HEMOGLOBIN A1c/A2/F
BIO-RAD D-10 DUAL PROGRAM

CLINICAL SIGNIFICANCE

Hemoglobin A1c
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. The complications of diabetes, involving the eyes, kidneys, nerves and the large blood vessels of the heart, brain and extremities, are common to both forms of the disease. Diabetes mellitus affects more than 5% of the world population. 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. 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. An accurate index of the mean blood glucose concentration may be established by the measurement of hemoglobin A1c (HbA1c) every two to three months. HbA1c, 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 A1c, or pre-A1c), a reversible reaction between the carbonyl group of glucose and the N-terminal valine of the β-chain of hemoglobin. Labile A1c formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A1c is converted (Amadori rearrangement) to form a stable ketoamine, HbA1c. The level of HbA1c is proportional to both the average glucose concentration and the life span of the red blood cell in the circulation. The measurement of HbA1c has therefore been accepted for the clinical management of diabetes through routine monitoring. Methods for the determination of HbA1c include electrophoresis, immunoassays, and chromatography. HbA1c determination with the D-10 Dual Program has been optimized to eliminate interferences from hemoglobin variants, labile A1c, and carbamylated hemoglobin. Please refer to Limitations of the Procedure and Performance Characteristics in the Bio-Rad D-10 Dual Program Instruction Manual for more information.

Hemoglobins A2 and F
A frequently occurring thalassemia, beta-thalassemia (β-thalassemia), is commonly found in the heterozygous state as β-thalassemia minor or β-thalassemia trait. Adult blood contains primarily hemoglobin A (HbA), a small percentage of hemoglobin A2 (HbA2), and trace amounts of fetal hemoglobin (HbF). Carriers of β-thalassemia typically have HbA2 levels of 4-9% and HbF levels of 1-5%. The D-10 Dual Program HbA2/F/A1c assay can be used for β-thalassemia screening by quantitation of HbA2 and HbF. The most commonly occurring hemoglobin variants include hemoglobins S, E, C and D. Presumptive identification of these hemoglobin variants is made using retention time windows, such as an “S-Window” and “C-Window.”
Final determination of specific variants eluting in the windows is left to the educated judgment of the user. For the positive confirmation of any particular hemoglobin variant, alternative separation methods are required.

**TEST PRINCIPLE**

The D-10 Dual Program is based on chromatographic separation of the analytes by 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 HbA2/F/A1c values. A sample report and a chromatogram are generated for each sample. The A1c area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area.

**SPECIMEN REQUIREMENTS**

Blood is collected in a lavender top tube (EDTA) and refrigerated at 2-8°C. Whole blood is stable 4 days at 2-8°C or 24 hours at room temperature (15 – 30 °C). Lipemia up to a level of 5680 mg/dL of triglycerides does not interfere. Icterus up to a level of 20 mg/dL does not interfere. Hemolysis of the sample is not relevant, as whole blood is hemolyzed in the course of the analysis.

* Acceptable container sizes are 5 mL, 7 mL and 10 mL
* Samples with less than 2.0 mL volume (or height less than 25 mm), or clotted samples, require pre-dilution before being placed on the D-10.

Allow sample tubes to reach room temperature (15–30 °C) before performing the assay. No sample preparation is required. **Mixing the tubes prior to loading is not necessary.**

**REAGENTS**

The D-10 Dual Program Kit (Reorder Pack Cat. No. 220-0201) contains supplies sufficient for 200 analyses of Hb A2/F/A1c.

**Elution Buffer 1 (220-0210)**
Two bottles containing 2000 mL of a Bis-Tris/Phosphate buffer, pH 6.0. Contains <0.05% sodium azide as a preservative. Store at 15-30° C.

