510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE AND INSTRUMENT TEMPLATE

A. 510(k) Number:
K041444

B. Purpose for Submission:
Clearance of device that combines the functions of three predicate devices

C. Analyte:
Hemoglobin A_{1c} (HbA_{1c}), Hemoglobin A_2 (HbA2), and Hemoglobin F (HbF)

D. Type of Test:
Quantitative HPLC assay

E. Applicant:
Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:
Bio-Rad D-10™ Dual Program
Bio-Rad D-10™ Dual Program Calibrator Set

G. Regulatory Information:

1. Regulation section:
   21 CFR § 864.7470, Assay, glycosylated hemoglobin
   § 864.7400, Hemoglobin A_2 Quantitation
   § 862.1150, Calibrator
2. Classification:
   Class II
3. Product Code:
   LCP, Glycosylated hemoglobin assay
   JPD, Hemoglobin A2 assay
   JIT, Calibrator, secondary
4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

The Bio-Rad D-10™ Dual Program is intended for the percent determination of hemoglobin A_{1c}, A_2 and F, and for the detection of abnormal hemoglobins in human whole blood using ion-exchange high performance liquid chromatography (HPLC).

The measurement of the percent hemoglobin A_{1c} is effective in monitoring long-term glucose control in individuals with diabetes mellitus, and measurement of the percent HbA_2 and HbF are effective in long-term monitoring of β–thalassemias (i.e., hereditary hemolytic anemias characterized by decreased synthesis of one or more types of abnormal hemoglobin polypeptide chains).

Detection of hemoglobin thalassemia variants such as hemoglobins S, C, D and E by HPLC is effective in presumptive identification of these variants. The Bio-Rad D-10™ Dual Program is intended for Professional Use Only. For in vitro diagnostic use.

2. Indication(s) for use:

See Intended Use above.

3. Special condition for use statement(s):

For professional use only in clinical chemistry laboratories.

4. Special instrument Requirements:

Bio-Rad D-10™ Hemoglobin Testing System

I. Device Description:

The Bio-Rad D-10™ Dual Program is a testing system that uses HPLC for the chromatographic separation of Hemoglobins A_{1c}, A_2, F and related hemoglobins that may be present in human blood. The kit contains supplies for 400 tests for hemoglobin A_{1c} or 200 tests for hemoglobins A_{1c}, A_2 and F and consists of an analytical cartridge, elution buffers, wash/diluent solutions, specific calibrators, calibrator diluent, whole blood primer and program parameters.

This Bio-Rad D-10™ Dual Program system is run on the Bio-Rad D-10™ Hemoglobin Testing System that utilizes ion-exchange high-performance liquid chromatography (HPLC). A dual-piston, low pulsation HPLC pump and a proportioning valve deliver the buffer solution to the analytical cartridge and detector. The samples are automatically diluted on a sampling rack. Whole blood samples undergo an automatic two-step dilution process in the sample rack and then are introduced into the analytical flow path of the HPLC.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Bio-Rad Dual-10 Hemoglobin A_{1c}
   VARIANT™ II Hemoglobin A_{1c} Program
   VARIANT™ II ß-thalassemia Short Program

2. Predicate K number(s):
   K031043
   K984268
   K991127

3. Comparison with predicate:

The device and its predicate(s) share similar assay principle, use of controls, traceability, column type, sample type, and sample volume.

Similarities		
Item	**Device**	**Predicate**
Intended Use	The Bio-Rad D-10™ Dual Program is intended for the percent determination of hemoglobin A1c, A2, and F, and for the detection of abnormal hemoglobins in human whole blood using ion-exchange high performance liquid chromatography (HPLC). The Bio-Rad D-10™ Dual Program is intended for Professional Use only. For in vitro diagnostic use.	The Bio-Rad VARIANT™ II Hemoglobin A_{1c} (HbA_{1c}) Program is intended for percent determination of HbA_{1c} in human whole blood using ion-exchange high performance liquid chromatography (HPLC). The VARIANT™ ß-Thalassemia (HbA_2) Program is intended for the percent determination of HbA_2 and HbF in human whole blood using ion-exchange high performance liquid chromatography (HPLC). For in vitro diagnostic use.
Differences		
Item	**Device**	**Predicate**
Analyte Measured	% Hemoglobin A_{1c}, % Hemoglobin A_2, % Hemoglobin F, and related hemoglobin variants that may be present in human blood in one combined device	% Hemoglobin A_{1c} and % Hemoglobin A_2 and % Hemoglobin F in separate devices
Analytes Reported	Hemoglobin A_{1a}, A_{1b}, A_{1c}, F, A_O, S, C, A_2, CHb/ LA_{1c}, P3 windows.	Hemoglobin A_{1a}, A_{1b}, A_{1c}, F, A_O, C, LA_{1c}, P3 and S window.
Time to process sample	6.5 minutes	3 minutes or 6.5 minutes

Calibration	2 point calibration, once every 24 hrs. or if the program is switched between the "3.0 Minute" and "6.5 Minutes" Programs.	1 point calibration, once every run.

