, Wash/Diluent Solution, calibrators, calibrator diluent, whole blood primer and pre-filter.  |
| Temperature                                       | 36°C   | 28°C   |
| Standards met & Electrical Safety issues met      | Specific NGSP, IFCC Standards and FDA [09/30/1991] Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs; and FDA Guidance for the Software Contained in Medical Devices[05/29/1998]; <u>New</u> : Off-the-Shelf Software Use in Medical Devices [09/09/1999]; Electrical: EN61010-1: 2001, EN61010-2-010, EN61010-2-101, EN61326/A2:2001, EN61326:1997. | Specific NGSP, IFCC Standards & FDA [09/30/1991] Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs; and FDA Guidance for the Software Contained in Medical Devices [05/29/1998]; Electrical: EN61010-1: 2001, EN61010-2-010, EN61010-2-101, EN61326/A2:2001, EN61326:1997. |
| Analysis Medium                                   | Bis-Tris/Phosphate Buffers A & B and modified polymeric cation exchange based resin packed analytical and guard cartridges.  | Bis-Tris/Phosphate Buffer A & B, polymeric cation exchange based resin packed cartridge.   |
| New design feature                                | New column holder for the VCS that accepts analytical and guard cartridges to give 2000 injections per analytical cartridge.   | Cartridge holder has both a prefilter assembly and holder. The cartridge gives 1000 injections per analytical cartridge.   |
| New design feature                                | Redesigned column heater to hold new cartridge holder and to give better temperature calibration.  | Column heater holds Brownlee design cartridge holder and prefilter.  |
| New design feature                                | Coil mixer has replaced the T-mixer to improve mixing and reduce gradient delay.   | T- mixer and dynamic mixer.  |
| New design feature                                | 2-groove sample probe allows simultaneous venting and sampling of patient tubes for faster speed.  | Stainless Steel Sample Probe.  |
| New design feature                                | No mixing of patient sample on the TURBO required.   | Patient sample mixing is performed by the instrument.  |
| Time to process sample                            | 97 seconds, providing for a doubling of throughput of blood samples  | 3 minutes  |
| Media to Update HbA <sub>1c</sub> kit information | CD ROM operating at 24x-48x and using software compatible for the VARIANT II TURBO Hemoglobin Testing System.  | CD ROM operating at 24x-48x and using software compatible for the VARIANT II Hemoglobin Testing System. [K984268]  |

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

Standards and Guidances used to evaluate the VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program device included: a) NGSP (National Glycohemoglobin Standardization Program), IFCC HbA<sub>1c</sub> Calibrators; b) FDA [09/30/1991] Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs; c) FDA Guidance for Software Contained in Medical Devices [05/29/1998]; d) *New*: Off-the-Shelf Software Use in Medical Devices [09/09/1999].

**L. Test Principle**

This VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the Sampling Station and injected into the analytical cartridge. The Chromatographic Station dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The absorbance at 415 nm is measured. An additional filter at 690 nm corrects the background absorbance. The Clinical Data Management software performs reduction of raw data collected from each analysis. Two-level calibration is used for adjustment of the calculated values

**M. Performance Characteristics (if/when applicable):****1. Analytical performance:**

*a. Precision/Reproducibility* - The following table provides comparison data on the precision between VARIANT II TURBO Hemoglobin A<sub>1c</sub> and VARIANT II Hemoglobin A<sub>1c</sub> Programs, each utilizing EDTA whole blood patient samples, and both tested against samples with moderate (5.4-6.2%) and high (12.5-13.7%) Hemoglobin A<sub>1c</sub> content. The method of precision analysis was performed using a protocol based on the NCCLS Evaluation protocol, Vol.12, No. 4, EP5-A (Feb. 1999) applied to the VARIANT II TURBO Hemoglobin A<sub>1c</sub>, and NCCLS Evaluation protocol, Vol.12, No. 4, EP5-T2 (Mar.1992) applied to the VARIANT II Hemoglobin A<sub>1c</sub> Program. The protocols for both the VARIANT II TURBO Hemoglobin A<sub>1c</sub> and VARIANT II Hemoglobin A<sub>1c</sub> Programs are similar. Using these protocols, 40 runs (2 per day) were performed on one VARIANT II TURBO (or VARIANT II) Hemoglobin Testing System over 20 working days. In each duplicate daily run, one aliquot of low HbA<sub>1c</sub> and one aliquot of high HbA<sub>1c</sub> patient samples were each analyzed per run. Although the precision samples are different, since they were run at different time periods, the precision results between the VARIANT II TURBO Hemoglobin A<sub>1c</sub> and the VARIANT II Hemoglobin A<sub>1c</sub> Program are substantially equivalent. A summary of these combined comparative precision results is presented in the precision table below.

