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Hemoglobin A1c in Whole Blood Using the Bio-Rad D-100 HPLC

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

Measure percent hemoglobin A1c in whole blood using the Bio-Rad D-100 HPLC (high performance liquid chromatograph) system and document autoverification rules and evaluation criteria for A1c chromatograms by Beaumont Laboratory.

II. INTRODUCTION:

A. Diabetes mellitus is a condition characterized by hyperglycemia resulting from defects in insulin secretion or insulin action. Therapy for diabetes requires the long-term maintenance of a blood glucose level as close as possible to normal, minimizing the risk of long-term vascular consequences. A single fasting blood glucose measurement is an indication of the patient's immediate past condition (hours) and may not reflect long-term blood glucose regulation. An accurate index of mean blood glucose concentration may be established by the measurement of hemoglobin A1c (HbA1c) every two to three months. Each 1% change in the HbA1c represents an approximate 25 to 35 mg/dl change in the average plasma glucose.

B. HbA1c is formed in two steps by the non-enzymatic glycation of hemoglobin A (HbA). The first step is the formation of an unstable aldimine (Schiff base, labile, or pre-A1c), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the b-chain of hemoglobin. Schiff base formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the Schiff base is converted (Amadori rearrangement) to form a stable ketoamine, HbA1c.

C. The level of HbA1c is proportional to both the average glucose concentration and life span of

hemoglobin in the circulating red cell. HbA1c has therefore been accepted for the clinical management of diabetes. The contribution of plasma glucose to glycated hemoglobin or HbA1c depends on the time interval, with more recent glucose values providing a larger contribution than earlier values to HbA1c. Plasma glucose in the preceding one month makes up 50% of the HbA1c whereas days 60-120 determine only 25% of the HbA1c value.

D. In general, HbA1c should be monitored at least twice a year in patients who have stable glycemic control and quarterly in patients whose treatment has changed or patients who are not meeting glycemic goals.

III. PRINCIPLE:

The D-100 HbA_{1c} test utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically diluted on the D-100 and injected into the analytical cartridge. The D-100 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, where changes in the absorbance at 415 nm are measured.

The D-100 software collects raw data from each analysis and calculates HbA_{1c} values based on a bi-level calibration curve. The HbA_{1c} area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and a chromatogram are generated for each sample.

IV. SPECIMEN COLLECTION AND HANDLING:

1 mL of whole blood collected in a tube with the preservative ethylenediaminetetraacetic acid (EDTA) .

Specimens may be stored up to seven days at 2-8^{O}C or 1 day at room temperature. Sample volumes less than 1 mL require pre-dilution before being placed on the instrument. Minimum volume is 100 mcL. Allow 30 minutes for specimen tubes to come to room temperature.

If the height of sample in tube appears to be less than 1mL, then the sample may need to be prediluted 1:300 prior to analysis:

A. Before pipetting, thoroughly mix the sample by gently inverting the tube.

B. To predilute, pipet 1.5 mL of Sample Diluent into a labeled 1.5 mL microvial, followed by 5mcL of the whole blood sample

C. Cap the microvial and mix thoroughly.

V. REAGENTS AND SUPPLIES:

Component	REF	Content	Preparation and Storage
D-100 HbA_{1c} Analytical Cartridge/ Calibrator Pack	290-1004	One pack consisting of: • Cation exchange cartridge 10,000 tests	The Analytical Cartridge is stable until the expiration date when stored at 2–8 °C. The Analytical Cartridge can be used immediately

Component	REF	Content	Preparation and Storage
		each • Calibrator Pack: 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.	after removing from refrigerator. When installed on the instrument, the Analytical Cartridge is stable for 90 days at 15–35 °C.
D-100 HbA_{1c} Calibrator Pack	290-1006	One pack consisting of 1 vial of Conditioner, 1 vial of Calibrator Level 1, and 1 vial of Calibrator Level 2. The vials contain lyophilized human whole blood with glycine and trehalose as preservatives.	The Calibrator Pack is stable until the expiration date when stored unopened at 2-8°C. The Calibrator Pack can be used immediately after removing from refrigerator. Once reconstituted by the system, the Calibrator Pack is stable for 24 hours after initial use when stored at 2-8°C. The Calibrator Pack may be used for a second calibration within this period.
D-100 Prefilters	290-1007	2000 tests each. Package of 5.	The Prefiler is stable until the expiration date when stores at 2-8°C. The Prefilter can be used immediately after removing from refrigerator. When installed on the instrument, the Prefilter is stable for 90 days at 15-35°C.
D-100 Cleaning Tube	290-1008	One microvial containing 1.5 mL of a liquid cleaning solution.	The Cleaning Tube is stable until the expiration date when stored unopened at 15-35°C. See Product Safety Information included with the Cleaning Tube for hazards and precautions.
D-100 Sample Diluent	290-1009	Each bottle contains 1 L of deionized water with <0.1% sodium azide as a preservative.	The Sample Diluent is stable until the expiration date when stored unopened at 15-35°C. After opening, the Sample Diluent is stable for 90 days when stored at 15-35°C.