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

NGSP (National Glycohemoglobin Standardization Program)
IFCC HbA_{1c} Calibrators
FDA Guidance Document for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs
FDA Guidance for Software Contained in Medical Devices
NCCLS C28-A2 - How to Define and Determine Reference Interval in the Clinical Laboratory

L. Test Principle:

The Bio-Rad D-10™ Dual Program system utilizes the principles of ion-exchange high-performance liquid chromatography (HPLC). A dual -piston, low pulsation HPLC pump and a proportioning valve deliver the buffer solution to the analytical cartridge and detector. Samples are automatically diluted on a sampling rack and injected into an analytical cartridge. Whole blood samples undergo an automatic two-step dilution process in the sample rack and then are introduced into the HPLC analytical flow path.

A programmed buffer gradient of increasing ionic strength delivers the sample to the analytical cartridge, where the hemoglobins are separated based upon their ionic interactions with the cartridge material. The separated hemoglobins then pass through the filter photometer flow cell where changes in the absorbance are measured. The absorbance at 415 nm is measured. An additional filter at 690 nm corrects the background absorbance. The software performs a reduction of raw data collected from each analysis that may include use of a calibrator factor. A sample report and chromatogram are generated for each sample. Two-level calibration is used for adjustment of the calculated values.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
    a. Precision/Reproducibility:

Assay imprecision was evaluated by running two patient samples in duplicate twice per day for 20 days (for n = 80 determinations). In each duplicate daily run, duplicate aliquots of low HbA_{1c} and of high HbA_{1c} patient samples were each analyzed per run. Results are summarized below.

Sample	HbA_{1c} (6.5 Min.)		HbA_2		HbF	
	Normal	**Diabetic**	**Low**	**High**	**Low**	**High**
Mean (%Hb)	5.9	13.1	2.2	5.4	2.1	8.7
Within run (%CV)	0.8	0.3	4.5	1.7	1.7	1.4
Total Precision (%CV)	1.8	0.9	5.3	3.1	3.3	2.0

b. Linearity/assay reportable range:

To assess assay linearity, high and low samples were spiked to the following levels and mixed together in the ratios below (high and low sample values determined on the predicate assay):

HbA_{1c} – Normal (Low) (4.0%) and Diabetic (High) (19%) at 4:1, 2:1, 1:1, 1:2, 1:4, and 1:9
HbA_2 - Low (1.8%) and High (9.7%) at 4:1, 2:1, 1:1, 1:2, 1:4, and 1:9
HbF - Low (0.1%) and High (19.3%) at 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, and 1:20
The mixtures were then measured in duplicate and observed values and calculated expected values are summarized below. (units = % Hb)

% Contribution		HbA_{1c} (6.5 Mins)		
Normal	**Diabetic**	**Theoretical**	**Observed**	**% Recovery**
100	0	3.8	3.8	100
90	10	5.3	5.3	100
80	20	6.8	6.7	98.5
67	33	8.8	8.6	97.7
50	50	11.3	11.1	98.2
33	67	13.8	13.7	99.3
20	80	15.7	15.7	100
0	100	18.6	18.6	100

% Contribution		HbA_2		
Low	**High**	**Theoretical**	**Observed**	**% Recovery**
100	0	1.7	1.7	100
90	10	2.5	2.5	100
80	20	3.4	3.1	91.2
67	33	4.5	4.2	93.3
50	50	6.0	5.8	96.7
33	67	7.4	7.2	97.3
20	80	8.6	8.5	98.8
0	100	10.3	10.3	100

% Contribution		HbF		
Low	High	Theoretical	Observed	% Recovery
100	0	0.4	0.4	100
95	5	1.4	1.5	107.1
90	10	2.4	2.7	112.5
80	20	4.5	4.8	106.7
67	33	7.2	7.7	106.9
50	50	10.8	11.1	102.8
33	67	14.4	14.6	101.4
20	80	17.4	17.5	100.6
0	100	22.0	22.0	100

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

The HbA_{1c} Calibrators provided with the D-10™ Dual Program are traceable to the IFCC reference standard. The calibrators are value-assigned by multiple runs which include calibrators, controls, and 5 IFCC calibration standard samples. The set consists of 2 levels of calibration materials with targeted values in the low and upper-to-mid range. Assigned values for each lot are described in the calibrator package insert.