### VARIANT II TURBO Hemoglobin A<sub>1c</sub> and VARIANT II Hemoglobin A<sub>1c</sub> Precision

|                        | VARIANT II TURBO Hemoglobin A <sub>1c</sub> |                                   | VARIANT II Hemoglobin A <sub>1c</sub> |                                   |
|------------------------|---|-----------------------------------|---------------------------------------|-----------------------------------|
|                        | Low Patient (HbA <sub>1c</sub> )            | High Patient (HbA <sub>1c</sub> ) | Low Patient (HbA <sub>1c</sub> )      | High Patient (HbA <sub>1c</sub> ) |
| n= (number of samples) | 80  | 80                                | 80                                    | 80                                |
| Mean                   | 6.2   | 12.5                              | 5.4                                   | 13.7                              |
| Within run             | 0.82% CV                                    | 0.54% CV                          | 1.46 % CV                             | 0.65 % CV                         |
| Total Precision        | 1.94% CV                                    | 2.58 % CV                         | 2.14 % CV                             | 1.68 % CV                         |

#### b. Linearity/assay reportable range:

The following table provides comparison data on the linearity and recovery analyses between VARIANT II TURBO Hemoglobin A<sub>1c</sub> and VARIANT II Hemoglobin A<sub>1c</sub> Programs, each utilizing eight EDTA-based blood standards (n=2 for each standard). The % Recovery for Hemoglobin A<sub>1c</sub> by the VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is essentially the same as the VARIANT II Hemoglobin A<sub>1c</sub> Program. The standard values chosen for the linearity studies represent % Hb A<sub>1c</sub> recovery values for normal [non-diabetic] patients, who might range from low [i.e., between 3.5–3.8 %] to moderate [i.e., between 4.7 -5.0 %] values of HbA<sub>1c</sub>, as well as recovery values for abnormal [diabetic] patients, who might range from slightly high [i.e., above the range 5.8-6.3 %] to extremely high [i.e., between 14.1-17.3 %] values of Hb A<sub>1c</sub>. These comparative recovery results are presented in the following linearity table.

### VARIANT II TURBO Hemoglobin A<sub>1c</sub> and VARIANT II Hemoglobin A<sub>1c</sub> Linearity

| % Contribution<br>Normal      Diabetic |     | VARIANT II TURBO Hemoglobin A <sub>1c</sub> |                              |            | VARIANT II Hemoglobin A <sub>1c</sub> |                              |            |
|--|-----|---|------------------------------|------------|---------------------------------------|------------------------------|------------|
|  |     | Theoretical % HbA <sub>1c</sub>             | Observed % HbA <sub>1c</sub> | % Recovery | Theoretical % HbA <sub>1c</sub>       | Observed % HbA <sub>1c</sub> | % Recovery |
| 100                                    | 0   | 3.8   | 3.8                          | 100        | 3.5                                   | 3.5                          | 100        |
| 90                                     | 10  | 5.0   | 5.0                          | 100        | 4.7                                   | 4.7                          | 100        |
| 80                                     | 20  | 6.3   | 6.1                          | 96.8       | 5.9                                   | 5.8                          | 98.3       |
| 67                                     | 33  | 8.0   | 7.9                          | 98.8       | 7.6                                   | 7.4                          | 97.4       |
| 50                                     | 50  | 10.2  | 10.0                         | 97.9       | 9.8                                   | 9.6                          | 98.0       |
| 33                                     | 67  | 12.5  | 12.4                         | 98.0       | 12.1                                  | 11.9                         | 98.3       |
| 20                                     | 80  | 14.4  | 14.3                         | 99.3       | 14.1                                  | 13.8                         | 97.9       |
| 0                                      | 100 | 17.3  | 17.3                         | 100        | 17.2                                  | 17.2                         | 100        |

#### c. Traceability (controls, calibrators, or method):

The Hemoglobin A<sub>1c</sub> Calibrators provided with the VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program are traceable to the approved IFCC reference method. The IFCC reference method is used to assign IFCC values to the secondary reference materials. These secondary reference materials are used to value assign product calibrators and determine the product calibration parameters through use of the IFCC/NGSP master equation.

$$\begin{aligned}\text{Master equation: } (\text{NGSP A}_{1c}) &= 0.9148 (\text{IFCC A}_{1c}) + 2.152 \\ (\text{IFCC A}_{1c}) &= 1.093 (\text{NGSP A}_{1c}) - 2.350\end{aligned}$$

The NGSP values were assigned by the relationship between IFCC and NGSP. The VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program is certified by the NGSP.

*d. Detection Limit:*

Based on pooled and individual patient-derived blood standards, the lower limit of linear detection for this VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program was 4.1% HbA<sub>1c</sub> as listed in the Instructions for Use (IFU). The upper limit of linear detection for this VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program was 16.8% HbA<sub>1c</sub>. This linear or detection range was chosen to span all typical diagnostic testing that would occur for normal, diabetic, and extremely diabetic patients.

