BIO-RAD

REF 220-0101

L20012105

D-10™

Hemoglobin A_{1c} Program

Instruction Manual

D-10™
Hemoglobin A_{1c} Program
Reorder Pack,
400 Tests

CE

IVD

EC REP ***UNITED STATES,*** *Bio-Rad Laboratories, Inc., Hercules, CA 94547*
FRANCE, *Bio-Rad, Marnes-la-Coquette*

Multiple-Language CD

This kit includes a multiple-language CD-ROM in the following languages:
English, German, French, Spanish, Italian, Portuguese, Swedish, Danish, Greek, and Norwegian.

In Vitro Diagnostic Directive (IVDD, 98/79/EC) Symbols

CE • European Conformity • EG-Konformität • Conformité européenne • Conforme a la normativa europea • Conformità Europea • Conformidade com as normas europeias • Uppfyller EU-direktiv • CE-mærkning • Συμμόρφωση με τα ευρωπαϊκά πρότυπα • Europeisk samsvar	 • Manufacturer • Hersteller • Fabricant • Fabricante • Produttore • Fabricante • Tillverkare • Producent • Κατασκευαστής • Produsent	EC REP • Authorized Representative in the European Union • Autorisierter Vertreter in der Europäischen Union • Représentant agréé pour l'Union Européenne • Representante Autorizado en la Unión Europea • Rappresentante autorizzato per l'Unione Europea • Representante Autorizado da União Europeia • Auktoriserad EU-representant • Autoriseret repræsentant i EU • Εξουσιοδοτημένος αντιπρόσωπος στην Ευρωπαϊκή Ένωση • Autorisert representant i EU
LOT • Lot Number • Chargenbezeichnung • Numéro de lot • Número de lote • Numero di lotto • Número do lote • Batchnummer • Lotnummer • Αριθμός παρτίδας • Lotnummer	 • Use by • Haltbar bis • Utiliser avant • Fecha de caducidad • Scadenza • Utilizar até • Användes före • Udløbsdato • Χρήση έως • Utløpsdato	IVD • For In Vitro Diagnostic Use • In-vitro-Diagnostikum • Utilisation comme test de diagnostic in vitro • Para uso en diagnóstico in vitro • Per uso diagnostico in vitro • Para uso em diagnóstico in vitro • För in vitro-diagnostiskt bruk • Til in vitro-diagnostisk brug • Για in vitro διαγνωστική χρήση • Til in vitro-diagnostisk bruk
 • Temperature Limit • Temperaturgrenze • Limite de température • Límite de temperatura • Limite di temperatura • Limite de temperatura • Temperaturgränser • Temperaturområde • Όριο θερμοκρασίας • Temperaturgrenser	REF • Catalog Number • Katalognummer • Référence • Número de catálogo • Numero di catalogo • Número de catálogo • Katalognummer • Katalognummer • Αριθμός καταλόγου • Katalognummer	 • Consult Instructions for Use • Gebrauchsanleitung beachten • Consulter la notice d'utilisation • Consulte las instrucciones de uso • Fare riferimento alle Istruzioni per l'uso • Consulte as instruções de utilização • Se bruksanvisning • Se brugsvejledningen • Συμβουλευθείτε τις οδηγίες χρήσης • Se bruksanvisning
DI H_2O • Deionized Water • Deionisiertes Wasser • Eau déionisée • Agua desionizada • Acqua deionizzata • Água desionizada • Avjoniserat vatten • Deioniseret vand • Απιονισμένο νερό • Deionisert vann	USE • For use with • Zur Verwendung mit • À utiliser avec • Uso con • Da usarsi con • Para utilizar com • För användning med • Til anvendelse sammen med • Για χρήση με • For bruk med	RECON • Reconstitute with • Rekonstitution mit • Reconstituer avec • Reconstituir con • Ricostituire con • Reconstituir com • Rekonstituera med • Rekonstitueres med • Ανασύσταση με • Rekonstitueres med