The Joint Committee on Traceability in Laboratory Medicine [JCTLM] has not identified a higher order reference method or reference material for quantitation of HbA_2 and HbF. Until recognized reference materials and methods for HbA_2 and HbF are created and approved that meet the criteria established in European harmonized standards (ISO 15193 and IS0 15194), the sponsor uses an existing calibrator value assignment process for these analytes. The calibrators are value-assigned by multiple runs which include calibrators, controls, and 5 internal master calibration samples. For these analytes, internal master calibrators are created by measurement on the predicate assay. The set consists of 2 levels of calibration materials with targeted values in the low and upper-to-mid range. Assigned values for each lot are described in the calibrator package insert.

d. Detection limit:

The upper and lower detection limits for this assay were determined by the linear range of the assay (as follows).

HbA1c – 3.8 % - 18.5% (short program)
3.7% - 18.4% (long program)
HbA2 – 1.5% - 11.4%
HbF – 0.8% - 16.5%

Users are instructed to report values above or below the reportable range of the assays as > or < the highest and lowest reportable values, respectively.

e. Analytical specificity:

$\underline{HbA_{1c}}$

In evaluating the specificity of the D-10™ Dual Program for $\%HbA_{1c}$ in EDTA-treated blood samples, two closely related but chemical derived analogs of HbA_{1c} where evaluated 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 2.0%. Also, influence of unstable labile hemoglobin A_{1c} was studied by spiking samples with glucose until unstable labile A_{1c} in hemoglobin reached 3.5%. As was the case for the predicate Bio-Rad VARIANT™ II HbA_{1c} system, the results with this new Rad D-10™ Dual Program system demonstrated that the final measurement of $\%HbA_{1c}$ at normal and diabetic levels was not significantly influenced by either added carbamylated hemoglobin or added glucose-labile hemoglobin A_{1c} at the above indicated limits.

Additional normal and diabetic 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: a) concentrated bilirubin was added to a final level of 20 mg/dL; b) concentrated lipids were added to a final level of 5680 mg/dL; and c) 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." For the measurement of normal and high diabetic HbA_{1c} in blood samples, the device was not influenced by these excess endogenous substances or excess EDTA anticoagulant, as illustrated below in the interference evaluation table.

Interfering Substances	**D-10™ Dual Program (6.5 Minutes) HbA_{1c}**
Bilirubin	No interference up to 20 mg/dL
Lipids (Triglycerides)	No interference up to 5680 mg/dL
EDTA	No interference up to 11X EDTA

$\underline{HbA_2}$

In evaluating for specificity of the D-10™ Dual Program for $\%HbA_2$ in EDTA-treated blood samples, additional normal, moderate and high blood $\%HbA_2$ 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: a) concentrated bilirubin was added to a final level of 20 mg/dL; b) concentrated lipids were added to a final level between 5680 mg/dL; and c) 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." For the final measurement of normal, moderate and high HbA_2 in blood samples, the device was not influenced by these excess endogenous substances or excess EDTA anticoagulant, as illustrated below in the interference evaluation table.

Interfering Substances	D-10™ Dual Program (HbA_2)
Bilirubin	No interference up to 20 mg/dL
Lipids (Triglycerides)	No interference up to 5680 mg/dL
EDTA	No interference up to 11X EDTA

HbF

The HbF assay was evaluated using the D-10™ Dual Program system as part of a detailed analytical specificity study. First the influence of an unstable complex of glucose & hemoglobin known as labile Hemoglobin A_{1c} (which elutes in proximity to HbF) was studied by spiking samples with glucose until labile A_{1c} in Hemoglobin reached 0-2.6%. Final measurement of HbF in these blood-based human specimens was not influenced significantly by labile Hemoglobin A_{1c} at the above-indicated limits.

In evaluating for specificity of this D-10™ Dual Program system for %HbF in EDTA-treated blood samples, additional normal, 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: a) concentrated bilirubin was added to a final level of 20 mg/dL; b) concentrated lipids were added to a final level between 5680 and 6000 mg/dL; and c) 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." For the final measurement of HbF, and HbA_{1c} & HbA_2, the device was not influenced by these excess endogenous substances or excess EDTA anticoagulant, as illustrated below in the interference evaluation table.

