

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

**A. 510(k) Number:** K051072

**B. Purpose for Submission:**

The Bio-Rad VARIANT™ nbs Sickle Cell Program system/device provides improvements over the a predicate chromatographic devices known as the: VARIANT™ Sickle Cell Short Program [K924813; cleared 01/14/93].

**C. Analyte:**

Hemoglobins F, A, S, D, C and E

**D. Type of Test:**

IVD Qualitative, Hemoglobin HPLC.

**E. Applicant:**

Bio-Rad Laboratories, Inc.,  
Clinical Systems Division  
4000 Alfred Nobel Drive  
Hercules, CA, U.S.A, 94547-1803

**F. Proprietary and Established Names:**

Bio-Rad VARIANT™nbs Sickle Cell Program  
Hemoglobin variants determination by HPLC

**G. Regulatory Information:**

1. Regulation section:  
21 CFR 864.7415 [Abnormal Hemoglobin Assay]
2. Classification:  
Class II
3. Product Code:  
GKA
4. Panel:  
Hematology (81)

**H. Intended Use:**

1. Intended use(s):  
The Bio-Rad VARIANT™ nbs Sickle Cell Program is intended as a qualitative screen for the presence of hemoglobins F, A, S, D, C and E in eluates of neonatal blood collected on filter paper by high performance liquid chromatography (HPLC).

The Bio-Rad VARIANT™ nbs Sickle Cell Program is intended for Professional Use Only. For In Vitro Diagnostic Use.

The Bio-Rad VARIANT™ nbs Sickle Cell Program is intended for use only with the Bio-Rad VARIANT™ nbs Newborn Screening System.

2. Indication(s) for use:  
This device, consisting of the reagents, apparatus, HPLC instrumentation, software and controls, is indicated for professional laboratory IVD use to isolate and identify genetically determined abnormal (S, D, C, E) and normal (F, A) hemoglobin types in neonatal blood samples.
3. Special condition for use statement(s):  
For professional IVD use only in clinical laboratory.
4. Special instrument Requirements:  
For Bio-Rad VARIANTnbs Newborn Screening System.

## I. **Device Description:**

This complete device consists of the **Bio-Rad VARIANT™ nbs Sickle Cell Program** (VnbsSCP) reagent kit, the **Bio-Rad VARIANT™ nbs Newborn Screening System** (VNBSS) instrument, and the **Bio-Rad Genetic Data Management Software** (GDM). The VNBSS instrument consists of a VARIANTnbs Neonatal Auto Sampler (VNAS) module for microwell plates and a VARIANTnbs Neonatal Chromatography Station (VNCS) module containing the high performance liquid chromatography (HPLC) hardware. The VnbsSCP reagent kit includes a specific analytical HPLC cartridge containing cation exchange resin, as well as two (2) buffer reagents for establishing an HPLC gradient. The GDM software is designed to execute the VnbsSCP assay protocol on the VNBSS instrument using the VnbsSCP reagent kit components for the purposes of qualitatively screening for the presence of normal hemoglobins F and A, as well as the abnormal hemoglobins S, D, C and E from neonatal heel stick blood, as collected on filter paper that is punched and eluted with deionized water. The VNBSS processes each sample individually. An eluted sample is aspirated directly from a microwell plate in the VNAS module with the punched filter paper disc still present, and transferred into the sample loop in the VNCS module. The contents of the sample loop are subsequently injected into the flow path of the VNCS module. The hemoglobins of interest are retained on the analytical cartridge in the presence of Elution Buffer 1. The ionic strength is subsequently raised by adding increasing amounts of Elution Buffer 2. The pre-programmed gradient is designed to have the hemoglobins of interest elute from the cartridge with retention times that fall within pre-determined windows characteristic of known normal and abnormal hemoglobins. The eluted hemoglobins are sequentially detected with a dual-wavelength filter photometer, which monitors hemoglobin absorbance at 415 nm and corrects for any gradient induced absorbance changes at 690 nm. The software processed HPLC data is outputted in a printed report that contains: 1) sample identification, 2) date and time of analysis, 3) a table of peaks that includes: observed peak identification (hemoglobin type) name(s), retention time(s), peak height(s), peak area(s), and relative area percent(s), 4) total chromatogram area, 5) complete chromatographic display and 6) any error message(s) relating (if needed) to such data. Also reported is an optional “pattern assignment” for each hemoglobin based upon “pattern rules” derived from diagnostic hemoglobin literature.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Bio-Rad VARIANT™ Sickle Cell Short Program
2. Predicate K number(s):  
K924813 [cleared: 01/14/1993]
3. Comparison with predicate:

<b>Summary of Technological Characteristic - Similarities to Predicate Device</b>		
<b>Features</b>	<b><u>New Device:</u> Bio-Rad VARIANT™ nbs Sickle Cell Program</b>	<b><u>Predicate Device:</u> Bio-Rad VARIANT™ Sickle Cell Short Program (K#924813)</b>
Intended Use	The Bio-Rad VARIANTnbs Sickle Cell Program is intended as a qualitative screen for the presence of hemoglobins F, A, S, D, C and E in eluates of neonatal blood collected on filter paper by high performance liquid chromatography (HPLC).	The VARIANT Sickle Cell Short Program is designed as a qualitative screen for the presence of hemoglobins F, A, S, D, C and E in eluates of neonatal blood collected on filter paper by high performance liquid chromatography.
	For In Vitro Diagnostic Use.	For In Vitro Diagnostic Use.
	For Professional Use Only.	For Professional Use Only.
Target Population	Neonates.	Neonates.
Design – Assay principle	Cation exchange high performance liquid chromatography.	Cation exchange high performance liquid chromatography.
Design – Assay Detection	Heme absorbance at 415 nm with background correction at 650 nm.	Heme absorbance at 415 nm with background correction at 650 nm.
Design – Analytes Identified	Six retention time windows for hemoglobins F, A, E, D, S and C.	Six retention time windows for hemoglobins F, A, E, D, S and C.
Design – Sample Type	Neonatal dried blood spots on filter paper collection cards.	Neonatal dried blood spots on filter paper collection cards.
Design – Punched Disc	One 1/8” disc.	One 1/8” disc.
Design – Manual Worklists	Accepts manual worklists.	Accepts manual worklists.
Materials - Components	Elution Buffer 1. Elution Buffer 2. Wash Solution. Analytical Cartridge. Lyophilized Whole Blood Primer. Lyophilized Retention Time Marker 1 (FAES). Lyophilized Retention Time Marker 2 (FADC).	Elution Buffer 1. Elution Buffer 2. Wash Solution. Analytical Cartridge. Lyophilized Whole Blood Primer. Lyophilized Retention Time Marker 1 (FAES). Lyophilized Retention Time Marker 2 (FADC).
Performance – Precision	Peak retention time precision is <1% for all hemoglobin peaks.	Peak retention time precision is <1% for all hemoglobin peaks.
Compatibility with Environment	U.S. FCC EMI and E.U. EMC standard compliant.	U.S. FCC EMI and E.U. EMC standard compliant.
Human Factors	For in vitro diagnostic use. For professional use only.	For in vitro diagnostic use. For professional use only.
Energy Used	Auto-switching 110 V and 220 V.	110 V and 220 V models.