In Vitro Diagnostic Directive (IVDD, 98/79/EC) Symbols

ANLT CRTR	BUF 1	BUF 2	CAL DIL
• Analytical Cartridge • Analytische Kartusche • Cartouche analytique • Cartucho de análisis • Cartuccia analitica • Coluna analítica • Analyskolonn • Analysekolonne • Αναλυτική μικροστήλη • Analysekolonne	• Elution Buffer 1 • Elutionspuffer 1 • Tampon d'élution 1 • Tampón de elución 1 • Tampone di eluizione 1 • Tampão de eluição 1 • Elueringsbuffert 1 • Elueringsbuffer 1 • Ρυθμιστικό διάλυμα έκλουσης 1 • Elueringsbuffer 1	• Elution Buffer 2 • Elutionspuffer 2 • Tampon d'élution 2 • Tampón de elución 2 • Tampone di eluizione 2 • Tampão de eluição 2 • Elueringsbuffert 2 • Elueringsbuffer 2 • Ρυθμιστικό διάλυμα έκλουσης 2 • Elueringsbuffer 2	• Calibrator Diluent • Kalibratorverdünnungslösung • Diluant des étalons • Diluyente del calibrador • Diluente del calibratore • Diluente Calibrador • Spädningsvätska för kalibrator • Kalibratorfortynder • Αραιωτικό μέσο βαθμονομητή • Kalibratordiluent
CAL DIL SET	**CAL 1**	**CAL 2**	**CAL SET**
• Calibrator/Diluent Set • Kalibrator-/Diluent-Set • Gamme étalon / diluant • Juego de calibradores/diluyente • Set del calibratore/diluente • Conjunto calibrador/diluente • Kalibrator/spädningsset • Kalibrator/fortyndingssæt • Σετ βαθμονομητή/ αραιωτικού μέσου • Kalibrator-/diluentsett	• Calibrator Level 1 • Kalibrator Level 1 • Étalon de niveau 1 • Calibrador Nivel 1 • Calibratore livello 1 • Calibrador Nível 1 • Kalibrator, nivå 1 • Kalibrator niveau 1 • Βαθμονομητής επιπέδου 1 • Kalibratornivå 1	• Calibrator Level 2 • Kalibrator Level 2 • Étalon de niveau 2 • Calibrador Nivel 2 • Calibratore livello 2 • Calibrador Nível 2 • Kalibrator, nivå 2 • Kalibrator niveau 2 • Βαθμονομητής επιπέδου 2 • Kalibratornivå 2	• Calibrator Set • Kalibratorset • Gamme étalon • Juego de calibradores • Set del calibratore • Conjunto calibrador • Kalibratorset • Kalibratorsæt • Σετ βαθμονομητή • Kalibratorsett
DISK	**RESIN**	**SAMP VIAL**	**SODIUM AZIDE**
• Floppy Diskette • Diskette • Disquette informatique • Disquete • Dischetto • Disquete • Diskett • Diskette • Δισκέτα • Diskett	• Resin • Harz • Résine • Resina • Resina • Resina • Resin • Resin • Ρητίνη • Resin	• Sample Vials • Probengefäße • Microtubes échantillons • Viales • Microprovette per campione • Frascos de amostras • Provrör • Prøvekopper • Φιαλίδια δείγματος • Prøveglass	• Sodium Azide • Natriumazid • Azide de sodium • Azida sódica • Sodio azide • Azida de sódio • Natriumazid • Natriumazid • Αζίδιο του νατρίου • Natriumazid
WSH DIL SOLN	**WB PRM**	**<0.05 %**	**Σ**
• Wash/Diluent Solution • Wasch-/Verdünnungslösung • Solution de lavage/dilution • Solución de lavado/diluyente • Soluzione di lavaggio/diluizione • Solução de lavagem/diluente • Tvätt-/spädningsvätska • Vaske-/fortyndingsreagens • Διάλυμα έκπλυσης/αραίωσης • Vaske-/diluentløsning	• Whole Blood Primer • Vollblut-Primer • Sang total de conditionnement • Cebador de sangre total • Primer di sangue intero • Iniciador de sangue total • Helblodsprimer • Fuldblodsprimer • Εκκινητής ολικού αίματος • Fullblodsprimer	• <0.05% • <0,05 % • <0,05 % • <0,05% • <0,05% • <0,05% • <0,05 % • <0,05 % • <0,05% • <0,05 %	• Number of Tests • Anzahl der Tests • Nombre de tests • Número de pruebas • Numero di analisi • Número de testes • Antal tester • Antal test • Αριθμός δοκιμασιών • Antall tester

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INTENDED USE

The Bio-Rad D-10™ Hemoglobin A_{1c} Program is intended for the percent determination of hemoglobin A_{1c} in human whole blood using ion-exchange high-performance liquid chromatography (HPLC). The Bio-Rad D-10 Hemoglobin A_{1c} Program is intended for Professional Use Only.

For In Vitro Diagnostic Use.

SUMMARY AND EXPLANATION OF THE TEST

Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and therefore, blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, either the pancreas does not make enough insulin or the body is unable to use insulin correctly.[1] The direct and indirect effects of hyperglycemia on the human vascular system are the major source of morbidity and mortality in both Type 1 and Type 2 diabetes. These effects include macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy).[2] Diabetes mellitus affects approximately 7% of the world population.[3]

Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to a normal level, minimizing the risk of long-term vascular consequences.[4,5] A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours), but may not represent the true status of blood glucose regulation.[6,7] The measurement of hemoglobin A_{1c} (HbA_{1c}) every two to three months has been accepted as a measure of glycemic control in the care and treatment of patients with diabetes mellitus.

HbA_{1c}, the glycohemoglobin of interest, is formed in two steps by the nonenzymatic glycation of HbA. The first step is the formation of an unstable aldimine (labile A_{1c}, or pre-A_{1c}), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A_{1c} formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A_{1c} is converted (Amadori rearrangement) to form a stable ketoamine, HbA_{1c}.[8]

The D-10 Hemoglobin A_{1c} Program is based on chromatographic separation of HbA_{1c} on a cation exchange cartridge. Separation is optimized to minimize interferences from hemoglobin variants, labile A_{1c}, and carbamylated hemoglobin. Please refer to *Limitations of the Procedure* and *Performance Characteristics* for more information. The D-10 Hemoglobin A_{1c} Program also offers automatic sampling from a primary whole blood tube, followed by sample dilution, and an analysis time of three minutes per sample.

PRINCIPLE OF THE PROCEDURE

The D-10 Hemoglobin A_{1c} Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-10 and injected into the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobins then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured.

The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantitation of the HbA_{1c} values. A sample report and a chromatogram are generated for each sample. The A1c peak is shaded. This area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A_{1c} and carbamylated peak areas from the A1c peak area.

The D-10 Hemoglobin A_{1c} Program is for use only with the Bio-Rad D-10 Hemoglobin Testing System.

KIT COMPONENTS

REF 220-0101, D-10 Hemoglobin A_{1c} Reorder Pack

The reorder pack contains supplies for 400 tests:

REF	*Description*
220-0110*	**Elution Buffer 1.** Two bottles containing 2000 mL of a Bis-Tris/Phosphate buffer, pH 6.0. Contains <0.05% sodium azide as a preservative.
220-0111*	**Elution Buffer 2.** One bottle containing 1000 mL of a Bis-Tris/Phosphate buffer, pH 6.7. Contains <0.05% sodium azide as a preservative.
220-0112*	**Wash/Diluent Solution.** One bottle containing 1600 mL of deionized water with <0.05% sodium azide as a preservative.
220-0113*	**Analytical Cartridge.** One cation exchange cartridge, 4.0 mm ID x 30 mm.
220-0115*	**Floppy Diskette** with D-10 Hemoglobin A_{1c} Program parameters.
220-0118*	**Calibrator/Diluent Set.** One set consisting of three vials of Calibrator Level 1, three vials of Calibrator Level 2, and one bottle of Calibrator Diluent. The calibrator vials contain lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Calibrator Diluent contains 100 mL of deionized water with <0.05% sodium azide as a preservative.
220-0148*	**Whole Blood Primer.** Four vials of lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 1.0 mL per vial.
220-0149	**Sample Vials.** 100 polypropylene vials with pierceable caps, 1.5 mL.
	Thermal Paper. One roll.