Component	REF	Content	Preparation and Storage
D-100 HbA_{1c} Elution Buffer A	290-1010	Each bottle contains 2600 mL of a succinate/sodium perchlorate buffer. Contains < 0.1% sodium azide as a preservative.	Stable until the expiration date when stored unopened at 15–35 °C or for 90 days at 15-35 °C after installing on the instrument.
D-100 HbA_{1c} Elution Buffer B	290-1011	Each bottle contains 1400 mL of a succinate/sodium perchlorate buffer. Contains <0.1% sodium azide as a preservative.	Stable until the expiration date when stored unopened at 15–35 °C or for 90 days at 15-35 °C after installing on the instrument.
D-100 Wash Solution	290-1012	Each bottle contains 3300 mL of deionized water with <0.1% sodium azide as a preservative.	Stable until the expiration date when stored unopened at 15–35 °C or for 90 days at 15-35 °C after installing on the instrument.

VI. EQUIPMENT:

BioRad™ D-100 Hemoglobin Testing System
Pipettes, 5 mcL, 0.5 mL, 1 mL, 1.5 mL

VII. CALIBRATIONS:

Calibration must be performed upon installation of every new analytical cartridge. Additional calibration should be performed after major instrument maintenance or repair, or when quality control does not fall within acceptable criteria. See calibration procedure below.

VIII. QUALITY CONTROL:

Bio-Rad Lyphochek Diabetes Control levels 1 and 2 should be tested at the beginning of a run (at least once every 8 hours) and every 100 samples. These controls are stable until the expiration date when stored unopened at 2-8° C. Once the control is reconstituted with 0.5 mL DI H_2O, the control is stable for 7 days when stored tightly capped at 2-8° C.

A. Prepare each level of Bio-Rad Diabetes controls by pipetting 1.5 mL of wash/diluent into a sample dilution vial followed by 5 mcL of well-mixed control. Cap vial and mix.
B. Return stock controls to 2-8° C once the dilutions have been made.
C. Use the pre-labeled vial holders to run your controls. The Bio-Rad D-100 instrument can use these barcodes to transfer data to QC management software.
D. Barcodes on the cap-pierceable vials are intended for use with Bio-Rad ® D-100™ instruments. To use the barcode, align the barcode on the vial(s) with the barcode reader on the instruments.

IX. PRECAUTIONS:

A. Handle all calibrators, controls, waste and patient samples as potentially BIOHAZARDOUS material.

B. All elution buffers and wash solutions contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metallic azides.

C. Do not use reagents after their expiration date.

X. PROCEDURE:

A. **Installing a New Analytical Cartridge**
The Analytical Cartridge must be changed every 90 days or 10,000 injections. Priming, calibration, and temperature adjustment are performed automatically by the D-100 before first analysis with a new analytical cartridge.

1. Verify that the instrument is in Sleeping State (Utilities/Manual Operations/General/Sleep)
2. Open cartridge holder door.
3. Remove old cartridge and discard.
4. Insert new cartridge.
5. Close the cartridge door. The test parameters are automatically updated.

B. **Installing a New Prefilter**
Replace the prefilter every 90 days or after 2,000 injections.

1. Ensure that the instrument is in Sleeping State (Utilities/Manual Operations/General/Sleep).
2. Open the prefilter holder door.
3. Remove the old prefilter and discard.
4. Insert the new prefilter.
5. Close the prefilter door. The prefilter information is automatically updated.

C. **Calibration**
Calibration must be performed upon installation of every new analytical cartridge. If using Calibration pack to troubleshoot, open a case with service to be reimbursed for used Calibration pack.

1. Ensure the instrument is in Sleeping or Standby State.
2. To retrieve the Stat rack, touch **Open** on the home screen.
3. Insert Diabetes Controls (in barcoded adapters) in positions 1 and 2 and insert the D-100 Calibrator Pack in the dedicated position with the barcodes facing out towards operator. Note: Racks containing patient samples can be placed in the input area to be automatically processed after the calibration has passed.
4. Touch **Load**.