**Elution Buffer 2 (220-0211)**
One bottle containing 1000 mL of a Bis-Tris/Phosphate buffer, pH 6.7. Contains <0.05% sodium azide as a preservative. Store at 15-30° C.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash/Diluent Solution (220-0112)</td>
<td>One bottle containing 1600 mL deionized water, with &lt;0.05% sodium azide as a preservative; Store at 15-30°C.</td>
</tr>
<tr>
<td>Analytical Cartridge (220-0212)</td>
<td>One cation exchange cartridge (4.0 mm ID x 30 mm). Store at 15 – 30 °C.</td>
</tr>
<tr>
<td>Calibrator/Diluent Set, Hb A2/F/A1c (220-0218)</td>
<td>Set includes:</td>
</tr>
<tr>
<td></td>
<td>Calibrator Level 1: Three vials containing:</td>
</tr>
<tr>
<td></td>
<td>Lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Store lyophilized and reconstituted calibrator at 2-8 °C.</td>
</tr>
<tr>
<td></td>
<td>Calibrator Level 2: Three vials containing:</td>
</tr>
<tr>
<td></td>
<td>Lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 7 mL per vial. Store lyophilized and reconstituted calibrator at 2-8 °C.</td>
</tr>
<tr>
<td></td>
<td>Calibrator Diluent: One bottle 100 mL Deionized H2O with &lt;0.05% sodium azide as a preservative. Store at 2 – 8 °C.</td>
</tr>
<tr>
<td>Sample Vials (220-2149)</td>
<td>Two packs (one hundred each) sample vials with piercable caps, 1.5ml each.</td>
</tr>
<tr>
<td>Floppy Diskette (220-0215)</td>
<td>Contains kit-specific D-10 Dual Program Parameter information.</td>
</tr>
<tr>
<td>Whole Blood Primer (220-0148)</td>
<td>Four vials of lyophilized human red blood cell hemolysate with gentamicin, tobramycin, and EDTA as preservatives. Reconstituted volume is 1.0 mL per vial.</td>
</tr>
<tr>
<td>Thermal Paper (220-0375)</td>
<td>Box of 10 rolls</td>
</tr>
</tbody>
</table>

**UPDATE KIT, CARTRIDGE, PRIME AND CALIBRATION**

**Frequency of update, prime and calibration:** Every installation of a new cartridge (200 injections). The software will prompt the user when 200 injections have been reached (analysis is not permitted past the allowed number of injections). Calibration must be performed after priming a new analytical cartridge and after installing a new lot of buffer. Thereafter, calibration should be repeated every 24 hours.
**Whole Blood Primer Preparation:**
Reconstitute Whole Blood Primer by adding 1 mL of CLRW to the vial. Allow to stand for 10 – 15 minutes at 15-30°. Swirl gently to dissolve and ensure complete mixing. Reconstituted Whole Blood Primer is stable for 24 hours when stored at 2-8 ° C.

**Calibrator Preparation:**
Reconstitute Hb A1c/A2/F Calibrator Levels 1 and 2 with 7 mL of cold Calibrator Diluent using a volumetric pipette or other accurately calibrated device. Allow the calibrators to stand for 5-10 minutes. Swirl gently to dissolve. Label the bottles with preparation and expiration dates. Reconstituted calibrators are stable for 10 days at 2-8 ° C. The calibrators are ready for use without further preparation.

**Installing a New Reorder Pack Lot (Update Kit Floppy Disk):**
Prior to first use of the kit components for Dual Program assays, the kit lot specific parameter values stored on the floppy diskette included with this kit must be loaded into the D-10 system. Perform the following steps:
1. Go to the LOT INFO screen.
2. Press the UPDATE KIT button.
3. Place the UPDATE KIT floppy diskette in the A:\ drive.
4. Follow the instructions on the screen to proceed with the Update Kit Procedure.
5. Remove the floppy diskette from the A:\ drive once the procedure is completed.

**Analytical Cartridge Priming Procedure**
Priming must be performed once following the installation of every new analytical cartridge. Priming must also be performed after decontaminating the D-10 system.
Perform the following steps:

- Prepare a D-10 sample rack to contain all required samples in the order listed below:

<table>
<thead>
<tr>
<th>POSITION</th>
<th>SAMPLE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PRIMER</td>
<td>1 mL reconstituted Whole Blood Primer in microvial/barcoded adapter</td>
</tr>
</tbody>
</table>