Components are not available for individual sale.

REF 220-0109, D-10 Hemoglobin A_{1c} Supplemental Reagent Pack

The reagent pack is used as a supplement to the D-10 Hemoglobin A_{1c} Reorder Pack, providing low-test-volume users with buffers and wash/diluent solution to achieve 400 tests.

REF	*Description*
220-0110*	**Elution Buffer 1.** Two bottles containing 2000 mL of a Bis-Tris/Phosphate buffer, pH 6.0. Contains <0.05% sodium azide as a preservative.
220-0111*	**Elution Buffer 2.** One bottle containing 1000 mL of a Bis-Tris/Phosphate buffer, pH 6.7. Contains <0.05% sodium azide as a preservative.
220-0112*	**Wash/Diluent Solution.** One bottle containing 1600 mL of deionized water with <0.05% sodium azide as a preservative.

Components are not available for individual sale.

NOTE: *The D-10 calculates buffer levels and waste level assuming test volumes of 200 or more tests per month and 4 or more samples per run. Low-test-volume users will run out of buffers and fill the external waste tank before the applicable warning messages are displayed. Visually check the buffer levels and waste tank level prior to each run.*

ADDITIONAL ITEMS, AVAILABLE FROM BIO-RAD

REF	*Description*
220-0297	**Sample Vial Adapter**, 10 x 1.5 mL
220-0375	**D-10 Thermal Paper**, 10 rolls
740	**Lyphochek® Diabetes Bi-level Control**, 6 x 0.5 mL
120	**Lyphochek® Hemoglobin A_{1c} Linearity Set (1 each of 4 levels)**, 4 x 0.5 mL
171	**Liquichek™ Diabetes Control, Level 1,** 6 x 1.0 mL
172	**Liquichek™ Diabetes Control, Level 2,** 6 x 1.0 mL
173	**Liquichek™ Diabetes Control, Level 3,** 6 x 1.0 mL
172X	**Liquichek™ Diabetes Control, Trilevel MiniPak,** 3 x 1.0 mL

ADDITIONAL REQUIRED ITEMS, NOT AVAILABLE FROM BIO-RAD

Pipettes, 5 µL, 0.5 mL, 1 mL, 7 mL
Deionized Water
Disposable Gloves

PRECAUTIONS/WARNINGS

1. For in vitro diagnostic use.
2. Wear personal protective equipment while handling all reagents and samples and while operating the D-10 system.
3. Dispose of all waste in accordance with applicable national and/or local regulations.
4. Some reagents contain sodium azide, which may react with copper or lead plumbing to form explosive metal azides. Use caution in disposing of these reagents. If disposing to drain, flush with large volumes of water to prevent azide buildup.
5. Waste material containing patient samples or biological products should be considered biohazardous when disposing or treating.
6. Chemical reagents should be handled in accordance with Good Laboratory Practices.
7. Clean up all spills immediately and thoroughly. Disinfect the area for any spills involving biohazardous materials. Dispose of all contaminated materials appropriately.
8. Do not interchange vial or bottle caps and stoppers; this will lead to cross-contamination of reagents.
9. Never mix the contents from different bottles of the same reagent. Doing so may lead to reagent contamination and compromise the performance of the product.
10. 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. No test method can offer complete assurance that products containing human source materials will be absent of these and other infectious agents. In accordance with good laboratory practice, all human source material should be considered potentially infectious for all infectious agents; therefore, handle the calibrators and whole blood primer with the same precautions used with patient specimens.
11. Adherence to the protocol specified herein is necessary to ensure proper performance of this product.
12. The Calibrator vial stoppers contain dry natural rubber.
13. Do not use the Calibrator Diluent for prediluting patient samples.

SPECIMEN COLLECTION AND HANDLING

Specimen Type

Whole blood.

Specimen Collection Precaution

Consider any materials of human origin as infectious and handle them using typical biosafety procedures.

Specimen Additives, Preservatives

The whole blood specimens should be collected in a vacuum collection tube containing EDTA.

Specimen Storage

Whole blood specimens may be stored up to 7 days at 2–8 °C or 3 days at room temperature (15–30 °C).

Specimen Preparation

1. Allow sample tubes to reach room temperature (15–30 °C) before performing the assay. No sample preparation is required. Mixing has no impact on HbA_{1c} value as long as the total area is in range. The sample tubes are loaded into the D-10 sample rack and placed in the D-10. Ensure that the sample barcodes are facing towards the back of the instrument. Use special rack inserts for 12, 13, and 14 mm diameter tubes. Remove all inserts for 16 mm diameter tubes. Tubes with a height of 75 mm to 100 mm are acceptable for use.
2. If the sample is in an abnormal size/type tube, or if there is less than 2.0 mL of sample in the tube, then the sample must be prediluted. Before pipetting, thoroughly mix the sample by gently inverting the tube. To predilute, pipet 1.5 mL of Wash/Diluent Solution into a labeled 1.5 mL vial, followed by 5 µL of the whole blood sample. Cap the sample vial and mix thoroughly. Use a sample vial adapter for 1.5 mL vials.

NOTE: *Studies indicate that the use of MONOJECT™ (REF 8881311446, Covidien, Mansfield, MA, 02048 USA) collection tubes on the D-10 Hemoglobin Testing System can result in elevated total area. In some cases, blood collects under the cap and causes oversampling, resulting in high total area that exceeds the acceptable range. To use this tube type, users must either manually dilute the sample or ensure that blood does not collect under the cap before loading the sample on the D-10 Hemoglobin Testing System. Results with total area within the acceptable range are reportable.*

Specimen Shipping

All samples of human origin must be shipped in accordance with national and international transportation regulations.