5. Touch **Calibrate Now**.
6. Touch **Open** after all samples in the Stat area have been processed.
7. Remove the samples from the Stat rack.
8. Calibration packs are good for 24 hours after opening or two uses. If a Calibration pack is to be used again, select box “Calibrate without reconstituting (calibrate without prediluted material).” This will prevent the instrument from diluting the Calibration pack again.

D. **Routine Run Setup**

Anytime the instrument has returned to Sleeping or Standby modes, the routine run set-up must be followed.

1. Verify in the upper right corner of the screen that the D-100 is logged into Operator mode. (Service mode does not track reagent usage.)
2. Check cartridge counts, buffer and waste levels, and check for leaks. The D-100 waste bottle is detached from the waste line by carefully retracting the metal sleeve on the connector.
3. Verify temperature is acceptable on maintenance sheet with a functionality check once per day. Note: If the instrument temperature is out of range, an error message will appear and the instrument will be inoperable. The temperature is shown on the main screen of the instrument in the bottom right hand corner.
4. When installing wash or buffer bottles, insert the new bottle ¾ of the way into the reagent compartment and position the bottle lock on the top of the bottle, near the center. Apply pressure on the top of the lock with one hand and push in the bottle handle with the other. An audible “thunk” will be heard when the bottle is secure. Verify on the home screen that the bottle has been read and is pressurized.
5. Mixing the tubes prior to loading is unnecessary. However, if the samples have been sitting for more than 8 hours, it is acceptable to mix by 1 quick inversion prior to loading onto the instrument. Over-mixing specimens can lead to low total area counts.
6. When loading sample racks, avoid leaving three (3) consecutive empty positions when feasible. The instrument tallies these to determine when to initiate a cleaning procedure, so avoiding this rack configuration can minimize unnecessary cleanings.
7. If a sample is in an abnormal size tube, the height of sample in the tube is less than 25 mm, or clotted, then the sample must be pre-diluted as described above. (Acceptable tube sizes include 12,13 and 14 mm diameter x 75 to 100 mm height.)
8. Controls are prepared by pipetting 1.5 mL of wash/diluent into a vial followed by 5 mcL of well mixed control. Cap the vial and mix.
9. Press Run and the instrument will automatically perform warm up functions. Set up a routine run as follows:
    a. Control 1
    b. Control 2
    c. Patient Samples

d. Repeat both levels of controls every 100 samples.

XI. REPORTING:

A. Total area required for the D-100 chromatogram is 50,000 – 350,000. The instrument performs an automatic repeat if below this range. However, samples still out of the area range after this repeat should be manually diluted with more or less sample as appropriate. See Procedure / Routine Run Setup.

B. HbA1c chromatograms are evaluated by the instrument using the modified D100 Advisor rules listed in Appendix A. These rules evaluate peak shape, baseline characteristics, and potential hemoglobin variants. A1c results acceptable according to D100 Advisor rules are released automatically from the D-100 to the LIS and are autoverified.

C. **IMPORTANT: A1c results released automatically or manually from the D-100 instruments are autoverified in the lab information system (LIS) and reported immediately in the electronic medical record. Flagged results must not be released without proper review by Pathologist or PhD or medical technologist as described below.**

D. When a variant is present that does not interfere, including Hb S, Hb C, Hb D, Hb E, or by Hb F < or = 30%, a D-100 instrument message is received by the LIS, which automatically adds the A1C1 comment: “A hemoglobin variant was detected. This variant should not interfere with Hgb A1c measurement.”

E. A small peak (approx. 10% or less) not typically identified in the peak table may elute after the Ao peak in the E-window or as an Unknown in some calibrator, control, or patient samples. This will not interfere with A1c quantitation. However, if the peak appears frequently in a given run, then a service call should be placed with Bio-Rad. The analytical cartridge may need to be replaced if recommended by Bio-Rad.

F. Chromatograms not meeting D-100 Advisor criteria will be flagged/held for review by a medical technologist. Follow up may include manual dilutions, barcode read errors, system leaks or failures, which may require Bio-Rad service by phone or on-site.

G. Samples with the following notes or comments, or any unusual or questionable chromatograms, should be flagged/held by the D-100 for review by Pathologist, PhD, or medical technologist who has been trained to address these exceptions:

1. No HbA1c (or HbA0) peak
2. HbA1c result out of range
3. High HbA1c
4. Possible variant interference
5. Elevated HbF
6. A1c (or A0) peak shape
7. Ramping baseline
8. LA1c-A1c unusual separation

Print the chromatogram, record patient name and MRN on the chromatogram, and place in the review binder. Also write patient name, MRN, and sample ID number on

the review log sheet.

