VARIANT- HBA1C KIT 2.0 ASSAY

INTENDED USE

The Bio-Rad VARIANT™ II TURBO HbA1c Kit - 2.0 is intended for the determination of the percent of A1c using ion-exchange high-performance liquid chromatography (HPLC).

SUMMARY AND EXPLANATION OF THE TEST

The measurement of hemoglobin A1c (HbA1c) every two to three months is accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus. HbA1c is formed in two steps by the nonenzymatic glycation of HbA. The first step is formation of an unstable Labile- A1c, which is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the Labile- A1c is converted to form HbA1c.

PRINCIPLE OF THE PROCEDURE

The Bio-Rad VARIANT II TURBO HbA1c Kit - 2.0 utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). Samples are diluted on the Sampling Station (VSS) and injected into the analytical cartridge. The pumps in the Chromatographic Station (VCS), deliver a programmed buffer gradient of increasing ionic strength to the cartridge. The hemoglobins are separated based on their ionic interactions with the cartridge resin, and then pass through the flow cell where changes in absorbance at 415 nm are measured.

KIT COMPONENTS

Whole Blood Primer	Lyophilized human red blood cell hemolysate
Elution Buffer A	Each bottle contains 2500 mL of a sodium perchlorate buffer.
Elution Buffer B	Each bottle contains 2000 mL of a sodium perchloratebuffer.
Calibrator/Diluent Set	Calibrators contain lyophilized human red blood cell hemolysate. Calibrator Diluent contains 100 mL of deionized water with < 0.05% sodium azide as a preservative.
Kit CD-ROM	Contains VARIANT II TURBO HbA1c Kit - 2.0 test parameters.
Analytical Cartridge	Cation exchange cartridge (2500 tests)
Pre-filters	Five pre-filters (500 tests each)
Sample Vials	Microvials with pierceable caps, 1.5 mL

ADDITIONAL REQUIRED ITEMS

Wash/Diluent 2.0	Contains 2500 mL of deionized water with preservatives.
Tube Adapters	Used with microvial cups
Pipettes / DI water	5 µL, 0.5 mL, 1 mL, 1.5 mL, 7 mL
Protective Items	Gloves, Face shield

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PRECAUTIONS/WARNINGS

1. For in vitro diagnostic use.

2. Whole blood used in the manufacture of the calibrators and primer was tested by FDA accepted methods and found non-reactive for HIV-1, HIV-2, Hepatitis B (HBV), Hepatitis C (HCV), and syphilis.

3. Dispose of all waste in accordance with applicable national and/or local regulations.

4. Reagent buffers and Wash Diluent contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. When disposing, flush with large volumes of water to prevent azide buildup.

SPECIMEN COLLECTION AND HANDLING

SPECIMEN TYPE: EDTA Whole blood.

SPECIMEN STABILITY: 7 days at 2–8 °C or 1 day at room temperature (15–30 °C).

SPECIMEN PREPARATION:

1. No sample preparation is required. Gently mix the tubes prior to loading. Use special rack inserts for 12, 13, and 14 mm diameter tubes.

2. If the height of the sample in the tube is less than 25 mm, the sample must be prediluted 1:300 prior to analysis using a microvial sample tube.

PREPARATION AND STORAGE OF REAGENTS AND CARTRIDGES

1. Refer to the Calibrator and QC package inserts for value assignments, and ranges.

2. When changing to a new lot/shipment of reagents/cartridges, the corresponding kit CD-ROM must be installed to ensure optimum performance.

REAGENT	INSTRUCTIONS	STABILITY
Whole Blood Primer	Reconstitute vial with 1mL of DI water. Allow to stand 10 minutes. Swirl gently to dissolve. Reagent is interchangeable between lots.	1 day at 2-8 deg° C.
Calibrators (Two Levels)	Reconstitute vial with 7mL of cold calibrator diluent. Let stand 2 minutes; swirl to dissolve.	24 hours at 2-8º C. Do not freeze.

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Liquichek Diabetes Control (Two Levels)	Dilute 1:200 prior to analysis. (5 uL control in 1.0 of Wash/Diluent 2.0 solution.	14 days at 2-8º C.
Elution buffers A and B	Bring Buffers to room temp before use. Mix gently prior to installation. **See IMPORTANT NOTE following this table**.	Stable until bottle expiration if unopened and stored at 5-30º C. Open expiration: Buffer A : 30 days Buffer B: 90 days
Wash/Diluent	Reagent is interchangeable between lots. No preparation required.	Expiration: Manufacturer's dating.
Analytical Cartridge	Cartridge must be primed and calibrated after installation.	Stable 90 days or 2500 tests after installation. Store at 2-8 º C.
Pre-filter Adapter and Pre-Filter	Non-disposable Adapter is installed with black O-ring directed upward. The pre-filter is snapped over the adapter and is non-directional.	Pre-filter is stable for 500 injections. Store in refrigerator in the A1c supply basket.

NOTE: (1) Buffers are compatible within a resin lot. Buffer and cartridge labels are coded using alphabetical letters to indicate compatibility. A compatible set of buffers and cartridge will have the same letter code on each label. Do not use combinations of cartridges and buffers with different letter codes.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

• If reagents were frozen during shipment, mix by gently inverting before installation.
• Do not use if discolored, cloudy, precipitated, or if it shows signs of leakage.
• Do not use the calibrators or primers if the pellet is brown or the vial is broken. If the lyophilized material contains insoluble matter, discard.
• If the system overpressures due to excessive particulates (e.g., sample clots or precipitates), the cartridge pre-filter should be replaced.