PREPARATION AND STORAGE OF REAGENTS

Refer to the insert included with the current lot of calibrators and controls for value assignment and ranges. When changing to a different lot of reagents and/or cartridge, the parameters from the matching floppy diskette must be installed to ensure optimum performance of the program.

To install or change Elution Buffers, Wash/Diluent Solution, and Analytical Cartridge, follow the procedures described in the *D-10 Operation Manual, Section 4.2*.

Elution Buffers and Wash/Diluent Solution

1. Allow the Elution Buffers and Wash/Diluent Solution to reach room temperature (15–30 °C) before performing the assay. Mix each bottle by gently inverting prior to installation.
2. The Elution Buffers and Wash/Diluent Solution will be stable until the expiration date when stored unopened at 15–30 °C. After opening the bottles, these reagents are stable for 8 weeks when stored at 15–30 °C.
3. With a new reorder pack, install one bottle of each reagent and follow the procedure for *Installing a New Reorder Pack Lot* in the *Procedure* section. After 200 injections, install a fresh bottle of Elution Buffer 1. Reset the volume in the LOT INFO/Buffer 1 screen after installing this reagent.

   NOTE: *When using the optional D-10 Rack Loader, the two bottles of Elution Buffer 1 are installed simultaneously. Manually resetting the volume is not required.*
4. The Wash/Diluent Solution is interchangeable between Reorder Pack lots.

Whole Blood Primer

Use fresh aliquots of Whole Blood Primer when installing a new cartridge.

1. The Whole Blood Primer will be stable until the expiration date when stored unopened at 2–8 °C.
2. Prepare the Whole Blood Primer by adding 1 mL of deionized water to the vial.
3. Allow to stand for 10–15 minutes at 15–30 °C.
4. Swirl gently to dissolve and ensure complete mixing.
5. Write the reconstitution date on the label. The reconstituted Whole Blood Primer is stable for 1 day when stored at 2–8 °C.
6. The Whole Blood Primer is interchangeable between lots.

Hemoglobin A_{1c} Calibrators

Reconstitute and store the HbA_{1c} Calibrators as directed in the *Calibrator/Diluent Set Insert*.

Extracted Standards

This HPLC method does not use extracted standards.

Controls

- Reconstitute and store the controls according to the manufacturer's package insert.
- Bio-Rad Lyphochek Diabetes Controls must be diluted 1:300 prior to analysis. Pipet 1.5 mL of Wash/Diluent Solution into a labeled 1.5 mL vial, followed by 5 µL of the reconstituted control. Cap each control vial and mix thoroughly.
- Bio-Rad Liquichek Diabetes Controls must be diluted 1:200 prior to analysis. Pipet 1.0 mL of Wash/Diluent Solution into a labeled 1.5 mL vial, followed by 5 µL of the control. Cap each control vial and mix thoroughly.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- If reagents were frozen during shipment, mix each bottle by gently inverting before installing on instrument.
- Do not use any reagents which have any indications of discoloration, cloudiness or precipitation.
- Do not use any reagents that show any signs of leakage.
- Do not use the calibrator or whole blood primer if the pellet is brown or the vial is broken. If the lyophilized material contains insoluble matter, discard the material and reconstitute a new vial.

PROCEDURE

Method Selection

From the LOT INFO screen:

- Press METHOD on the touch screen.
- Select the desired method (HbA_{1c}).
- Press EXIT.
- Press YES to confirm the method change.
- Press EXIT.
- The selected method is indicated to the left of the status bar.

There is no need to perform a system flush unless you are installing a new lot of reagents.

Installing a New Reorder Pack Lot (Update Kit Floppy Diskette)

- Go to the LOT INFO screen.
- Press the UPDATE KIT button.
- Place the UPDATE KIT floppy diskette in the A:\drive.
- Follow the instructions on the screen to proceed with the *Update Kit Procedure*.
- Remove the floppy diskette from the A:\drive once the procedure is completed.

Analytical Cartridge Priming Procedure

- Pipet 1 mL of reconstituted Whole Blood Primer into a sample vial. Put the sample vial into a sample vial adapter labeled with a Primer barcode, then place the adapter into sample rack position 1.
- Start a run. When finished, the cartridge is ready for calibration.

Calibration

Calibration must be performed once, following the installation and priming of every new analytical cartridge. Additional calibration may be performed at the discretion of the laboratory.

- Prepare samples as described in *Specimen Preparation* section.
- Place calibrators and controls on the D-10. Use sample vial adapters for 1.5 mL vials with calibrators and controls. The adapters should have barcodes identifying sample type.

Sample order with calibrators:

Sample #	Reagent	Adapter
1	HbA_{1c} Calibrator, Level 1	Calibrator 1
2	HbA_{1c} Calibrator, Level 2	Calibrator 2
3	Control, Level 1	A_{1c} Low Control
4	Control, Level 2	A_{1c} High Control
5–10	Patient Samples	

NOTE: *The analyte values printed on the calibrator reports are the calibrator assigned values. In the case of calibration failure, the reports will indicate these assigned values; however, the statement "Calibration Failed" will be printed at the bottom of the calibrator report. If the alert setting "Stop if calibration fails" is NOT selected (see Section 2.4.4 of the D-10 Operation Manual), the run will continue, using the calibration factors from the previous acceptable calibration run. For troubleshooting advice, refer to Section 6.1 of the D-10 Operation Manual.*

Routine Run

Once the cartridge has been calibrated, use the following run configuration. Refer to *QC Requirements* for information on control sample frequency.