<b>Summary of Technological Characteristic - Similarities to Predicate Device</b>		
<b>Features</b>	<b><u>New Device:</u> Bio-Rad VARIANT™ nbs Sickle Cell Program</b>	<b><u>Predicate Device:</u> Bio-Rad VARIANT™ Sickle Cell Short Program (K#924813)</b>
Chemical Safety	Sodium azide concentration <0.05%. Gentamicin Sulfate concentration <0.1%. Tobramycin concentration <0.1%. Warnings provided in labeling as required, including State of California Proposition 65 Warning.	Sodium azide concentration <0.05%. Gentamicin Sulfate concentration <0.1%. Tobramycin concentration <0.1%. Warnings provided in labeling as required, including State of California Proposition 65 Warning.
Electrical, Mechanical and Thermal Safety	System certification to US and Canadian product safety standards and EU low voltage safety standards.	System certification to US and Canadian product safety standards and EU low voltage safety standards.
Standards Met	<ul style="list-style-type: none"> <li>• EN375:2002</li> <li>• EN591:2001</li> <li>• EN980:2003</li> <li>• EN1658:1996</li> <li>• EN13485:2003</li> <li>• EN13640:2002</li> <li>• EN14971:2001</li> <li>• EN61010-1:2001</li> <li>• EN61010-2-101:2002</li> <li>• EN61326:2001</li> </ul>	<ul style="list-style-type: none"> <li>• EN375:2002</li> <li>• EN591:2001</li> <li>• EN980:2003</li> <li>• EN1658:1996</li> <li>• EN13485:2003</li> <li>• EN13640:2002</li> <li>• EN14971:2001</li> <li>• EN61010-1:2001</li> <li>• EN61010-2-101:2002</li> <li>• EN61326:2001</li> </ul>

<b>Summary of Technological Characteristic - Differences to Predicate Device</b>		
<b>Features</b>	<b><u>New Device:</u> Bio-Rad VARIANT™ nbs Sickle Cell Program</b>	<b><u>Predicate Device:</u> Bio-Rad VARIANT™ Sickle Cell Short Program (K#924813)</b>
Design – System Configuration	Separate chromatography and auto sampler modules and separate PC workstation with software.	Single integrated unit with chromatography, auto sampler and software functionalities.
Design – Media	CD-ROM.	ROM Card.
Design – Container, Elution Volume, Reconstitution Volume, Sample Loop, Column loading	Plastic 96 microwell plate. 250 µL sample elution volume. 500 µL primer and retention time marker reconstitution volume. 10 µL sample loop. Column loading is 1/25 of eluted sample volume and 1/50 of reconstituted material.	Plastic sample vial. 500 µL sample elution volume. 1000 µL primer and retention time marker reconstitution volume. 20 µL sample loop. Column loading is 1/25 of eluted sample volume and 1/50 of reconstituted material.
Design – Aspiration Probe Tip, Punched Disc Disposition	Beveled aspiration probe tip. Dried blood spot punched disc left in microwell during sample aspiration.	Blunt aspiration probe tip. Dried blood spot punched disc removed before sample aspiration.
Design – Additional retention time windows.	Seven (7) additional retention time windows: F1, “Other (1)”, “Other (2)”, “Other (3)”, “Other (4)”, “Other (5)” and “Other (6)”.	Feature not available.
Design – Automated Worklists	Accepts automated worklists from spot punchers.	Feature not available.
Design – Pattern Assignment	Optional pattern assignment feature uses pattern rules derived from literature.	Feature not available.

<b>Summary of Technological Characteristic - Differences to Predicate Device</b>		
Features	<u>New Device:</u> Bio-Rad VARIANT™ nbs Sickle Cell Program	<u>Predicate Device:</u> Bio-Rad VARIANT™ Sickle Cell Short Program (K#924813)
Performance – Variants Limit of Detection	The limit of detection for S, D, C and E is 1% of the total area of the sample when the total area is 1.5 million microvolt-second.	Limit of detection for hemoglobins S, D, C and E is 1% of the total area when the total area of the sample is 1.0 million microvolt-second.
Performance – Guideline for Interpretation of Results	Total area must be between 900,000 to 6.3 million microvolt-second.	Total area should range from 1,000,000–3,000,000 microvolt-second.
Performance – Total Area Limit of Detection	900,000 microvolt-second.	Not addressed in instruction manual.
Performance – Bilirubin interference	Bilirubin up to 20 mg/dL does not interfere.	Not addressed in instruction manual.
Performance – Triglyceride interference	Triglyceride up to 6000 mg/dL does not interfere.	Not addressed in instruction manual.
Performance - Eluate stability	Eluates are stable for 48 hrs on the cooled auto sampler and at 2-8 °C and stable for 24 hrs at 15-30 °C.	Eluates are stable for 24 hrs at 2-8 °C.
Guidances Met	<ul style="list-style-type: none"> <li>FDA 2002 - General Principles of Software Validation; Final Guidance for Industry and FDA Staff.</li> <li>FDA 1999 - Guidance for Off-the-Shelf Software Use in Medical Devices; Final.</li> <li>FDA 1998 - Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; Final.</li> <li>FDA 2003 – Device Advice; Content of a 510(k)</li> </ul>	Not addressed.
Standards Met	<ul style="list-style-type: none"> <li>NCCLS EP-05A 1999.</li> <li>NCCLS EP-05A2 2004.</li> </ul>	Not addressed.

