

Laboratory Procedure Manual

Analyte: **Glycohemoglobin (*HbA1c*)**

Matrix: **Whole Blood**

Method: **Trinity Biotech Boronate Affinity HPLC**

Method No.: Not applicable for this procedure

Revised:

As performed by: *Diabetes Diagnostic Laboratory*
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Important Information for Users

University of Missouri periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
GHB_J	LBXGH	Glycohemoglobin (%)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Test Principle – Glycated proteins (hemoglobins and plasma proteins) differ from non-glycated proteins by the attachment of a sugar moiety(s) to the former at various binding sites by means of a ketoamine bond. GHb and GPP thus contain 1,2-cis-diol groups not found in non-glycated proteins. These diol groups provide the basis for separation of glycated and non-glycated components by boronate affinity chromatography. In this analytical technique, a boronate such as phenylboronic acid is bonded to the surface of the column support. When a solution of proteins (hemolysate or diluted plasma) is passed through the column, the glycated component is retained by the complexing of its diol groups with the boronate. After the unretained nonglycated component elutes from the column, the glycated component is eluted from the column with a reagent that displaces it from the boronate. The Premier Hb9210™ employs the principles of boronate affinity and high-performance liquid chromatography (HPLC). The pumps transfer reagents through the analytical column which contains aminophenylboronic acid bonded to a porous polymer support (gel). Hemolysed samples for HbA1c analysis are automatically injected onto the column during the flow of Premier Hb9210™ Reagent Buffer A. The glycated component binds to the boronate, while the non-glycated component passes through the column to the spectrophotometric detector, where it is detected at 413 ± 2 nm. After the elution of the non-glycated component, the system pumps Premier Hb9210™ Reagent Buffer B, which displaces the glycated component from the column. The glycated component then passes through the detector. See below for a diagram of the binding of glycated hemoglobin in the Premier Hb9210™ System. The compositions of Elution Buffers A & B are designed to exhibit virtually identical absorption in the 413 ± 2 nm range to ensure a stable baseline. The detector signal is also referenced by the split-beam technique. In the final stage of the cycle, the column is re-equilibrated with Elution Reagent A. All reagent selection occurs in a timed sequence designed to allow complete elution of non-glycated and glycated components. All functions are controlled by the computer. The computer processes the signal from the spectrophotometric detector and calculates the concentration of glycated hemoglobin or plasma protein as a percentage of the total detected. Integration is by peak area in Absorbance Units (AU)-seconds. The computer produces printed reports by analyzing signal as it is

received by the detector. A Batch Summary Report is printed at the end of the run. The software, especially designed for HbA1c analysis, controls the four basic system operation functions of sample identification, instrument operation, and calculation of results as well as printing and storing complete reports. Based on computer hard disc capacity, many years' worth of data (chromatograms and batch summary reports) can be automatically archived.

Calculation of the percentage of GHb in the sample is by the following formula, with peak area in AU/seconds:

$$\frac{\text{Peak 2 Area}}{(\text{Peak 1 Area} + \text{Peak 2 Area})} * 100$$

The final result is obtained by comparison to reference samples using a 2-point calibration. The Premier Hb9210™ is traceable to the IFCC method and is also NGSP certified in order to ensure A1c levels are quantitated correctly.

Clinical Significance – Glycated hemoglobin (HbA1c) is of particular clinical interest in diabetes mellitus. Measurement of glycated hemoglobin is a clinically useful means of assessing glycemic control in diabetics. HbA1c values reflect blood glucose levels over the circulatory half-life of the erythrocyte (about 60 days) and correlate significantly with mean blood glucose levels during that time. Therefore, measurement of glycated hemoglobin provides a means, independent of multiple measurements such as patient records of self-monitored blood glucose, for assessing the overall efficacy of therapy. Factors such as diet, exercise, insulin regimen and stress can affect glycemic control, and therefore HbA1c values. In uncontrolled or poorly controlled diabetics, glycated hemoglobin values may be two or three times as high as in non-diabetics, while meticulously controlled diabetics may have HbA1c values near or in the normal range. Uncontrolled or poorly-controlled diabetics brought under better control will exhibit a gradual drop in HbA1c values, reaching a new equilibrium in approximately eight weeks. There is significant evidence that maintaining good glycemic control has a positive impact on the development of the long-term complications of diabetes.