- After priming is complete, prepare D-10 sample rack(s) to contain all required samples in the order listed in the table below. If desired, also prepare any optional patient samples, and optional duplicate control samples. Predilutions are made using 1.5 mL D10 Wash/Diluent solution and 5 uL of QC material/patient sample.
<table>
<thead>
<tr>
<th>POSITION</th>
<th>SAMPLE</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CALIBRATOR 1</td>
<td>1 mL reconstituted Calibrator Level 1 in microvial/barcoded adapter</td>
</tr>
<tr>
<td>2</td>
<td>CALIBRATOR 2</td>
<td>1 mL reconstituted Calibrator Level 2 in microvial/ barcoded adapter</td>
</tr>
<tr>
<td>3</td>
<td>DIABETES CONTROL 1 (DB1)</td>
<td>Prediluted 1:300 in microvial/ barcoded adapter</td>
</tr>
<tr>
<td>4</td>
<td>DIABETES CONTROL 1 (DB1)</td>
<td>Prediluted 1:300 in microvial/ barcoded adapter</td>
</tr>
<tr>
<td>5-10</td>
<td>PATIENT SAMPLE(S)</td>
<td><strong>Barcodes must face rear opening in sample rack</strong> (samples are not rotated in racks, nor are they mixed prior to sampling)</td>
</tr>
<tr>
<td></td>
<td>(Note a STOP tube is not needed for the D-10)</td>
<td></td>
</tr>
</tbody>
</table>

***NOTE: The D-10 sample racks and microvial adapters ARE NOT INTERCHANGEABLE with those on the Variant II and Variant II Turbo. USE THE DESIGNATED SAMPLE RACKS on the D-10 ONLY. SAMPLE BARCODES MUST FACE THE REAR OF THE SAMPLE RACK SO THE D-10 READER CAN READ THEM.***

**QUALITY CONTROL**

It is recommended that controls be run once per shift to check the performance of the assay at both the diabetic and non-diabetic levels of Hb A1c. Place one set of controls at the beginning and end of each run.

Bio-Rad LYPHOCHEK Diabetes Control Levels 1 and 2 (Cat. No. 740), stored at 2-8°C, is stable until the manufacturer's expiration date. Reconstitute each vial with 0.5 mL of CLRW. After reconstitution, it is stable 7 days when stored at 2-8°C.

**PROCEDURE**

**Sample Preparation**
The D-10 instrument requires a minimum sample volume of 2.0 mL for patient specimens collected in standard size tubes (75mm - 100mm length, 12-16 mm diameter). Whole Blood specimens collected in irregular tube sizes, or samples with insufficient volume (< 2.0 mL or 25 mm height) must be prediluted before use.

**Standard Sample Tube Preparation** – To prepare standard size sample tubes containing sufficient sample volume proceed as follows:
1. Allow sample tubes to reach room temperature (15-30 °C) before performing the assay. No other sample preparation is required. Mixing the tubes prior to loading is not necessary.
2. Load the sample tubes into the D-10 sample racks so that the barcodes will be facing the back of the instrument.
**Patient Specimens Requiring Pre-Dilution** – To prepare samples requiring pre-dilution use the 1.5 mL sample vials provided in the kit for all predilutions. Prepare samples as follows:

1. Label a new 1.5 mL sample vial with the patient CID.
2. Pipette 1.5 mL Wash/Diluent Solution and 5 µL whole blood specimen into the vial.
3. Cap the vial and mix thoroughly.
4. Insert the capped vial into an unlabeled D-10 vial adapter. Insert the adapter with vial into the D-10 sample rack.

Prior to starting a patient run and after completion of daily maintenance procedures, the analyzer must be started up from SLEEP. Press the START UP button to begin this process, which will take approximately five minutes. A “Daily Report” will print out at the completion of start-up.

Insert the rack into the slot on the right side of the analyzer. Patient/QC IDs will appear on the screen after they have been scanned by the barcode reader. For prediluted samples, you must enter in the ID by highlighting its rack position on the screen and pressing the EDIT button, which will bring you to an alphanumeric touch screen keypad. Press the DONE button after you have entered each patient ID.