H. Pathologist/PhD/MLS will review the D-100 results and chromatogram, the available patient history, and previous lab results to determine whether the D-100 method provides an accurate hemoglobin A1c result. Examples of possible reporting steps or other follow up actions include:

1. Run the sample on the Variant beta-Thal HPLC Short Program and/or hemoglobin electrophoresis. This is to obtain additional information on potentially interfering hemoglobin variants. The Variant results are not reported but are solely to aid A1c result interpretation.
2. When the D-100 A1c result is considered accurate for glucose monitoring in the patient, the pathologist/PhD/MLS enters the D-100 A1c result in the LIS. The manual data entry of the A1c result and/or comment is checked a second time by the pathologist/PhD/MLS using the LIS final verification functionality, and the result is released to the medical record.
3. Pathologist/PhD/MLS enters a standard comment into the A1c result comment field (see below) according to the potential interference or clinical situation. Note that in some cases, the A1c result is reported in the comment field only, with a cautionary statement. After a second check on result entry using LIS functionality, result is verified.
4. Pathologist/PhD/MLS may enter a custom comment into the A1c result comment field based on specific findings in the patient record. After a second check on result entry using LIS functionality, result is verified.
5. The sample may be sent to a reference lab for further testing. Prepare an aliquot for sendout. Record date and time the sample was given to the sendout department. Enter a comment into the LIS indicating the sample was sent to a reference laboratory.

I. **Chromatograms from Dearborn Laboratory: Workflow for Pathologist/PhD/MLS review of A1c exceptions**

1. Dearborn and Royal Oak laboratories must use the same D-100 Advisor rules.
2. Dearborn chromatograms not released from the D-100 (see section XI.G. above) will be labeled with patient's first and last name and Beaumont medical record number (MRN) and faxed each morning Mon-Fri to Royal Oak Special Chemistry for review.
3. The samples will be kept at Dearborn Laboratory in an exception rack until the chromatograms have been reviewed and results reported.
4. Pathologist/PhD/MLS at Royal Oak will review each case daily (Mon-Fri) and disposition as described above. A1c results will be reported in the LIS from Royal Oak, or
    a. If additional follow up actions are needed, instructions for each sample will be faxed or emailed to the Dearborn Laboratory lead medical technologists.
    b. If clinicians request immediate review of an A1c exception, a Dearborn medical technologist may call the Royal Oak D-100 workstation number

(248-551-8071) or general Special Chemistry number (248-551-4103) to ask for the Royal Oak pathologist or PhD to be paged.

XII. REPORT COMMENTS:

The following standard comments are available in the LIS and should be applied to A1c reports as indicated in brackets.

A. **A1CB** [*LIS attaches to all A1c results for adults > or = 18 years*]
Increased risk for diabetes (prediabetes): 5.7-6.4%
Diabetes: > or =6.5%
When using Hemoglobin A1c to diagnose diabetes, an elevated Hemoglobin A1c should be confirmed with a repeat measurement, fasting glucose, or other test for diagnosing diabetes. All hemoglobin A1c methods are affected by conditions that increase or decrease red blood cell survival. Falsely high results may be seen with iron deficiency or splenectomy. Falsely normal or low results may be seen with hemolytic anemias, unstable hemoglobins, end-stage renal disease, recent or chronic blood loss, or following transfusions.

B. **A1CA** [*LIS attaches to all A1c results for patients < 18 years*]
Hemoglobin A1c criteria for diagnosing diabetes have not been established for patients who are <18 years of age. All hemoglobin A1c methods are affected by conditions that increase or decrease red blood cell survival. Falsely high results may be seen with iron deficiency or splenectomy. Falsely normal or low results may be seen with hemolytic anemias, unstable hemoglobins, end-stage renal disease, recent or chronic blood loss, or following transfusions.

C. **A1C1** [*LIS rules add this comment to A1c results when the D-100 detects heterozygous Hgb S, C, D, E, or F < or = 30%*]
A hemoglobin variant was detected. This variant should not interfere with Hgb A1c measurement.

