



**Bio-Rad  
Laboratories**

*Diagnostics Group  
4000 Alfred Nobel Drive  
Hercules, California 94547  
Telephone: 510724-7000  
Fax: 510741-5824*

**SUMMARY OF SAFETY AND EFFECTIVENESS**

**Submitter:** Bio-Rad Laboratories, Inc.  
Clinical Systems Division  
4000 Alfred Nobel Drive  
Hercules, California 94547  
Phone 1-510-741-6015  
FAX 1-510724-5024

*K944048*

**Contact Person:** John W. Nelson  
Manager, Regulatory Affairs

*NOV 20 1995*

**Date Prepared:**

**Product Trade Name:** Variant™ Hemoglobinopathy Program

**Common Name:** Variant Hemoglobin Testing System

**Classification Name:** Abnormal Hemoglobin Assay, 81GKA

The Variant Hemoglobinopathy Program is designed for use on the fully automated Variant analyzer. The analytical system consisting of instrument and reagent kit provides an assay for the detection of normal and abnormal hemoglobins by High Performance Liquid Chromatography, HPLC.

To establish substantial equivalence to an existing device, and thus establish the safety and effectiveness of the Variant hemoglobinopathy Program, the hemoglobinopathy program has been compared to the Isolab RESOLVE®-Hb test (K84001 1). A review of the intended use of each system shows them to be essentially the same. The intended use of the Variant Hemoglobinopathy Program is stated as: *An aid in the detection of normal and abnormal hemoglobins by High Pressure Liquid Chromatography.* The intended use of the Isolab RESOLVE®-Hb test is stated as: *Designed for the separation of hemoglobin variants in adult and neonatal blood hemolysates by isoelectric focusing electrophoresis.*

The Variant Hemoglobinopathy Program utilizes the principles of cation exchange high performance liquid chromatography (HPLC). A hemolysate is prepared by mixing whole blood with a hemolysis reagent included in the kit. The prepared hemolysate is placed into the instrument's auto sampler. The sampler injects a specified amount of hemolysate onto the cartridge. The separation of normal and abnormal hemoglobins is accomplished on the cation exchange cartridge using a linear buffer gradient. A dual wavelength photometer (415 and 690 nm) monitors the elution of the separated hemoglobins from the cartridge, detecting absorbance changes at 415 nm. The 690 nm secondary filter corrects the baseline for effects caused by the mixing of buffers of different ionic strengths as the gradient is formed.

**Retention** time windows (ranges) are established for the presumptive identification of normal and abnormal hemoglobin based on their characteristic retention times. The Variant **Hemoglobinopathy** Program utilizes a Retention Time Marker containing hemoglobins D, S, C, E/A<sub>2</sub>, A<sub>0</sub> and F. The initial retention times for the Variant **Hemoglobinopathy** Program Retention Time Marker were established using patient samples with confirmed normal and abnormal hemoglobins. In an assay, the retention times of the hemoglobins in the marker are **compared** to the retention times of the hemoglobins in a sample to determine the hemoglobin phenotype of the individual from whom the sample was obtained.

The **Isolab RESOLVE®-Hb** test utilizes isoelectric focusing (IEF) to separate the hemoglobins in a prepared **hemolysate**. Mixtures of low molecular weight amphoteric molecules with varying pi's are **contained** in an **agarose** gel matrix and generate a stable **pH** gradient when an electric field is applied. When a sample is placed in this **pH** gradient generated by an electric field, it will migrate towards the anode or cathode until it reaches its isoelectric point. Once this happens, diffusion is counteracted by the electric field, and hemoglobin variants appear as discrete **bands** at their different isoelectric points. After separation is complete the bands are fixed, stained and compared by "eye" to known hemoglobins run under the same conditions for **identification**.

The performance of the Variant **Hemoglobinopathy** Program was evaluated for precision, accuracy, and limits of detection. The precision studies were done according to **NCCLS** Vol. 12, No. 4, **EPS-T2, p31**, and are summarized below in Table 1. Accuracy was determined by a correlation study against the **Isolab RESOLVE®-Hb** test the results of which are shown below in Table 2. Table 3 compares the general characteristics of the two methods.

**Table 1  
Precision Study**

Sample Hemoglobin	Mean Retention Time (min.)	Within-Run %CV	Total %CV
F	6.32	0.3	0.9
A <sub>0</sub>	9.76	0.2	0.7
A <sub>2</sub>	<b>10.55</b>	<b>0.2</b>	<b>0.7</b>
E	<b>10.43</b>	<b>0.2</b>	<b>0.6</b>
D	<b>11.60</b>	<b>0.2</b>	<b>0.4</b>
S	12.00	0.2	0.4
C	15.55	0.2	0.4

**Table 2  
Accuracy Study**

Hemoglobin Phenotype	N	Hemoglobinopathy Program		Isolab Resolve	
		+	-	+	-
AA	71	71	0	71	0
AS	14	14	0	14	0
AC	13	13	0	13	0
AE	7	7	0	7	0
EE	3	3	0	3	0
SS	4	4	0	4	0
SC	2	2	0	2	0
AD	2	2	0	2	0
DS	1	1	0	1	0
AzAz	1	1	0	1	0
ACG <sup>Philadelphia</sup>	1	1	0	1	0
<b>Total</b>	<b>119</b>	<b>119</b>	<b>0</b>	<b>119</b>	<b>0</b>

Note: "+" denotes a positive identification, "-" denotes no hemoglobin detected.

**Table 3  
Comparison of General Characteristics**

Comparison of Methods		
Parameter	Variant Hemoglobinopathy Program	Isolab RESOLVE-Hb
Analytes	Normal and abnormal hemoglobins	Normal and abnormal hemoglobins
Measurement	Qualitative	Qualitative or quantitative using a densitometer
Basic Principle	Charge based separation using liquid chromatography	Charge based separation using isoelectric focusing electrophoresis
Instrumentation	Variant Hemoglobin Testing System	Isoelectric focusing electrophoresis unit
Analysis Medium	Cation exchange high performance liquid chromatography	Agarose gel electrophoresis
Temperature	25 °C	15 °C

Comparison of Methods (Continued)		
Parameter	Variant Hemoglobinopathy Program	Isolab RESOLVE-Hb
Sample	Whole Blood	Whole Blood
Sample Volume	5 $\mu$ l	25 $\mu$ l
Reagents	Elution Buffers (1&2), Analytical cartridge (column), Retention Time Marker/Diluent, Hemolysis Reagent	IEF Gels, Anode Solution, Cathode Solution, Sample Prep Reagent, IEF Electrode Wicks, Sample Application Template, Blotting Paper

It can be concluded from the correlation study between the **Bio-Rad Variant Hemoglobinopathy Program** and the **Isolab RESOLVE<sup>®</sup>-Hb** test, and the similarities of the general characteristics, that the two assays are substantially equivalent. Based on the establishment of substantial equivalence, the safety and effectiveness of the **Bio-Rad Variant Hemoglobinopathy Program** is confirmed.