Press the START button to begin the analysis. Press YES when asked if you are sure you want to start the run. One full rack of ten samples will take approximately 65 minutes to complete (6.5 minutes per sample). The rack can only be ejected when the instrument returns to STANDBY, at which time a subsequent rack for analysis can be loaded in the same manner as above. It is recommended to start a timer for 65 minutes so subsequent racks can be loaded as soon as each previous rack is completed and the analyzer returns to STANDBY; if there is no activity for 30 minutes after returning to STANDBY, the analyzer will return to SLEEP. Should this occur, be aware that starting the analyzer up a second time, necessitating another startup gradient run, will result in a decrease in usable buffer volume.

**CALCULATION OF RESULTS**

The D-10 software performs reduction of raw data collected from each analysis. Two-level calibration is used for quantitation of the HbA2/F/A1c values. A sample report and a chromatogram are generated for each sample. The A1c area is calculated using an exponentially modified Gaussian (EMG) algorithm that excludes the labile A1c and carbamylated peak areas from the A1c peak area.

**RESULT REVIEW**

Observe the following guidelines to ensure acceptable results:

1. The D-10 has passed calibration. For your reference, the slope and intercept acceptable ranges are provided in the D-10 Dual Program Calibrator/Diluent Set package inserts.
2. Total area of each analysis should range from 1.0 to 4.0 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 Dual Program Analytical Cartridge package insert.

4. Quality Control values are in range. Refer to the quality control chart for the specified controls for value range assignment.

REFERENCE INTERVALS

The non-diabetic reference range for D10 Hemoglobin A1c is 4.3 to 5.6 %.

All hemoglobin A1c results will automatically be appended with the following table:

HbA1c cutoffs for diagnosing diabetes:
4.3% - 5.6% = normal
5.7% - 6.4% = increased risk for diabetes
>6.4% = diabetes

HbA1c goals in treatment of diabetes:
Ages 0-6 years: 7.6% - 8.4%
Ages 6-12 years: <8%
Ages 13-19 years: <7.5%
Adults: <7%

REPORTABLE RANGE

D-10 Dual Extended Program Reportable Ranges are (see Note below):
• for HbA1c: 3.7% – 18.4%

NOTE: These ranges were established based on data presented in the Performance Characteristics section of the D-10 Dual Program Instruction Manual.

When a result falls outside of the reportable range, the value is asterisked (*). HbA1c values that fall outside of the reportable range should not be reported.

TECHNICAL NOTES

1. Labile A1c (LA1c) levels up to 3.5% have no clinically significant effect on A1c determination.

2. Labile A1c (LA1c) levels up to 2.6% have no clinically significant effect on Hgb F determination.

3. Carbamylated hemoglobin (CHb) up to 2% have no clinically significant effect on A1c determination.
4. Hemoglobin Variants:
HbA1c values determined using the D-10 Dual Extended Program for HbE, HbD, HbS, and HbC trait specimens showed no clinically significant difference versus values determined by an NGSP certified boronate affinity method.

- Append ETC MUHB "Mutant hemoglobin present" to the A1c result when a variant hemoglobin is identified.
- In the rare homozygous forms (SS or CC), there is no HbA present and therefore, no HbA1c value can be determined.
- A degradation peak, which co-elutes with the HbA2 peak, may appear in HbS samples which have not been stored appropriately. This degradation peak interferes with the quantitation of HbA2. Review the HbA2 peak shape for all HbS samples before reporting. Do not report results if the peak shape is abnormal.
- A degradation peak that interferes with the A1c quantitation may appear in HbE and HbD samples which have not been stored appropriately. Do not report the A1c result if an "Unknown" peak is identified between the A1c and P3 peaks.
- The effect of other hemoglobin variants on the quantitation of A1c, A2, and F has not been fully evaluated.

LIMITATIONS OF THE PROCEDURE:
The D-10 Dual Extended Program provides an area percent determination of hemoglobins A2, F, and A1c, as well as qualitative separation of normal and commonly occurring abnormal hemoglobins. Other less frequently occurring variants may also elute within the established analyte identification windows.