*e. Analytical Specificity:*

Three closely related but chemical derived analogs of HbA<sub>1c</sub> were analytically evaluated using this VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program as part of a detailed analytical specificity study. The influence of carbamylated hemoglobin was studied by spiking specimens with sodium cyanate until the carbamylated hemoglobin levels increased to a range of 1-1.5%. The influence of acetylated hemoglobin was studied by spiking specimens with acetylsalicylic acid until acetylated hemoglobin levels increased to a range of 1-2%. The influence of labile hemoglobin A<sub>1c</sub> was studied by spiking samples with glucose until labile A<sub>1c</sub> in hemoglobin reached 1-3%. Thus, final measurement of HbA<sub>1c</sub> in these blood-based human specimens was not influenced by carbamylated, acetylated or labile hemoglobin A<sub>1c</sub> at the above indicated limits.

Additional low, moderate and high blood samples were obtained as patient bloods that were anticoagulated with EDTA in the standard manner. In three separate trials of patient pools or individual blood samples, concentrated bilirubin was added to a final level of 20 mg/dL; concentrated lipids were added to a final level between 5680 and 6000 mg/dL; and additional dipotassium EDTA was added to a concentration of ~1980 mg/dL (11x the normal level) to determine the effect of high concentrations of EDTA that can occur in cases of “short draws.” The table below shows that, for the final measurement of Hb-A<sub>1c</sub>, neither the TURBO VARIANT II nor cleared predicate VARIANT II device was influenced by these excess biochemicals or excess EDTA anticoagulant, as illustrated in the following table.

| Interfering Substance  | VARIANT II TURBO Hemoglobin A <sub>1c</sub> | VARIANT II Hemoglobin A <sub>1c</sub> |
|------------------------|---|---------------------------------------|
| Bilirubin              | No interference up to 20 mg/dL              | No interference up to 20 mg/dL        |
| Lipids (Triglycerides) | No interference up to 5680 mg/dL            | No interference up to 6000 mg/dL      |
| EDTA                   | No interference up to 11X EDTA              | No interference up to 11X EDTA        |

*f. Assay Cut-off/Decision Level:*

This VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program spans the typical cut-off limits (generally at < 6.0% HbA<sub>1c</sub> for normal, and well above the suggested diabetic action level at > 8.0% HbA<sub>1c</sub>).

## **2. Comparison studies:**

### *a. Method Comparison & Correlation Analyses with Predicate for Accuracy:*

Method correlation between VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program and VARIANT II Hemoglobin A<sub>1c</sub> Program was evaluated using n= 201 EDTA whole blood patient samples that ranged from 3.9% to 17.5% HbA<sub>1c</sub>. The coded patient blood specimens used in this study were blood samples that remained after earlier clinical results were reported. The results of this non-clinical correlation study are presented in the following regression table.

| <b>Regression Method</b> | <b>n</b> | <b>r<sup>2</sup></b> | <b>Slope</b> | <b>Intercept</b> |
|--------------------------|----------|----------------------|--------------|------------------|
| Least Squares            | 201      | 0.9946               | 0.9792       | 0.185            |

By this comparison, a linear regression equation was obtained as:  $Y = 0.9792X - 0.185$ , and an  $r^2 = 0.9946$ , showing close correlation and substantial equivalence between the new VARIANT II TURBO (Y) device and the predicate VARIANT II (X) device.

### *b. Matrix Comparison:*

EDTA whole blood is the only sample type indicated.

## **3. Clinical studies:**

### *a. Clinical sensitivity:*

NA

### *b. Clinical specificity*

NA

### *c. Other clinical supportive data (when a and b are not applicable)*

NA

## **4. Clinical cut-off**

NA

## **5. Expected value/Reference Range:**

The Expected Value range was established from the article Rohlfing et al, published in the reference article entitled “Use of GHb (HbA<sub>1c</sub>) in screening for Undiagnosed Diabetes in the U.S. Population”. This study used the Bio-Rad DIAMAT Hemoglobin A<sub>1c</sub> Program. The recommended weighted mean HbA<sub>1c</sub> for patients with normal fasting plasma glucose (n=5,694) was 5.17% with the standard deviation of 0.45%, while the 95% confidence limits (mean  $\pm$ 2SD) were 4.27%-6.07 HbA<sub>1c</sub>. The VARIANT II TURBO Hemoglobin A<sub>1c</sub> Program matched this expected result range and was so certified by the NGSP.

## **N. Conclusion:**

Based upon a Third Party Review of the information provided in this 510(k), the submitted information in this premarket notification is complete and supports a substantial equivalence decision.