Sample order without calibrators:

Sample #	Reagent
1	Control, Level 1
2	Control, Level 2
3–10	Patient Samples

Installing a Supplemental Reagent Pack

- When installing reagents from the Supplemental Reagent Pack, Buffer 1 and Buffer 2 must always be used as a matched set. Never use a buffer bottle from the Reorder Pack with a buffer bottle from the Supplemental Reagent Pack.
- Users will need to reset the buffer volume when new bottles are installed. (Refer to *Section 2.4.5, LOT INFO Screen,* in the *D-10 Operation Manual*.)
- The Buffer and Wash/Diluent lot and expiration information must be manually entered into the system.
- When installing the second bottle of Buffer 1, reset the Buffer 1 volume.

Certification/Traceability to Reference Material and Method

The D-10 Hemoglobin A_{1c} Program is traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The D-10 Hemoglobin A_{1c} Program is certified by the NGSP as having documented traceability to the Diabetes Control and Complications Trial (DCCT) reference method. The purpose of the NGSP is to standardize glycohemoglobin test results so that clinical laboratory results are comparable to those reported in the DCCT, where relationships to mean blood glucose and risk for vascular complications have been established.[9]

The IFCC Working Group on HbA_{1c} Standardization developed and maintains the reference measurement procedure for HbA_{1c} to be used as the analytical anchor for traceability of HbA_{1c}. The IFCC reference method separates the glycated and non-glycated N-terminal peptides of the β-chain by reversed-phase HPLC and quantifies the analyte by mass spectrometry or capillary electrophoresis.[10]

The IFCC reference method is used to assign IFCC values to secondary reference materials. These materials are used by manufacturers to assign values to product calibrators.[11]

HbA_{1c} results have traditionally been reported in either conventional percent (100 × HbA_{1c}/total Hb) or in SI percent units (HbA_{1c}/total Hb). In May 2007, the American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and IFCC issued a consensus statement on the worldwide standardization of the HbA_{1c} measurement. They recommended use of the IFCC SI units (mmol/mol).[12] To implement the use of the newly accepted SI units, the originally published master equations between the IFCC reference and the designated comparison methods have been modified as follows:

Master Equation for Designated Comparison Method (DCM):

DCM	Conversion from IFCC to DCM[10]
NGSP (USA)	NGSP = (0.09148 × IFCC) + 2.152

Examples of Patient Results:

IFCC	NGSP
43 mmol/mol	6.1%
48 mmol/mol	6.5%
53 mmol/mol	7.0%

QC Requirements

In keeping with good laboratory practice, diabetic and non-diabetic control specimens should be included in the run once per 24 hours. A repeat run is indicated when expected control values are not obtained.

GUIDELINES FOR THE INTERPRETATION OF RESULTS

Observe the following guidelines to assure acceptable results:

1. The D-10 has passed calibration. For your reference, the slope and intercept acceptable ranges are provided in the *D-10 Hemoglobin A_{1c} Program Calibrator/Diluent Set Insert*.
2. Total area of each analysis should range from 1.0 to 5.0 million µvolt • second. Results should not be reported if the area is outside this range.
3. The peaks are correctly identified. For your reference, the analyte retention time windows are provided in the *D-10 Hemoglobin A_{1c} Analytical Cartridge Insert*.
4. Quality Control values are in range.
5. The reportable range for HbA_{1c} was established based on data presented in *Performance Characteristics, Linearity/Recovery*. If the HbA_{1c} result falls outside the reportable range, it should not be reported.

	Reportable Range
NGSP % HbA_{1c}	3.8–18.5
IFCC mmol/mol HbA_{1c}	18–179

6. Any sample with >15% or >140 mmol/mol HbA_{1c} should be suspected of having a hemoglobin variant.[13]
7. Any sample with a combined area of ≥60% in the Variant, S, and C windows should be suspected of having a homozygous variant or a variant–β-thalassemia phenotype. The HbA_{1c} result should not be reported for these samples.[14]

P3 Peak Resolution

The P3 peak may split due to improved resolution. When this occurs, an "Unknown" peak will be listed in the peak table following the P3 peak (see Figure 4). The presence of this "Unknown" peak has no effect on the HbA_{1c} quantitation.

LIMITATIONS OF THE PROCEDURE

Sample Dilution

Normal total hemoglobin concentration corresponds to a total area of approximately 2.5 million µvolt•second. Low, medium, and high whole blood patient samples were diluted to achieve total areas from 1.0 to 5.0 million µvolt•second. These samples were run on the D-10 Hemoglobin A_{1c} Program to confirm that the total area does not affect the result. The recommended total area range is from 1.0 to 5.0 million µvolt•second.

If the sample area is outside of the expected range, manually predilute the sample following the *Specimen Preparation* guidelines.

If the sample area is still outside of the expected range, the sample should be rediluted to within the 1.0 to 5.0 million total area count range and rerun.

NOTE: *For some high total area, high HbA_{1c} samples (e.g., 15% or 140 mmol/mol HbA_{1c} with 5 M total area), the A1c peak may elute outside of the established retention time window. Predilute the sample to approximately 2.5 M total area and rerun.*

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.[15]

Hemoglobin Variants

HbA_{1c} values determined using the D-10 Hemoglobin A_{1c} Program for HbS trait and HbC trait specimens showed no clinically significant difference from values determined by an NGSP certified boronate affinity method. Typical chromatograms for HbS and HbC trait specimens are provided in Figures 7 and 8. In the rare homozygous forms (SS or CC), there is no HbA present; therefore, no HbA_{1c} value can be determined.

Other abnormal hemoglobin variants have not been evaluated on the D-10 Hemoglobin A_{1c} Program. For the positive confirmation of any particular hemoglobin variant, alternative separation methods are required.