For the positive confirmation of any particular hemoglobin variant, alternative separation methods are required.
• If a sample contains greater than 16.5% HbF, the HbF may elute in the LA1c/CHb or A1c window and no HbF will be reported. Specimens with HbA1c results greater than 18.4% should be tested for the possible presence of hemoglobin variant interference.
• Hemoglobin F quantitation may be adversely affected in the presence of LA1c greater than 2.6%.
• Elevated levels of HbA2 may be masked by concurrent iron deficiency anemia.
• In laboratory confirmation of a β-thalassemia trait diagnosis, HbA2 levels should be considered in conjunction with family history plus laboratory data including serum iron and iron binding capacity, red cell morphology, hemoglobin, hematocrit, and mean corpuscular volume (MCV).
• Hemoglobins D and E have been observed to co-elute with HbA2. Specimens with HbA2 results greater than 10% should be tested for the possible presence of hemoglobin variant interference.
PERFORMANCE CHARACTERISTICS:

Precision
The precision of the D-10 Dual Program was evaluated based on the NCCLS EP5-A guideline (for the Extended Program), "Evaluation of Precision Performance of Clinical Chemistry Devices." In these studies, 40 runs were performed over 20 working days. In each run, aliquots of low and high specimens were analyzed in duplicate. The results of the precision study are summarized in the tables below.

Precision results for HbA1c, Extended Program:

<table>
<thead>
<tr>
<th></th>
<th>Low Patient</th>
<th>High Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean(%HbA1c)</td>
<td>5.9</td>
<td>13.1</td>
</tr>
<tr>
<td>Within Run (%CV)</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Between Day (%CV)</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Between Run (%CV)</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Precision (%CV)</td>
<td>1.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Precision results for HbA2, Extended Program:

<table>
<thead>
<tr>
<th></th>
<th>Low Patient</th>
<th>High Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean(%HbA2)</td>
<td>2.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Within Run (%CV)</td>
<td>4.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Between Day (%CV)</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Between Run (%CV)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total Precision (%CV)</td>
<td>5.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Precision results for Hb F, Extended Program:

<table>
<thead>
<tr>
<th></th>
<th>Low Patient</th>
<th>High Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean(%Hb F)</td>
<td>2.1</td>
<td>8.7</td>
</tr>
<tr>
<td>Within Run (%CV)</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Between Day (%CV)</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Between Run (%CV)</td>
<td>1.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Total Precision (%CV)</td>
<td>3.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

REFERENCES

11. Procedure Manual - IFCC Network of Reference Laboratories for HbA1c. Dr. Cas Weykamp, IFCC Network Coordinator, c.w.weykamp@skbwinterswijk.nl, website: www.ifccba1c.com
Appendix 1

BioRad D-10 Hemoglobin A2/F/A1C
Reagent Lot and Cartridge Change

1) A floppy diskette is packaged with every D-10 Hemoglobin A2/F/A1C Kit. The diskette contains information related to lot numbers and test parameters. The diskette is loaded once for each kit in order to update the reagent lot numbers and cartridge injection count.

2) One Analytical cartridge is packaged with every D-10 200-test Hemoglobin A2/F/A1c kit. Each Analytical cartridge is good for 200 injections of patient samples (calibrators and controls are excluded from the count). The new Analytical cartridge needs to be primed once before calibration. Once calibrated, the calibration is good for 24 hours.

3) To install floppy diskette parameters:

   Under the LOT INFO screen,
   a) Select “UPDATE KIT”.
   b) Insert floppy diskette into the drive located on the left side of the analyzer.
   c) Select “UPDATE NOW”.
   d) Exit and remove diskette when done.
   e) In the LOT INFO screen, verify the lot numbers of the cartridges and reagents.

4) When a new buffer lot is installed, a system flush must be performed. When the same buffer lot is installed, a system flush is not necessary. To perform a System Flush (AFTER the new buffers have been installed), under the MAINTAIN screen, select “SYSTEM FLUSH” and allow process to complete.

5) Reconstitute one bottle of Whole Blood Primer by adding 1 mL of CLRW to each bottle. Swirl gently to dissolve. Allow the primer to stand for 10 minutes; swirl again to ensure complete mixing. The reconstituted primer is stable for 24 hours when stored at 2-8°C.

6) Reconstitute Calibrators Level 1 and 2 by adding 7 mL of cold Calibrator Diluent to each bottle. Allow the calibrators to stand for 5 to 10 minutes; swirl gently to dissolve. The reconstituted calibrators are stable for 10 days at 2-8°C. The calibrators are ready for use without further preparation.