D. **A1C2** [*Pathologist/PhD/MLS adds A1C2 to the result comment field and manually enters the D-100 result <u>into the A1C2 comment only</u>. Data entry checked again before final verification in LIS.*]
Hemoglobin A1c = *** % NOTE: A hemoglobin variant was detected but not identified. Interpret the Hgb A1c result with caution in this patient. Hemoglobin variants may interfere with accurate A1c quantitation or alter red cell lifespan. This hemoglobin A1c assay has been validated to provide accurate results in the presence of the most common hemoglobin variants when hemoglobin A is also present. Fructosamine (glycated serum proteins) suggested as an alternative monitor of glycemic control. Consider hemoglobinopathy evaluation if clinically indicated.

E. **A1C3** [*Pathologist/PhD/MLS adds A1C3 to the result comment field and manually enters the D-100 result <u>into the A1C3 comment only</u>. Data entry checked again before final verification in LIS.*]
Hemoglobin A1c = *** % NOTE: A hemoglobin variant was detected, consistent with a prior hemoglobinopathy evaluation (***/***/***) which identified a variant not associated with clinical or hematologic abnormalities. This variant should not interfere with hemoglobin A1c measurement by the D-100 high performance liquid chromatography method, but may interfere with other hemoglobin A1c assay methods. Interpret the Hgb A1c result with caution. Fructosamine (glycated serum proteins) suggested as an alternative monitor of

glycemic control.

F. **A1C4** [*Pathologist/PhD/MLS adds A1C4 to the A1c result comment field. The D-100 result is not reported. Comment checked again before final verification in LIS.*]
Hemoglobin A1c could not be determined due to the presence of one or more interfering hemoglobins. Fructosamine (glycated serum proteins) suggested as an alternative monitor of glycemic control. Consider hemoglobinopathy evaluation if clinically indicated.

G. **A1C5** [*Pathologist/PhD/MLS adds A1C5 to the A1c result comment field, along with the date and findings of the prior hemoglobinopathy evaluation. The D-100 result is not reported. Data entry checked again before final verification in LIS.*]
Hemoglobin A1c could not be determined due to the presence of interfering hemoglobin variant(s). Prior hemoglobinopathy evaluation (***/***/***) identified ***. Fructosamine (glycated serum proteins) suggested as an alternative monitor of glycemic control.

H. **A1C6** [*Pathologist/PhD/MLS adds A1C6 to the A1c result comment field. Insert clinical situation as appropriate for the patient (e.g., hemolytic anemia, end stage renal disease, recent or chronic blood loss, recent transfusion). The D-100 result is not reported. Comment checked again before final verification in LIS.*]
Hemoglobin A1c may not accurately reflect glycemic control in this patient due to ***. Fructosamine (glycated serum proteins) suggested as an alternative monitor of glycemic control.

I. **A1C7**
Sent to reference laboratory.

XIII. D-100 MAINTENANCE:

A. Waste Container Cleaning (Weekly)
    1. Remove waste bottles from any non-plumbed instrument. The D-100 waste bottle is detached from the waste line by carefully retracting the metal sleeve on the connector.
    2. Empty the bottle and rinse with a 10% bleach solution, then re-attach to waste line.

B. Database Backup Procedure (Monthly)
    1. Make sure the system is in Sleep Mode (Utilities/Manual Operations/General/Sleep Mode).
    2. Under the Utilities tab select Data. In the Backup area, select Full and Exclude patient demographics.
    3. Select the output path to USB in the Save To area. The USB port is located on the right side of screen on the instrument's monitor.
    4. Unlock Aegis Secure Key (encrypted flash drive) by pressing the green unlock icon. Red LED will glow solidly. Enter the User Pin located on the back of key and press the green unlock icon again. If the pin is accepted, the green LED will blink quickly four times, then continue to blink slowly until it is plugged into the USB port. (If not plugged into the USB port within 30 seconds, the key will automatically lock itself again.)
    5. Touch Backup. No calibration is needed after Database Backup.

Uploading from a Database Backup Procedure: To view historical data that has been stored on a backup USB, insert USB drive and select "View Archive" from the results menu. DO NOT RESTORE backup data unless directed by a supervisor.

C. Clean Procedure (Monthly)

1. Make sure the system is in Sleep Mode (Utilities/Manual Operations/General/Sleep Mode).
2. Under the Utilities tab select Clean System.
3. Place a D-100 Cleaning Tube (REF 290-1008) in the STAT area and select Clean Now.
4. The instrument will perform the clean and will eject tube when finished.

Note: It is recommended that the clean is run every 30,000 or after every three cartridge changes in addition to fluidics errors. The instrument will prompt the user to run the clean procedure when such fluidics errors occur.