REFERENCE VALUES

Hemoglobin A_{1c} Ranges[16]

The following HbA_{1c} ranges may be used for interpretation of results; however, factors such as duration of diabetes, adherence to therapy, and the age of the patient should also be considered in assessing the degree of blood glucose control. These values are for nonpregnant individuals. "Action Suggested" depends on individual patient circumstances. Such actions may include enhanced diabetes self-management education, co-management with a diabetes team, referral to an endocrinologist, change in pharmacological therapy, initiation or increased self-monitoring of blood glucose, or more frequent contact with the patient.

Hemoglobin A_{1c} (%)	Degree of Glucose Control
> 8	Action Suggested†
< 7	Goal‡
< 6	Non-Diabetic Level

† *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) HbA_{1c} in this area.*

Non-Diabetic Reference Interval

The Third National Health and Nutrition Examination Survey in the U.S. included subjects 20 years of age or older with normal fasting plasma glucose and with no previous diagnosis of diabetes. HbA_{1c} values were measured on the Bio-Rad DIAMAT™ System.

The weighted mean HbA_{1c} for patients with normal fasting plasma glucose (n = 5,694) was 5.17% with a standard deviation of 0.45%. The 95% confidence limits (mean ± 2SD) were 4.27–6.07% HbA_{1c}.[9]

Since the D-10 Hemoglobin A_{1c} Program is certified by the NGSP, this reference interval could be used as a point of reference until the laboratory has analyzed a sufficient number of samples to determine its own non-diabetic and diabetic reference intervals representative of the regional population being tested.

PERFORMANCE CHARACTERISTICS

Precision

The precision of the D-10 Hemoglobin A_{1c} Program was evaluated in a study based on the NCCLS EP5-T2 guideline "Evaluation of Precision Performance of Clinical Chemistry Devices" as adapted by the NGSP for use in the certification of glycohemoglobin methods. In this study, 40 runs were performed on one D-10 System over 20 working days. In each run, aliquots of normal and diabetic specimens were analyzed in duplicate. The results of the precision study are summarized in Table 1.

	Normal Patient	Diabetic Patient
Mean (% HbA_{1c})	5.7	9.4
Within Run (% CV)	0.78	0.46
Between Day (% CV)	0.68	0.99
Between Run (% CV)	0.52	0.53
Total Precision (% CV)	1.16	1.22

Table 1. Results of Precision Study.

Accuracy

The D-10 Hemoglobin A_{1c} Program was compared to the VARIANT™ II Hemoglobin A_{1c} Program (REF 270-2101). A comparison study was performed on 40 patient samples. See Figure 1.

slope = 0.9743
intercept = 0.3078
R^2 = 0.9945

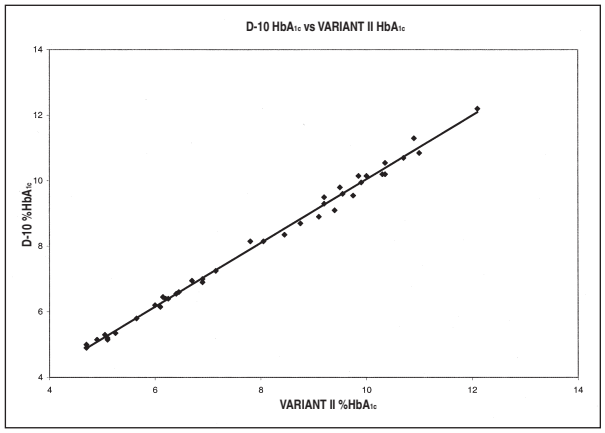


Figure 1. Correlation: D-10 Hemoglobin A_{1c} Program vs. VARIANT II Hemoglobin A_{1c} Program (REF 270-2101)

Linearity/Recovery

To demonstrate linearity of HbA_{1c} measurement on the D-10 Hemoglobin A_{1c} Program throughout the reportable range, a normal and a diabetic HbA_{1c} specimen were derived as follows:

Normal: Whole blood from a normal patient sample was supplemented with Bio-Rad Lyphochek Hemoglobin A_{1c} Linearity Set Level 1 to yield relative HbA_{1c} level of 3.8%.

Diabetic: Whole blood from a diabetic patient sample was supplemented with Bio-Rad Lyphochek Hemoglobin A_{1c} Linearity Set Level 4 to yield relative HbA_{1c} level of 18.5%.

The diabetic specimen was diluted with the normal specimen in varying ratios to yield specific relative HbA_{1c} percentages (theoretical percent HbA_{1c}). These diluted samples were analyzed with the D-10 Hemoglobin A_{1c} Program (observed percent HbA_{1c}). Percent recovery was determined by dividing the observed percent HbA_{1c} by the theoretical percent HbA_{1c} and multiplying the result by 100. Results from the Linearity Study are shown in Table 2.

% Contribution		Theoretical % HbA1c	Observed % HbA1c	% Recovery
Normal	Diabetic			
100	0	3.8	3.8	100
80	20	6.6	6.6	100
67	33	8.6	8.5	99
50	50	11.0	11.0	100
33	67	13.5	13.2	98
20	80	15.4	15.2	99
0	100	18.5	18.5	100

Table 2. Results of Study on Linearity and Recovery.

Interfering Substances

- Icterus, as indicated by bilirubin concentrations up to 20 mg/dL, does not interfere with the assay.
- Lipemia, as indicated by triglyceride concentrations up to 5680 mg/dL, does not interfere with the assay.
- Hemoglobin F concentrations up to 10% do not interfere with the assay.
- Labile A_{1c} (LA1c/CHb-1) concentrations up to 4% do not interfere with the assay.
- Carbamylated hemoglobin (LA1c/CHb-2) concentrations up to 3.5% do not interfere with the assay.