7) Lyphochek Diabetes Control Levels 1 and 2 must be diluted 1:300 prior to analysis. Pipette 1.5 mL of Wash/Diluent Solution into a labeled microvial, followed by 5 µL of the reconstituted control. Cap each control vial and mix thoroughly.

8) Lyphochek Hgb A2 Control Levels 1 and 2 must be diluted 1:300 prior to analysis. Pipette 1.5 mL of Wash/Diluent Solution into a labeled microvial, followed by 5 µL of the reconstituted control. Cap each control vial and mix thoroughly.

9) Installing new Elution Buffers, Wash/Diluent Solution, and Analytical Cartridge:

   a) Ensure system is in SLEEP.
   b) Mix each bottle of reagent by gentle inversion.
   c) Remove the reagents ONE AT A TIME.
d) Do not touch the reagent lines below the caps, and do not wipe the lines.

c) After replacing each reagent, place each bottle in its proper position on the reagent tray.

f) If any reagent is replaced before a new cartridge is installed, update reagent inventory under the LOT INFO tab. Select the volume of the desired reagent. Select RESET at the next screen to update inventory (analyzer counts down remaining injections available based on reagent volume).

g) Open the lower of the two panels at the front of the analyzer. Open the cartridge heater door (lower right side of compartment). Remove the cartridge holder.

h) Remove the old cartridge from the holder and discard. Install the new analytical cartridge with the arrow facing to the right. Left side of cartridge should be flush with the left side of the holder.

i) Install the cartridge holder and close the heater door securely. Close the front panel.

j) A new Analytical cartridge is primed once before the calibration run.

k) Fill a microvial with 1 ml Whole Blood Primer.

l) Place the vial in the white adapter labeled “PRIMER” and place the adapter in a D-10 sample rack. Ensure barcode is facing the back of the rack. **NOTE: D-10 sample racks are NOT interchangeable with those for the Variant II TURBO. Do not use Variant II TURBO racks on the D-10, or D-10 racks on the Variant II TURBO.**

m) Insert rack into analyzer; wait for barcode to be read. DO NOT INCLUDE ANY OTHER SAMPLES ON THE RACK WITH THE PRIMER.

n) Press RUN to start prime. Entire process takes approximately 13 minutes.

o) When the prime is complete, select EJECT to remove rack.

10) Calibration:

a) Place the following barcoded vial adapters in positions 1 through 4 of a D-10 sample rack: Calibrator 1, Calibrator 2, Level 1 or Low Control, Level 2 or High Control.

b) Ensure barcodes are facing the back of the rack.

c) Pipette 1.0 mL of each Calibrator level into the designated vial.

d) Controls require 1:300 dilution with Wash/Diluent Solution.

e) Insert rack into analyzer; wait for barcodes to be read. If they do not read correctly samples must be manually programmed into the worklist.

f) From the RUN screen, select START.

g) The calibration report will print automatically after completion of analysis. Acceptable slope and intercept ranges are listed on the appropriate calibrator/diluent package insert.

h) Select EJECT from the RUN screen to remove the rack.

i) Calibration curve is valid for 24 hours.
Appendix 2
Bio-Rad D-10 Maintenance

Daily Maintenance

1) Check buffers and wash solution levels
   • Reset number of injections if new bottle(s) installed (LOT INFO tab)
   • Confirm correct line goes to the appropriate bottles

2) Check cartridge injection count (LOT INFO tab)
   • Reset number of injections if new cartridge installed

3) Check and empty external waste tank

4) Check pump pressure (MAINTAIN tab/START PUMP)
   • Flow rate should be the same as during analysis (1.5 mL/min). Pressure should be 15-75 kg/cm².

5) Check for leaks during pressure check
   • Open the lower front panel and visually inspect the compartment for the presence of liquid.

6) Check paper supply; replace roll as necessary.

Weekly Maintenance

1) Shut down the D-10 software and power off the instrument.
   • From the RUN screen, press the SHUT DOWN button. Wait for the message indicating that it is safe to turn off the system.
   • Turn off the power switch.
   • Wait 10 seconds before turning on the power switch.

