

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

A. 510(k) Number:

k090699

B. Purpose for Submission:

Modification to a marketed device

C. Measurand:

Hemoglobin A_{1c} (HbA_{1c})

D. Type of Test:

Quantitative ion-exchange high-performance liquid chromatography (HPLC)

E. Applicant:

Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:

VARIANT II TURBO HbA_{1c} Kit - 2.0

G. Regulatory Information:

1. Regulation section:
21 CFR § 864.7470, Glycosylated Hemoglobin Assay
2. Classification:
Class II
3. Product code:
LCP, Assay, Glycosylated Hemoglobin
4. Panel:
Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
The Bio-Rad VARIANT™ II TURBO HbA_{1c} Kit -2.0 is intended for the percent determination of hemoglobin A_{1c} in human whole blood using ion-exchange high-performance liquid chromatography (HPLC). Bio-Rad VARIANT™ II TURBO HbA_{1c} kit is intended for Professional Use Only.

Measurement of percent hemoglobin A_{1c} is effective in monitoring long-term glucose control in individuals with diabetes mellitus.

2. Indication(s) for use:
See intended use above.

3. Special conditions for use statement(s):
For Prescription Use Only
4. Special instrument requirements:
For use with the Bio-Rad VARIANT II TURBO Hemoglobin Testing System

I. Device Description:

The VARIANT II TURBO Hemoglobin Testing System provides an integrated method for sample preparation, separation and the percent determination of HbA_{1c} in EDTA human whole blood. The VARIANT II TURBO 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 versions 3.6T or 4.0.

The proposed device contains an analytical cartridge, 5 prefilters, Elution Buffers A and B, Calibrator Level 1, Calibrator Level 2, Whole Blood Primer, sample vials and a CD-ROM with test parameters.

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.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Variant II Hemoglobin A_{1c} Program, Bio-Rad Laboratories, Inc.
2. Predicate K number(s):
k070452
3. Comparison with predicate:

Item	Device	Predicate (k070452)
Similarities		
Intended use	Intended for the percent determination of HbA _{1c} in human whole blood using ion-exchange HPLC. Measurement of percent HbA _{1c} is effective in monitoring long-term glucose control in individuals with diabetes mellitus.	same
Assay principle	Cation exchange HPLC	same
Analytical	Resin formulation	same

cartridge						
Sample type	Anticoagulated whole blood (EDTA)	same				
Visible detection	415 nm	same				
Standardization	Traceable to the Diabetes Control and Complication Trial (DCCT) reference method and IFCC. Certified via the National Glycohemoglobin Standardization Program (NGSP)	same				
Calibrators	2 levels	same				
Interference	Bilirubin: no interference up to 20 mg/dL Lipids: no interference up to 6000 mg/dL EDTA: no interference up to 11X EDTA	same				
Differences						
Elution buffers	Sodium perchlorate buffer	Tris-bis buffer				
Calibration	Analytical cartridge must be calibrated after priming a new cartridge.	Analytical cartridge must be calibrated after priming a new cartridge, with each lot of buffers and every 30 days.				
Kit size	2500 tests	1000 tests				
Total area range	Total area range of 1.0 to 3.5 million $\mu\text{volt}\cdot\text{second}$	Total area range of 1.5 to 4.5 million $\mu\text{volt}\cdot\text{second}$				
Peak window names	In addition to A _{1a} , A _{1b} , F, LA _{1c} , A _{1c} , P ₃ , P ₄ , A _o and C, the candidate device has a variant window that includes HbE, C and S.	In addition to A _{1a} , A _{1b} , F, LA _{1c} , A _{1c} , P ₃ , P ₄ , A _o and C, the predicate has ED and S windows.				
Precision		Normal Patient	Diabetic Patient		Normal Patient	Diabetic Patient
	Mean (% HbA _{1c})	5.6	11.4	Mean (% HbA _{1c})	5.5	8.8
	Within run (% CV)	0.78	0.39	Within run (% CV)	0.90	0.59
	Within device (% CV)	1.15	0.91	Within device (% CV)	1.60	1.38
Linear range	3.5 to 19.0% HbA _{1c}		3.1 to 18.5 % HbA _{1c}			
Interference	Hemoglobin F: No interference up to 25% Hemoglobin variants: Two out of 7 hemoglobin AD-trait, 2 out of 11 hemoglobin AS-trait, 1 out of 12 hemoglobin AE-trait, and 3 out of 9 hemoglobin AC-trait patient samples at the clinically significant levels of 6% and 9% HbA _{1c} exhibited differences of more than $\pm 10\%$ from values obtained using boronate affinity reference method.			Hemoglobin F: No interference up to 15% Hemoglobin variants: no interference.		