SAMPLE REPORT FORMAT

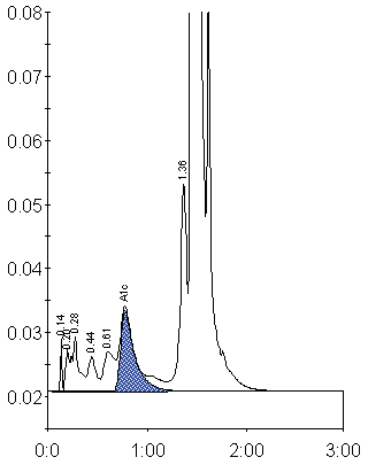
Several of the following sample report examples include both IFCC (mmol/mol) and NGSP (%) units for HbA_{1c}. Beginning with D-10 software version 3.60, your lab's preferred reporting units for standardized HbA_{1c} results (i.e., NGSP only or NGSP and IFCC) can be configured in the D-10 Service Software by a Bio-Rad representative.

NOTE: *The sample report examples with the HbA_{1c} result in % only were generated using D-10 software version 3.50.*

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 09/23/2010
D-10	TIME: 09:16 AM
S/N: #DA3G222606	Software version: 3.57
Sample ID:	RACKB3-3-23-22-9-2010
Injection date	09/22/2010 11:45 AM
Injection #: 23	Method: HbA1c
Rack #: B3	Rack position: 3



Peak table - ID: RACKB3-3-23-22-9-2010

Peak	R.time	Height	Area	Area %
Unknown	0.14	8245	9082	0.3
A1a	0.20	6676	17769	0.5
A1b	0.28	8455	40358	1.2
F	0.44	5438	30425	0.9
LA1c/CHb-1	0.61	6180	46070	1.4
A1c	0.78	12575	125339	5.1
P3	1.36	32535	149519	4.5
A0	1.45	734587	2909750	87.4
Total Area:	3328311			

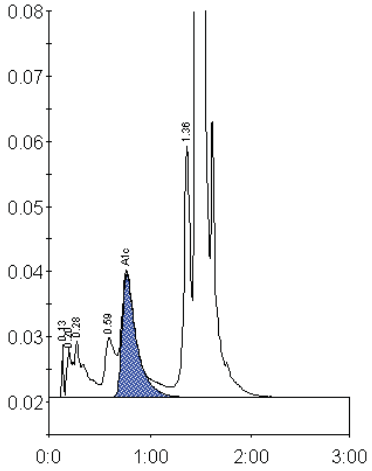
Concentration:	%	mmol/mol
A1c	5.1	32

Figure 2. Non-Diabetic (Normal) Sample

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 09/22/2010
D-10	TIME: 04:53 PM
S/N: #DA3G222606	Software version: 3.57
Sample ID:	RACK03-7-19-20-9-2010
Injection date	09/20/2010 11:52 AM
Injection #: 19	Method: HbA1c
Rack #: 03	Rack position: 7



Peak table - ID: RACK03-7-19-20-9-2010

Peak	R.time	Height	Area	Area %
Unknown	0.13	8151	9565	0.4
A1a	0.20	7532	21104	0.8
A1b	0.28	8670	69405	2.6
LA1c/CHb-1	0.59	9253	66063	2.5
A1c	0.77	18925	192773	9.2
P3	1.36	38585	176994	6.6
A0	1.46	530542	2149611	80.0
Total Area:	2685517			

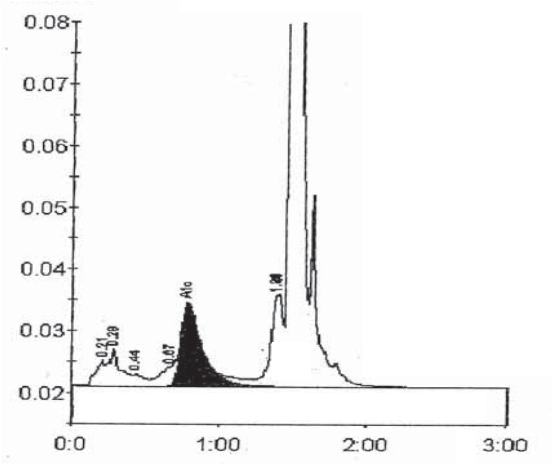
Concentration:	%	mmol/mol
A1c	9.2	77

Figure 3. Diabetic Sample with an Elevated HbA_{1c} Level

SAMPLE REPORT FORMAT

Control report

Bio-Rad	DATE: 03/23/2007
D-10	TIME: 04:03 PM
S/N: #DA5H505313	Software version: 3.50-1
Control ID:	33712
Injection date	11/08/2006 10:00 AM
Injection #: 5	Method: HbA1c
Rack #: D4	Rack position: 5



Peak table - ID: A1CTRH

Peak	R.time	Height	Area	Area %
A1a	0.21	3951	17524	1.0
A1b	0.28	5794	31276	1.8
F	0.44	1852	11829	0.7
LA1c/CHb-1	0.67	3008	18783	1.1
A1c	0.79	13142	131154	10.3
P3	1.39	14754	56407	3.2
Unknown	1.41	14990	30733	1.8
A0	1.48	382268	1445140	82.9
Total Area:	1742844			

Concentration:	
% A1c	10.3

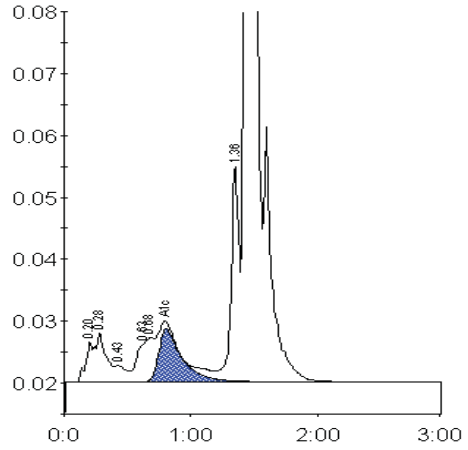
Control OK

Figure 4. Split P3 Peak

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 01/25/2008
D-10	TIME: 04:03 PM
S/N: #DA5H505327	Software version: 3.50-A1
Sample ID:	00010
Injection date	01/25/2008 03:50 PM
Injection #: 14	Method: HbA1c
Rack #: C1	Rack position: 2