2. SAFETY PRECAUTIONS

No test method can offer complete assurance that Hepatitis B (and C) Virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent. Therefore, all human blood products (including controls) as a hazardous biological material and should be considered potentially infectious.

All healthcare personnel shall routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of an patient is anticipated. All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious disease. Wear appropriate Personal Protective Equipment (PPE), including facial protection, gloves, and protective clothing. When handling biological material and reagents. Wash hands thoroughly after handling specimens and kit reagents.

Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of analysis. Dispose of all liquid hazardous waste in properly hazardous waste container.

Refer to the MSDS for each reagent for specific precautions.

Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where it exists, *for the USA*: Center for Disease Control/National Institutes manual "Biosafety in Microbiological and Biomedical Laboratories", 1984. Place all biologically contaminated liquid waste in a regular sink (contains no mercury) and contaminated solid waste into the biohazard waste. Wear disposable gloves, laboratory coats, facial protection and other appropriate protective devices

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Data are maintained on a secured Microsoft Access/ Microsoft SQL server client-server system in a 128-bit authenticated Windows domain environment.

1. Laboratory services are requested through the Westat system operations via an email notification containing a unique manifest list of the samples and sample analysis type (e.g. GHB), which confirms that specimens have been shipped to DDL.
2. Each Manifest Form should include and be verified against each sample received:
 - a. Patient Sample ID #
 - b. Test Name
 - c. Date Collected
 - d. Shipment ID #
 - e. Shipment Date
 - f. Lab Name
 - g. Lab ID
 - h. Survey Year
3. Once specimens are received and verified the corresponding file is imported electronically into the SQL server database via secure transfer.
4. After analysis the results, date analyzed and tech initials are imported from the instrument into the SQL server database via secure transfer.
5. Data check sheets are printed out and checked against the instrument printouts by the supervisor or delegate.
6. After results are cleared by the supervisor a results file in the specified format is exported and uploaded to Westat via secure transfer.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- i. Specimen Collection and Handling

Each specimen should be labeled with a sample ID and test name. Sample IDs should be verified against the sample manifest that list the below;

- a. Patient Sample ID #
 - b. Test Name
 - c. Date Collected
 - d. Shipment ID #
 - e. Lab Name
- ii. Specimen Type and Stability:
- a. EDTA Whole Blood mixed thoroughly.
 - b. HbA1c in whole blood:¹
 - i. Room Temperature for 6 days
 - ii. 2 to 8°C for 30 days
 - iii. 37°C for 1 day
 - iv. Freezer (-20 to -15°C) for 15 days
 - v. Ultra-low Freezer (-85 to -60°C) for 2 years
 - vi. Refer to the Premier validation binder for in-house specimen stability studies.
 - c. Minimum volume
 - i. Offline dilution ~ 50 µL
- iii. Offline Dilutions: All whole blood must be diluted with diluent prior to HPLC separation
- Fresh and previously frozen EDTA whole blood
1. Ensure all specimens are well-mixed
 2. Using an autodilutor set to dilute 12 µL of whole blood with 1488 µL diluent, dispense dilutions for patient specimens into clear 13 x 75mm clear polystyrene tubes. Controls should always be prepared alongside patient specimens, and calibrators and controls use blue-tinted 12 x 75mm polystyrene tubes.
 3. Dilution ratio may be altered in order to obtain a concentration of hemoglobin, as determined by total area of the resulting chromatogram, within the desirable range of 600,000 to 1,250,000.