Monthly Maintenance

1) Exterior and interior surface cleaning
   • Use damp gauze to wipe the exterior surface of the system.
   • Wipe up any fluid inside the chromatography station.
   • Clean the interior base plate with damp gauze.

2) Clean and decontaminate the sampling fluid path
   • Make sure the system is in SLEEP.
   • Remove the analytical cartridge and replace it with the plastic PEEK dummy cartridge.
   • Place green end caps onto the analytical cartridge and store for reuse, if <200 injections.
   • Under the SETTINGs tab, change “Auto Printout” to NO (saves paper).
   • Under the LOT INFO tab, change the “Method” to “Decont”; press EXIT, then YES in the confirmation box.
   • From the RUN screen, press STARTUP to wake up the analyzer.
   • Prepare a D-10 sample rack with 10 non-barcoded sample vial adapters: place 5 sample microvials of undiluted bleach in the first five positions, and 5 sample microvials of CLRW in the last five positions.
• After startup has completed, load the aforementioned D-10 sample rack into the analyzer.
• You must manually enter a sample ID for ALL ten positions (any ID will do, e.g. 1, 2, 3, 4, etc.) otherwise the samples will not run. Select DONE after each of the 10 entries.
• From the RUN screen, press START. Press EJECT to remove the rack when completed.
• When the run is complete and the status returns to STANDBY, perform the following with the dummy cartridge still in place: Pipette 1 mL of reconstituted whole blood primer into a sample vial, and put the sample vial into an adapter labeled with the PRIMER barcode. Place the adapter into position #1 of a D-10 sample rack and insert the rack into the analyzer. Press START to begin the run. When the run is complete, press EJECT to remove the rack from the analyzer and dispose of the used vial.
• When the Decon run is completed and the rack has been removed as in the previous step, from the RUN screen, press the SLEEP button.
• While the analyzer is in SLEEP, remove the cartridge holder and the plastic PEEK dummy cartridge. Place a paper towel under the cartridge heater. Rinse the connection points in the heater with CLRW. Wipe the wet surface with a paper towel.
• Remove the plastic PEEK cartridge from the cartridge holder. Rinse the cartridge holder with CLRW, and dry with a paper towel.
• Reinstall analytical cartridge (in use if <200 injections; new if time for replacement).
• Under the LOT INFO tab, select “Method”, then “Hb A2/F/A1c”. Press EXIT, then YES at the confirmation screen.
• Under the SETTINGS tab, re-enable “Auto Printout”.
• Note that a whole blood prime is required if the analytical cartridge is new.

3) Clean the dilution chamber
• Under the MAINTAIN tab, select “Service”
• Select “Access Wash st.” After you hear the “click”, you have 5 (five) seconds to open the upper front door before it locks itself again and the sample needle returns to its home position.
• Disconnect the color-coded luer fittings.
• Push latch to unlock dilution chamber; lift chamber up and remove it from analyzer.
• Rinse with CLRW to remove any residue and wipe completely dry.
• Re-install the chamber and reconnect the color-coded luer fittings.
• Close the upper front door by pushing it down GENTLY, and the sample probe will automatically return to its home position.

4) Clean and inspect sample racks

5) Clean internal waste bottle
• The internal waste bottle must be cleaned monthly to prevent the buildup of particles from the primary sample tube caps; if allowed to build up, these particles can block tubing, leading to leaks.
• While the system is in SLEEP, open the door on the right side of the analyzer to access the internal waste bottle.
• Remove the internal waste bottle from its C-clip by gently pulling the bottle towards you.
• Unscrew the cap and place on an absorbent towel.
• Properly dispose of the liquid waste as directed by lab safety procedures. Rinse the internal waste bottle with undiluted bleach.
• Screw the cap back onto the internal waste bottle and place back in the C-clip housing.
• Under the MAINTAIN tab, select “Service”. Select “Check Waste bottle” to test the seal of the internal waste bottle. This takes approximately 20 seconds; while the test is running, the button reads “Check is running”; if the test passes, the button changes back to “Check Waste bottle”. If the test doesn’t pass, the button will read “Waste Check failed – Click again”. Ensure the bottle cap is tightly screwed on and all luer fittings are tight. Repeat the test.