Peak table - ID: 00010

Peak	R.time	Height	Area	Area %
A1a	0.20	6503	24054	0.9
A1b	0.28	7981	47991	1.8
F	0.43	2600	16423	0.6
LA1c/CHb-1	0.63	6114	42262	1.6
LA1c/CHb-2	0.68	6802	54664	2.0
A1c	0.81	8536	109265	5.4
P3	1.36	34764	153264	5.7
A0	1.44	527758	2231123	83.3
Total Area:	2679047			

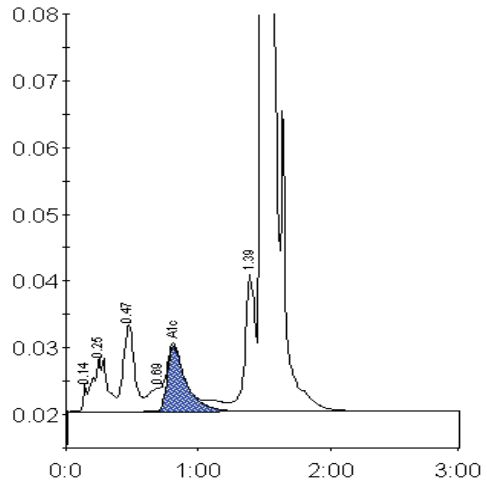
Concentration:	
% A1c	5.4

Figure 5. Labile A_{1c} and Carbamylated Hemoglobin

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 12/15/2007
D-10	TIME: 04:04 AM
S/N: #DA4C323319	Software version: 3.50-A1
Sample ID:	F-INTERFERENCE
Injection date	10/29/2006 04:58 PM
Injection #: 102	Method: HbA1c
Rack #: E3	Rack position: 2



Peak table - ID: F-INTERFERENCE

Peak	R.time	Height	Area	Area %
Unknown	0.14	4111	8862	0.4
A1a	0.25	8240	59580	2.4
F	0.47	12960	78089	3.1
LA1c/CHb-1	0.69	3470	22827	0.9
A1c	0.81	9755	97424	5.7
P3	1.39	20716	107715	4.3
A0	1.49	565969	2119338	85.0
Total Area:	2493835			

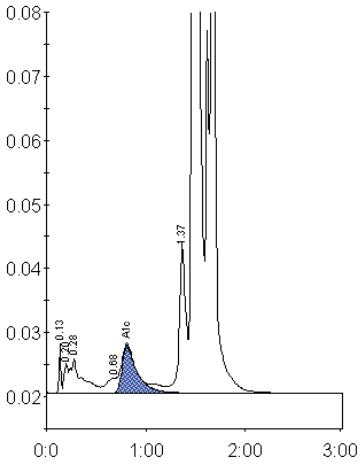
Concentration:	
% A1c	5.7

Figure 6. Patient Sample with Elevated HbF

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 09/23/2010
D-10	TIME: 02:57 PM
S/N: #DA3G222606	Software version: 3.57
Sample ID:	RACKC2-1-24-17-9-2010
Injection date	09/17/2010 11:18 AM
Injection #: 24	Method: HbA1c
Rack #: C2	Rack position: 1



Peak table - ID: RACKC2-1-24-17-9-2010

Peak	R.time	Height	Area	Area %
Unknown	0.13	7678	9422	0.4
A1a	0.20	4591	13413	0.5
A1b	0.28	5343	43577	1.7
LA1c/CHb-1	0.68	2329	17693	0.7
A1c	0.81	7404	77290	6.3
P3	1.37	23435	104787	4.1
A0	1.48	357969	1407810	55.7
S-Window	1.67	489999	853066	33.8
Total Area:	2527057			

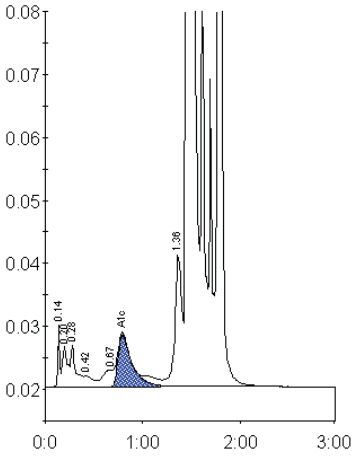
Concentration:	%	mmol/mol
A1c	6.3	46

Figure 7. Patient Sample with Hemoglobin S Trait, or Sickle Cell Trait (AS)

SAMPLE REPORT FORMAT

Patient report

Bio-Rad	DATE: 09/23/2010
D-10	TIME: 02:58 PM
S/N: #DA3G222606	Software version: 3.57
Sample ID:	RACKB3-1-13-23-9-2010
Injection date	09/23/2010 11:15 AM
Injection #: 13	Method: HbA1c
Rack #: B3	Rack position: 1



Peak table - ID: RACKB3-1-13-23-9-2010

Peak	R.time	Height	Area	Area %
Unknown	0.14	10208	13877	0.4
A1a	0.20	6542	23873	0.8
A1b	0.28	6573	24966	0.8
F	0.42	1810	12280	0.4
LA1c/CHb-1	0.67	2654	20186	0.7
A1c	0.79	8243	82393	5.1
P3	1.36	20991	104655	3.4
A0	1.46	470153	1979912	63.9
C-Window	1.79	407012	834752	27.0
Total Area:	3096895			

Concentration:	%	mmol/mol
A1c	5.1	32

Figure 8. Patient Sample with Hemoglobin C Trait (AC)

PRODUCT SAFETY INFORMATION

Whole Blood Primer and Hemoglobin A_{1c} Calibrator 1 and 2

WARNING: These products contain a chemical known to the State of California to cause birth defects or other reproductive harm. Contains <0.1% Gentamicin Sulfate and <0.1% Tobramycin.

TRADEMARK INFORMATION

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NOTES:

TECHNICAL ASSISTANCE

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