4. Dilution volume may be lowered (with the same dilution ratio) if using a conical bottom polystyrene or blue polypropylene tube.
 5. If the sample does not have a barcode, place lab tape on the back side of the rack so that the instrument will detect the presence of a sample in that spot.
 6. The use of offline dilutions prepared more than 8 hours previous should be avoided.
- iv. Unacceptable specimen criteria:
1. Avoid coagulated blood specimens.
 2. Specimens collected in tubes with preservatives/anticoagulants other than EDTA.
 3. Leaking specimens.
 4. Unlabeled samples (specimens are to be labeled with the specimen ID and test name).
 5. The below must be followed for unaccepted specimen collections:
 - a. Document each unacceptable specimen in the Preanalytical & Problem Resolution Log by filling out the test name, date received/collected, sample ID, reason for rejection, form of contact, and tech's initials.
 - b. Avoid specimens which have leaked their contents (document the specimen IDs of leaky specimens and contact NHANES immediately).

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

- i. Equipment
 1. Trinity Biotech Premier Hb9210 Automated HPLC System
 - a. Computer with Trinity Premier Software

- b. Touchscreen monitor
 - c. Printer
 - d. Barcode Scanner
 2. Autodilutor with 2.5 mL and 25 μ L syringes.
- ii. Reagents (Obtained from Trinity)
 1. Boronate Affinity Column
 - a. p/n 09-06-0046
 - b. 500 Injections
 2. Buffer A Reagent
 - a. p/n 01-03-0095
 - b. 940 mL
 - c. 30 days on-board/ open stability at room temperature
 3. Buffer B Reagent
 - a. p/n 01-03-0096
 - b. 940 mL
 - c. 30 days on-board/ open stability at room temperature
 4. DIL Reagent (for Rinse Station and Syringe)
 - a. p/n 01-03-0097
 - b. 3.8 L
 - c. 30 days on-board/ open stability at room temperature
 5. WASH Reagent
 - a. p/n 01-03-0098
 - b. 940 mL
 - c. 30 days on-board/ open stability at room temperature
 6. Pump Rinse Water
 - a. Type I water (From Millipore Synergy)
 - b. 940 mL
 - c. Changed weekly
 7. DIL reagent for Hamilton Autodilutor
 - a. Daily use
 - b. Pour-off from DIL or Syringe bottle currently on instrument
 - c. Discard at end of each day

iii. Other materials

1. Sample Vials

- a. 13 x 75 mm untreated polystyrene test tubes (clear) for hemolysates (Request as needed from Trinity)
- b. 12 x 75 mm untreated polystyrene test tubes (blue) for controls and calibrators (Request as needed from Trinity).

iv. Quality Control Materials

- i. Quality Control Preparation: Donors are recruited and compensated for their blood. Blood products are pooled together, mixed for at least 30 min, and aliquoted under refrigerated conditions, see below.

1. Pooled Low Control

- a. Single level low control was prepared from EDTA whole blood whole blood drawn from known non-diabetic individuals (Normal level HbA1c). Refer to the QC and Calibrations' Manual the current QC lot in use.
- b. The blood specimen were pooled, dispensed in 50 μ L aliquots into 400 μ L microtubes under refrigerated conditions. Batches of low controls (aliquots) were assigned a lot number, preparation date (date the controls were collected), and stored at -70°C on the same day (working stock for daily usage, no more than 50 tubes). The remaining aliquots were assigned the same lot number/date and placed in a cryogenic (liquid nitrogen) tank at -196°C in freezer boxes on the same day (long term storage).

2. Pooled High Control

- a. The elevated (Abnormal) HbA1c level pooled whole blood (EDTA) controls were purchased from Aalto Scientific. Refer to Aalto Scientific product insert for additional details. Refer to the QC and Calibrations' Manual the current QC lot in use.
- b. The blood was dispensed into 250 μ L aliquots. Batches of high control, were assigned a lot number, date these controls were made and stored at -70°C on the same day. The remaining high

control aliquots we assigned the same lot number/date and were placed in a cryogenic (liquid nitrogen) tank at -196°C.

v. Calibrators

Calibrator Preparation: Donors are recruited and compensated for their donation of blood. EDTA whole blood tubes are pooled together, mixed for at least 30 min, and aliquoted under refrigerated conditions, see below.