QC REQUIREMENTS

Two levels of Quality Control material are performed at the start and end of each run. For very large batches, one level of QC may be included within the run every 50 samples.

In the presence of poor chromatography and/or baseline ramping within a run, stop the run, and perform troubleshooting. Do not continue running patient specimens unless you feel the issue has been resolved. When resolved, perform a look-back by reviewing and/or repeating affected samples.

If the QC expected control values are not obtained, stop the run, and perform troubleshooting. Perform a look-back for affected or suspect samples after the issue has been resolved by reviewing specimens which were performed after the last acceptable QC sample.

LIMITATIONS OF THE PROCEDURE

Sample Dilution

Normal total hemoglobin concentration corresponds to a total area of approximately 2.5 million µvolt•second. The required total area range for the VARIANT II TURBO HbA1c Kit - 2.0 is 1.0 million to 3.5 million µvolt•second. Specimens with area counts outside these limits should have appropriate dilutions made to achieve the acceptable limits.

Abnormal Red Cell Survival

Samples from patients with hemolytic anemias will exhibit decreased glycated hemoglobin values due to the shortened life span of the red cells. This effect will depend upon the severity of the anemia. Samples from patients with polycythemia or post-splenectomy may exhibit increased glycated hemoglobin values due to a somewhat longer life span of the red cells.

Hemoglobin Variants

●In a study to assess interference from hemoglobin variants, some patients with Hg AD-trait, Hg AS-trait, Hg AE-trait, Hg AC-trait, at the clinically significant levels of 6% and 9% HbA1c exhibited differences of more than ±10% from values obtained using a boronate affinity reference method. This should be taken into consideration when evaluating results from patients with a hemoglobin variant trait.

●NOTE: Hemoglobins E, D, and S elute in the Variant Window.

●In the rare homozygous forms of these hemoglobins (e.g., SS, CC), there is no HbA present; therefore, no HbA1c value can be determined.

●Other abnormal hemoglobin variants and their effects on the percent determination of HbA1c have not been evaluated on the VARIANT II TURBO HbA1c Kit - 2.0. For the positive confirmation of any particular hemoglobin variant, alternative separation methods are required.

REFERENCE RANGE INTERPRETATION

> 8 Action Suggested†
< 7 Goal‡
< 6 Non-Diabetic Level

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† High risk of developing long-term complications such as retinopathy, nephropathy, neuropathy, and cardiopathy.
Action suggested depends on individual patient circumstances.
‡ Some danger of hypoglycemic reaction in Type I diabetics. Some glucose intolerant individuals and "sub-clinical" diabetics may demonstrate (elevated) HbA1c in this area.

LINEARITY

HbA1c linearity from 3.9−19.0%

INTERFERING SUBSTANCES

• HEMOGLOBIN F: Concentrations up to 25% do not interfere with the assay. In cases where concentrations are greater than 25% (e.g., thalassemia syndromes and hereditary persistence of fetal hemoglobin), the %A1c should not be reported.
• LABILE A1C: Concentrations up to 6% do not interfere with the assay.
• CARBAMYLATED HGB: Concentrations up to 4% do not interfere with the assay.
• ICTERUS: Bilirubin concentrations up to 20 mg/dL, does not interfere with the assay.
• LIPEMIA: Triglyceride concentrations up to 6000 mg/dL, does not interfere with the assay.
• HEMOGLOBIN VARIANTS: In a study to assess interference from hemoglobin variants, some patients with Hg AD-trait, Hg AS-trait, Hg AE-trait, Hg AC-trait, at the clinically significant levels of 6% and 9% HbA1c exhibited differences of more than ±10% from values obtained using a boronate affinity reference method. This should be taken into consideration when evaluating results from patients with a hemoglobin variant trait.

CERTIFICATION / TRACEABILITY TO REFERENCE MATERIAL AND METHODS

The VARIANT II TURBO HbA1c Kit - 2.0 has been certified by the NGSP as having documented traceability to the Diabetes Control and Complications Trial reference method.

RESULT REPORTING

●The A1c result is reported as a percentage.
●The Estimated Glucose Mean (eAG) is calculated by Cerner.
●The Estimated Glucose Mean is invalid for specimens with A1c levels less than 6.0%.
●For specimens with A1c levels outside of reportable limits, the Estimated Glucose Mean is invalid.
●For specimens with Homozygous Variant Hemoglobins, the results are not reported. A footnote is appended to the test, which directs the physician to consider confirmatory testing for identification of the Variant by alternative hemoglobin separation techniques.
●Results are not reported for specimens with interfering hemoglobins above established cut-off limits, such as Hgb F, Carbamylated hemoglobin, Labile-A1c, and for heterozygous Variants. These specimens are sent to our reference laboratory (PAML) for re-testing using an alternate HPLC reagent platform.

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CERNER REPORTING

Workcenter/Testsite: 130/130

For specimens sent to PAML: TRT the sample to WC/TS: 950/975. Place a send-out label on the specimen cap and deliver to out-patient processing.

TECHNICAL ASSISTANCE

Call toll-free 1-800-2BIORAD (224-6723), available 24 hours a day, 7 days a week. Instrument serial number will be required (Located inside the door of the Variant Chromatography Station (VCS).

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