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline--Second Edition (CLSI EP5-A2)
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
- Interference Testing in Clinical Chemistry; Approved Guideline--Second Edition (CLSI EP7-A2)

- Draft Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs.
- Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy
- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff
- Guidance for Industry and FDA Staff - Use of Symbols on Labels and in Labeling of *In Vitro* Diagnostic Devices Intended for Professional Use

L. Test Principle:

The candidate device is a well established method of measuring the level of HbA_{1c} in the red blood cell. The candidate device is based on chromatographic separation of HbA_{1c} 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 A_{1c}, hemoglobin F and carbamylated hemoglobin.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of the proposed device was evaluated using a protocol based on the CLSI EP5-A2 guideline. Normal patient, normal control, diabetic patient and diabetic control samples were run in the precision study. The precision study was performed on 6 instruments in 3 laboratories over 10 days. Each site was provided with the same sample set and directed to perform 2 replicates of each sample on each of 2 runs/day (morning and evening) for 10 days. Each site conducted the study on 2 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. Patient samples are EDTA human whole blood. The results are summarized below:

	Normal patient	Diabetic patient
Mean (% HbA _{1c})	5.6	11.4
Within-run (% CV)	0.78	0.39
Between-day (% CV)	0.66	0.69
Between-run (% CV)	0.53	0.45
Within-device (% CV)	1.15	0.91
Total precision (% CV)	2.01	1.93

	Normal control	Diabetic control
Mean (% HbA _{1c})	5.6	10.0
Within-run (% CV)	1.09	0.62
Between-day (% CV)	0.59	0.62
Between-run (% CV)	0.00	0.33
Within-device (% CV)	1.24	0.94
Total precision (% CV)	2.08	2.10

b. Linearity/assay reportable range:

The study was performed following the CLSI EP6-A guideline. Linearity across the reportable range was performed using low (2.5% HbA_{1c}) and high (19.6% HbA_{1c}) EDTA whole blood patient samples. These were mixed together in varying ratios. The measured values were compared to the theoretical values based upon the dilution factor. 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. The measured results for each dilution were within a maximum difference of 0.24% in this range. The results are summarized below:

% A _{1c} (theoretical)	Predicted 1 st order	Predicted 3 rd order	Difference
2.55	2.82	2.58	0.24
5.04	5.26	5.34	-0.08
7.10	7.28	7.45	-0.17
9.52	9.66	9.79	-0.13
11.08	11.20	11.26	-0.06
13.63	13.70	13.64	0.06
15.56	15.59	15.47	0.12
17.61	17.61	17.52	0.09
19.60	19.56	19.63	-0.07

The reportable range of the device is 3.5 to 19.0 % HbA_{1c}.

c. Traceability, Stability, Expected values (controls, calibrators, or methods): Calibrators were previously cleared in k070452. The recommended control materials were cleared in k070546 and k052838.

The proposed device has been certified by the National Glycohemoglobin Standardization Program (NGSP) as having documented traceability to the Diabetes Control and Complications Trial reference method.

d. *Detection limit:*

The reportable range is 3.5 to 19.0 % HbA_{1c} (see linearity section above).

e. *Analytical specificity:*

Icterus (bilirubin) and lipemia (triglyceride) interference: Two EDTA whole blood patient samples representing normal and diabetic HbA_{1c} samples were split into aliquots and treated with increasing concentrations of triglycerides and bilirubin. The treated and untreated samples were run in duplicate on a VARIANT II TURBO Hemoglobin Testing System and the differences in % HbA_{1c} between the samples were analyzed. Bilirubin up to 20mg/dL and triglycerides up to 6000 mg/dL had no significant effect on HbA_{1c} determination.

EDTA interference: In order to determine the effect of high concentrations of EDTA that can occur in cases of “short draws”, 2 whole blood patient samples representing normal and diabetic HbA_{1c} samples were split into aliquots and treated with 11 times (11X) the expected EDTA concentration in samples. The treated and untreated samples were run in duplicate on a VARIANT II TURBO Hemoglobin Testing System and the differences in % HbA_{1c} between the samples were analyzed. EDTA, up to 11X had no significant effect on HbA_{1c} determination.