Interfering Substance	D-10 ™ Dual Program (Extended) (HbA_{1c} & HbA_2/F)
Potential Labile Hb (glucose + Hb) Interference	No significant interference up to 3.5% Labile Hb on HbA_{1c}
Potential Labile Hb (glucose + Hb) Interference	No significant interference up to 2.6% Labile Hb on HbF
Bilirubin	No interference up to 20 mg/dL
Lipids (Triglycerides)	No interference up to 5680 mg/dL
EDTA	No interference up to 11X EDTA

f. Assay cut-off:
Not applicable

2. Comparison studies:
   a. Method comparison with predicate device:

HbA_{1c}
Method correlation between D-10™ Dual Program system and the predicate was evaluated using 40 EDTA whole blood patient samples (measured in duplicate) that ranged from 4.7% to 11.2% HbA_{1c}. By this comparison, a linear regression equation was obtained as: $Y = 0.9906*X + 0.4310$, and an $r^2 = 0.9843$, showing substantial equivalence between the new device and the predicate for HbA_{1c} analyses from human blood. Similar results were also seen at two additional sites using a total of 148 additional samples.

HbA_2
Method correlation between D-10™ Dual Program system and the predicate was evaluated using 40 EDTA whole blood patient samples(measured in duplicate) that ranged from 1.9% to 8.9% for HbA_2. Samples used in this study were both spiked and normal coded (stored) patient samples. By this comparison, a linear regression equation was obtained as: $Y = 1.0898*X - 0.2407$, and an $r^2 = 0.9832$, showing substantial equivalence between the device and the predicate for HbA_2 analyses from human blood.

HbF
Method correlation between D-10™ Dual Program system and the predicate was evaluated using 40 EDTA whole blood patient samples (measured in duplicate) that ranged from 0% to 12.9% for HbF. Samples used in this study were both spiked and normal coded (stored) patient samples. By this comparison, a linear regression equation was obtained as: $Y = 0.9497*X - 0.1785$, and an $r^2 = 0.9959$, showing substantial equivalence between the device and the predicate for HbF analyses from human blood.

b. Matrix comparison:

EDTA whole blood is the only sample type indicated.

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:

HbA_{1c}
The Expected Value range was established in the article by Rohlfing et al, as published in a reference article entitled *"Use of GHb (HbA1c) in Screening for Undiagnosed Diabetes in the U.S. Population"*. The recommended weighted mean the $\%HbA_{1c}$ 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_{1c}. The D-

10™ Dual Program system matched this expected result range and has been so certified by the NGSP.

HbA_2 and HbF

The Expected Value range for the D-10™ Dual Program system was established from a modified version of NCCLS C28-A2. The protocol did not follow the sample acquisition and identification requirements, since this was a non-clinical evaluation. A total of 53 EDTA whole blood samples from apparently healthy male and females in Northern America were analyzed for HbA_2 and HbF using the D-10™ Dual Program system. The range is a 95% non-parametric range obtained by eliminating the highest and lowest 2.5% of values. The mean HbA_2 value was 3.0%. The 95% confidence interval for HbA_2 was 2.2% to 3.7%. The mean hemoglobin F value was 0.2%. The 95% confidence interval for hemoglobin F was 0% to 0.8%.

N. Instrument Name:

Bio-Rad D-10™ Hemoglobin Testing System

O. System Descriptions:

1. Modes of Operation:
   Automatic
2. Software: see K031043 – no additional functional requirements or software were added

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ___3___ (see K031043) or No _____

3. Sample Identification:
   Barcode
4. Specimen Sampling and Handling:
   No sample preparation necessary. Whole blood (EDTA) tubes are used for sampling
5. Assay Types:
   Chemistry (ion exchange chromatographic separation)
6. Reaction Types:
   Photometric detection of elution peaks
7. Calibration:
   Two levels of calibration materials are supplied for each analyte. See traceability section above for more information on the calibrators.
8. Quality Control:
   The sponsor recommends that users perform quality control using low and high specimens once per 24 hours or when expected control values are not obtained.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "L. Performance Characteristics" Section Of The SE Determination Decision Summary:

The instrument and software for the two devices that were combined to result in this device were previously cleared. The sponsor states that no changes were made to the cleared software (K031043) to accommodate the new analytes as the cleared version was designed and validated to support the addition of these additional analytes.

Q. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.