1. Pooled Low Calibrator

- a. Single level low calibrator was prepared from K₂EDTA whole blood drawn by venipuncture from four non-diabetic individuals.
- b. The blood specimens were pooled, dispensed in 30µL aliquots into 400µL microtubes under refrigerated conditions. Batches of these low calibrators (aliquots) were assigned a lot number, preparation date and stored at -70°C on the same day (working stock for daily usage, not more than 50 tubes). The remaining aliquots were assigned the same lot number/date and placed in a cryogenic (liquid nitrogen) tank at -196°C in freezer boxes in the same day (long term storage).
- c. Low HbA_{1c} calibrator values were assigned by analyzing once at the beginning of a run and once at the end of a run for at least 10 analysis batches on 10 different days for a minimum of 20 analyses. The mean of these analyses was used as the assigned value, or rounded to the nearest tenth decimal place if the instrument allows only single decimal place precision for calibrator assigned values. Refer to quality control (QC) and Calibrator's binder and the controls and calibrator color key table (locate at the bench) for the current the controls and calibrator acceptable ranges.
- d. The acceptable calibrator values used should be within 2 standard deviations (SD) of the assigned value.

2. Pooled High Calibrator

- a. Single level high calibrator was prepared from pooled EDTA whole blood purchased from Aalto Scientific. Refer to Aalto Scientific product insert for additional details.
- b. The blood was dispensed into 250 μ L aliquots under refrigerated conditions. Batches of these high calibrators (aliquots) were assigned a lot number, date the high calibrators was made (and received from Aalto) and stored at -70°C on the same day (working stock for daily usage, no more than 50 tubes). The remaining aliquots were assigned the same lot number/date and were placed in a cryogenic (liquid nitrogen) tank at -196°C in freezer boxes on the same day (long term storage).
- c. High HbA1c calibrator values were assigned by analyzing once at the beginning of a run and once at the end of a run for at least 10 analysis batches on 10 different days for a minimum of 20 analyses.
- d. The mean of these analyses was used as the assigned value, or rounded to the nearest tenth decimal place if the instrument allows only single decimal place precision for calibrator assigned values.
- e. The acceptable calibrator values used should be within 2 standard deviations (SD) of the assigned value.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Preparation and Stability – Aliquots from Liquid Nitrogen to -70°C .

1. Removed one box of calibrators from the liquid nitrogen phase to the vapor nitrogen phase for at least 2 hours. After 2 hours, transfer box into the -70°C freezer for at least 2 hours to allow materials to acclimate. If box is already in the vapor nitrogen phase, move box into the -70°C freezer and allow materials to acclimate.
2. After the acclimation period, transfer 50 tubes (while working in the freezer) into a box labeled the material's name, date removed from liquid nitrogen, and with

an expiration date 60 days into the future (studies have shown whole blood stability for up to two years at -70°C).

3. Return the box with the remaining calibrator aliquots to the vapor nitrogen phase of the liquid nitrogen. Calibrators are stable for up to five years or longer in nitrogen.
 4. Low and high calibrator aliquots are pulled from freezer daily and placed in the refrigerator ($2-8^{\circ}\text{C}$) at start of the day. Prior to use, thaw materials at room temperature, and mix by inversion gently.
- vi. Frequency: Calibration is to be performed;
 1. When the instrument is initially installed.
 2. Daily (on the day the test is being performed)
 3. When a column is changed.
 4. If control values are out of the acceptable range.
 5. If drift in QC is observed.
 6. After a new reagent lot.
 7. After routine maintenance and a PM
- vii. Procedure
 1. Prepare at least 3.0 mL of hemolysate from each level
 2. Place into appropriate spots on control carousel
 3. Select the calibration checkbox when starting run
- viii. Acceptance
 1. Slope and Intercept must be within the parameters set on the instrument
 2. Normal and Abnormal controls analyzed immediately following calibration must be acceptable according to the parameters set on the instrument for the instrument to accept the calibration.
- ix. Calibration Verification
 1. Calibration verification is performed roughly every 6 months using CAP Linearity survey LN15.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