Labile A_{1c} (LA_{1c}) interference: Two EDTA whole blood patient samples representing normal and diabetic HbA_{1c} samples were split into aliquots and treated with increasing concentrations of a glucose solution (up to 1000 mg/dL) and incubated for 3 hours at 37 °C to facilitate the formation of LA_{1c}. The treated samples and untreated control samples were run in duplicate on two VARIANT II TURBO Hemoglobin Testing Systems and the differences in % HbA_{1c} between the samples were analyzed. LA_{1c} up to 6% had no significant effect on HbA_{1c} determination.

Carbamylated hemoglobin interference: A series of EDTA whole blood patient samples representing normal and diabetic HbA_{1c} were prepared with varying concentrations of a high carbamylated hemoglobin solution (prepared by treating the red blood cells of a normal patient sample and a diabetic patient sample with 2.6 mM potassium cyanate at 37 °C for 45 minutes until the carbamylated hemoglobin level increased to 6%). The carbamylated-containing samples and the control samples were run in duplicate on a VARIANT II TURBO Hemoglobin Testing System and the differences in % HbA_{1c} between the samples were analyzed. Carbamylated hemoglobin up to 4% had no significant effect on HbA_{1c} determination.

Hemoglobin F (HbF) Interference: A series of EDTA whole blood patient samples representing normal and diabetic HbA_{1c} were prepared with varying concentrations of a high HbF (>25 %) solution. The treated samples and untreated control samples were run in duplicate on two VARIANT II TURBO

Hemoglobin Testing Systems and the differences in % HbA_{1c} between the samples were analyzed. HbF up to 25% had no significant effect on HbA_{1c} determination.

Hemoglobin Variant Interferences: Hemoglobin AD, AE, AS and AC-trait patient samples (20 of each) with HbA_{1c} values distributed across the non-diabetic and diabetic range were analyzed by the proposed device in replicates of four and compared to values obtained by a NGSP secondary Reference Laboratory using a boronate affinity method. Two out of 7 hemoglobin AD-trait, 2 out of 11 hemoglobin AS-trait, 1 out of 11 hemoglobin AE-trait and 3 out of 9 hemoglobin AC-trait patient samples within the clinically significant range of 6% and 9% HbA_{1c} tested exhibited differences of more than $\pm 10\%$ from values obtained using the reference method.

The sponsor includes the following statements in the product insert:

- Icterus, as indicated by bilirubin concentrations up to 20 mg/dL, does not interfere with the assay.
- Lipemia, as indicated by testing a normal and a diabetic sample spiked with a lipemic clinical serum sample to achieve triglyceride concentrations up to 6000 mg/dL, does not interfere with the assay.
- Carbamylated hemoglobin concentrations up to 4% do not interfere with the assay.
- Hemoglobin F concentrations up to 25% do not interfere with the assay. In cases where hemoglobin F concentrations are greater than 25% , the % A_{1c} should not be reported.
- In a study to assess interference from hemoglobin variants, 2 out of 7 hemoglobin AD-trait, 2 out of 11 hemoglobin AS-trait, 1 out of 11 hemoglobin AE-trait and 3 out of 9 hemoglobin AC-trait patient samples within the clinically significant range of 6% and 9% HbA_{1c} tested exhibited differences of more than $\pm 10\%$ from values obtained using the reference method. Laboratories should take this into consideration when evaluating results from patients with a hemoglobin variant trait.

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

To demonstrate accuracy across the measuring range of 3.5 to 19% HbA_{1c}, the proposed device was compared to the predicate using 40 EDTA whole blood patient samples and 12 spiked EDTA whole blood samples. The samples were run in singlicate on the proposed device and on the predicate device. A linear regression analysis of the results calculated a slope of 0.9621, an intercept of 0.4443, and a correlation coefficient of 0.994.

A second study was performed to evaluate the proposed device compared to the previous VARIANT II TURBO Hemoglobin A1c method (cleared in k040872 and the software was modified and cleared in k063400). A comparison of 42 EDTA whole blood patient samples with values distributed from 4.8 to 12.5 % HbA_{1c} (on the proposed device) were run in singlicate on both systems. A linear regression analysis of these results calculated a slope of 1.0957, an intercept of 0.7375, and a correlation coefficient of 0.9952.

- 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 cited from the American Diabetes Association. Standards of Medical Care for Patients with Diabetes Mellitus. Diabetes Care 2001, 24 (Suppl. 1), 33-34.

Hemoglobin A _{1c} (%)	Degree of Glucose Control
> 8	Action Suggested
< 7	Goal
< 6	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.