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

Standards Met	<ul style="list-style-type: none"> <li>EN375:2002 - Information supplied by the manufacturer with in vitro diagnostic reagents for professional use.</li> <li>EN591:2001 – Instructions for use in vitro diagnostic instruments for professional use.</li> <li>EN980:2003 - Graphical symbols for use in the labeling of medical devices.</li> <li>EN1658:1996 - Requirements for marking of in vitro diagnostic instruments.</li> <li>EN13485:2003 - Quality systems - Medical devices - Particular requirements for the application of EN ISO 9001:1994.</li> <li>EN13640:2002 - Stability testing of in vitro diagnostic medical devices.</li> <li>EN14971:2001 - Medical devices – Application of risk management to medical devices.</li> <li>EN61010-1:2001 - Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements</li> <li>EN61010-2-101:2002 - Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.</li> <li>EN61326:2001 - Electrical equipment for measurement, control and laboratory use -</li> </ul>
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	EMC requirements <ul style="list-style-type: none"> <li>• NCCLS EP05A 1999 – Evaluation of Precision Performance of Clinical Chemistry Devices.</li> <li>• NCCLS EP05A2 2004 – Evaluation of Precision Performance of Quantitative Measurement Methods.</li> </ul>
Guidances Met	<ul style="list-style-type: none"> <li>• FDA 2002 - General Principles of Software Validation; Final Guidance for Industry and FDA Staff.</li> <li>• FDA 1999 - Guidance for Off-the-Shelf Software Use in Medical Devices; Final.</li> <li>• FDA 1998 - Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; Final.</li> <li>• FDA 2003 – Device Advice; Content of a 510(k)</li> </ul>

## L. Test Principle:

Cation exchange high performance liquid chromatography with visible light detection.

## M. Performance Characteristics (if/when applicable):

### 1. Analytical performance:

#### a. Precision/Reproducibility:

The Bio-Rad VARIANT™ Sickle Cell Program device was based on NCCLS Protocol EP-05A (Vol. 19, No. 2 [1999]) and EP-05A2 (Vol. 24, No. 25 [2004]). Two analytical runs were performed per day on 20 days for a total of 40 runs on each of 3 separate systems. Each run included 4 replicates of two retention time positional QC controls. Within-run precision and within-device precision (formerly total precision) were determined. The reported predicate device retention time precision protocol included the same two retention time positional QC controls. However, the protocol did not strictly conform to NCCLS Protocol EP5-A2 guidelines, nor was within laboratory (or within-device) precision reported, as this was strictly a qualitative assay. The results of within run precision for both predicate and new devices was less than 1% for Hemoglobins F, A, E, D, S and C, and are presented in the following tables:

Average Retention Time Summary								
		RT Window	0.59-0.71	0.79-0.89	0.95-1.03	1.03-1.13	1.15-1.25	1.63-1.77
			Retention Time Within-Run Precision (CV %)					
Device	Repli-cates	Sample	Peak F	Peak A	Peak E	Peak D	Peak S	Peak C
New System 1	160	QC Control 1	0.63	0.83	0.98		1.21	
New System 2	160	QC Control 1	0.63	0.83	0.98		1.21	
New System 3	160	QC Control 1	0.63	0.83	0.98		1.20	
New System 1	160	QC Control 2	0.63	0.83		1.08		1.70
New System 2	160	QC Control 2	0.63	0.83		1.08		1.70
New System 3	160	QC Control 2	0.63	0.83		1.08		1.70