D. Cleaning of Racks (Monthly)

1. Inspect racks for visible contaminants.
2. Clean with either 10% bleach solution or with alcohol pads.

XIV. CALCULATIONS:

The D-100 software collects raw data from each analysis and calculates HbA1c values based on a bi-level calibration curve. The HbA1c area is calculated using an exponentially modified Gaussian (EMG) algorithm. A sample report and chromatogram are generated for each sample. Chromatograms are evaluated based on rules programmed and validated for use with D-100 on-board integrated Advisor software. The LIS calculates and reports out an estimated Average Glucose (eAG) for each HbA1c result using the equation: 28.7 x HbA1c - 46.7

XV. REFERENCE INTERVALS:

Normal	4.0 – 5.6 % HbA1c
Increased Risk for Diabetes (Prediabetes)	5.7 – 6.4 % HbA1c
Diabetes	≥ 6.5% HbA1c

XVI. REPORTABLE RANGE:

3.6 - 20.0% A1c
Manual dilutions to bring total area counts into acceptable range are performed, as described herein. However, dilutions to bring % A1c into reportable range are not performed.

XVII. LIMITATIONS AND INTERFERING SUBSTANCES:

A. The HbA1c test is not intended for analysis of samples collected from newborns.

B. The HbA1c test should not be used to replace glucose testing in pediatric patients, pregnant women, or patients with Type 1 diabetes.

C. In cases of rapidly evolving Type 1 diabetes, the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentration and/or the typical clinical symptoms.

D. The HbA1c test should not be used to diagnose diabetes during pregnancy or to diagnose gestational diabetes. HbA1c reflects the average blood glucose levels over the preceding 3 months (the average life of a red blood cell), and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red cell survival.

E. The HbA1c test should not be used to diagnose diabetes in patients with the following conditions: (a) Any condition that alters the life span of the red blood cells, including recent blood loss, transfusion, significant iron deficiency, hemolytic anemia (including hereditary spherocytosis) or other hemolytic diseases, hemoglobinopathies and thalassemias, as the altered red blood cell turnover interferes with the relationship between mean blood glucose and HbA1c values. (b) Malignancies or severe chronic hepatic and renal disease.

F. The D-100 method demonstrates no significant interference from heterozygous Hb S, Hb C, Hb D, Hb E, or by HbF < or = 30%, LA1c < or = 7%, carbamylated Hb, acetylated Hb, and low amounts of P3 and other Unknown peaks.

G. β-thalassemia trait, as indicated by increased HbA2 concentrations, does not interfere with the test.

H. Common therapeutic drugs do not interfere (see manufacturer instructions).

I. Endogenous substances up to stated concentrations do not interfere:

Lipemia (Intralipid®)	6000 mg/dL
Conjugated bilirubin	60 mg/dL
Unconjugated bilirubin	60 mg/dL
Glucose	2000 mg/dL
Rheumatoid factor	750 IU/mL
Total protein	21 g/dL

XVIII. REFERENCES:

A. Bio-Rad D-100 Hemoglobin Testing System Operation Manual (2016)

B. Bio-Rad D-100 Hemoglobin Testing System HbA1c Advisor Handbook; Rule Set Version 1.4 (Apr 2020); LB001552revC.

C. Bio-Rad D-100 HbA1c Subsidiary Communication: Chromatography Training Information: Identification of Extra Peaks (Sep 2016); LB001607revA.

D. Bio-Rad D-100 HbA1c Subsidiary Communication: Advisor A0 Tau Rule Update (Sep 2018); LB002302revB.

E. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes – 2019. Diabetes Care.* 2019; 42(Suppl. 1):S13-S28.

F. Tietz Textbook of Clinical Chemistry, 6th edition (2018); pp. 1160,1188-94.

Attachments

Appendix A-Hemoglobin A1c D-100 Advisor Rules

Appendix B-Hemoglobin A1c D-100 Chromatogram Review

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	8/26/2022
	Ann Marie Blenc: System Med Dir, Hematopath	8/17/2022
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	8/17/2022
Policy and Forms Steering Committee Approval (if needed)	Leah Korodan: Mgr Laboratory	8/17/2022
	Caitlin Schein: Staff Physician	8/17/2022
	Steven Truscott: Tech Dir, Clin Chemistry, Path	8/8/2022
	Leah Korodan: Mgr Laboratory	8/8/2022
	Leah Korodan: Mgr Laboratory	8/8/2022

Applicability

Dearborn, Royal Oak