Retention Time Within Device Precision (Formerly Total Precision)								
Device	Replicates	Sample	Retention Time Within-Run Precision (CV %)					
			Peak F	Peak A	Peak E	Peak D	Peak S	Peak C
New System 1	160	QC Control 1	0.3	0.4	0.5		0.5	
New System 2	160	QC Control 1	0.6	0.6	0.4		0.6	
New System 3	160	QC Control 1	0.6	0.6	0.6		0.5	
Predicate	Not Reported							
New System 1	160	QC Control 2	0.3	0.4		0.4		0.2
New System 2	160	QC Control 2	0.7	0.6		0.4		0.3
New System 3	160	QC Control 2	0.6	0.6		0.5		0.3
Predicate	Not Reported							

*b. Linearity/assay reportable range:*

Refer to method correlation results in Section 2a,

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

Not applicable

*d. Detection limit:*

The Bio-Rad VARIANT™ Sickle Cell Program new device peak area limit of detection for hemoglobin variants (E, D, S and C) was determined using a total of 102 sample measurements bracketing the 1% peak area limit of detection for each variant, when the total chromatogram area was 1.5 million microvolt x second (=  $\mu\text{volts}\cdot\text{sec}$ ). The reported predicate device peak area limit of detection was 1%, when the total chromatogram area was 1.0 million  $\mu\text{volts}\cdot\text{sec}$ . The results re: peak area limit of detection for hemoglobin variants (E, D, S and C) was 1% for both predicate and new device, and is documented in the following table:

Variant Peak Area – Limit of Detection						
Device	Chromatogram Total Area (microvolt-second)	Peak Area % Limit of Detection				
		Peak E	Peak D	Peak S	Peak C	
New System 1	1.5	1%	1%	1%	1%	
Predicate	1.0	1%	1%	1%	1%	

*e. Analytical specificity:*

Refer to method correlation results in Section 2a.

*f. Assay cut-off:*

Not applicable

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

Method correlation between predicate and -Rad VARIANT™ Sickle Cell Program new device was evaluated using 1025 unknown samples prepared from retrospective neonatal dried blood spot collection cards. Using a total area range of 0.90–6.30 million  $\mu\text{volts}\cdot\text{sec}$ , there was 99.8% (1023/1025) agreement between test and predicate devices for all sample hemoglobin identifications. There was 100.0% (250/250) agreement between test and predicate devices for identifying hemoglobin S (HbS) in those samples that predicate device identified as containing HbS. In the two disagreements, the new device identified all hemoglobins identified by the predicate as well as one additional peak in each case. [See explanation below.] These results indicate satisfactory correlation between the predicate and test devices. The results are presented in the following tables



<b>Hemoglobin Identification Correlation Summary</b>				
	Number of Samples	Predicate Device	New device	
		F, A, E, D, S and (or) C Identified	Agree	Disagree
	591	FA	590	1 (FAS)
	52	FAE	52	0
	38	FAD	37	1 (FADC)
	247	FAS	247	0
	90	FAC	90	0
	3	FSC	3	0
	2	FC	2	0
	1	FE	1	0
	1	F	1	0
Total	1025		1023	2

In one case of disagreement the predicate device reported FA with an “unknown” peak in the S window while the test device reported FAS. Independent iso-electric focusing results identified FAS in support of the test device results.

FA and FAS		Test Device	
		FA	FAS
Predicate Device	FA	591	1
	FAS	0	247

In the other case of disagreement the predicate device reported FAD while the test device reported FADC with a 2.6% peak area of HbC. Inspection of the test device chromatogram showed a low broad background in the C window without a resolved peak suggesting baseline noise.

FAD and FADC		Test Device	
		FAD	FADC
Predicate Device	FAD	37	1
	FADC	0	0

2. Comparison studies (continued):

*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:

Not applicable

**N. Conclusion:**

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