i. Special safety precautions

1. Gloves, lab coat, and safety goggles are required for handling all human blood specimens, analyzer (and components), and reagents. When working in the biological safety cabinets, appropriate personal protective equipment (PPE) shall be worn.
2. Vials containing human blood should be opened in the biological safety cabinet with the sash in the correct position.
3. All plastic tips, sample cups, gloves, etc. that contact blood are considered contaminated and are to be placed in a biohazard waste container.
4. All hoods, telephones, doorknobs and work surfaces are wiped down with Oxyvir disinfectant or 10% bleach at least one time during each work shift. Any area in which blood is spilled is also to be cleaned and disinfected immediately with Oxyvir disinfectant or 10% bleach.

ii. Operation

1. From the standby mode screen, enter the username and password. (Default: Administrator, 7235)
2. Visually verify reagents are sufficiently full and within the expiration date (30 days since opening)
 - a. Reagents containing irritating chemicals. Always wear PPE when handling, and flush unused buffer down the drain with plenty of water.
3. Verify column can complete run within lifetime of 500 injections. This counts only samples, not controls, calibrators, or re-equilibrations (blanks).
 - a. Follow instructions on instrument to replace the column and frits (Chapter 10.2.6). When changing the frits, be certain to

place paper towels underneath the mixing valve to prevent the spilling of reagents into the sample loader.

4. If this is the first run of the week, replace the water in the water bottle.
 - a. Rotate T-Valve 45° counterclockwise. Remove lines from water bottle. Pour used water down the drain and refill with deionized water. Replace the bottle and lid. Rotate T-valve 45° clockwise.
5. Activate the instrument from the menu.
6. Address any warnings that pop up.
7. Once the pumps are running, make sure no leaks are present
8. When the instrument returns to “Standby”, record the pressure (Lower right hand corner of the Main screen).
9. Record the number of column injections remaining (Click on the Reagent Bottle from the Main screen).
10. Prepare dilutions
 - a. If possible, print labels for sample hemolysates to be prepared.
 - b. Ratio should be set at 7:993 μL or 12:1438 μL . The lower volume is sufficient for conical bottom tubes and the higher volume for round-bottom tubes.

Controls should be diluted into blue tubes, or, alternatively, into conical bottom clear tubes, but never into the clear round-bottom tubes. An additional dilution of each level should be made into a typical tube used for samples, as two controls will need to be run as samples on each run.
 - c. Try to use dilutions only on the day of preparation.

Manufacturer claims stability over 6 days if kept at 2 to 8°C. Please avoid using dilutions from prior days if possible.
11. Load freshly diluted controls and calibrators into the carousel.
 - a. Be careful to not pull on the brown PEEK tubing above the carousel, as it is easy to pull this out of its frit.
12. Load sample racks. Sample order is OPPOSITE of G8.

- a. Lab tape should be adhered to the back side of the hemolysates racks when a sample is present and does not have a barcode. This is to assure that the instrument detects the presence of the sample. Please print barcodes whenever possible.
13. Choose to Calibrate if this is first run of the day. Calibrations are performed daily.
14. Press Ok.
15. Press the database button to pull up samples from the current analysis day.
 - a. Sample ID numbers can be edited here, as required.
 - b. Sample ID's entered prior to the completion of the run will be included in the report printed, so please complete prior to the end of the run.
16. After the completion of the run, a PDF is automatically created. Press print from the PDF program to print out the chromatograms etc. After printing, close the PDF program.
17. Enter batch/run information and control values into A1c Dairy Sheet.
18. Select Premier 1
19. Verify that the default values are correct. For example Run #, calibrator values, buffer information with the accurate expiration date & date calibrated. The select next.
20. At the Premier Dairy Sheet Entry Form Sheet, enter the assay date; tech initials, and check to see reagents are not expired.
21. Select Control Values and enter the low and high QC results from the instrument run.
22. Under sample information, enter the samples that were analyzed. E.g. Pathology samples or under Study sample type in the study samples that were assayed.
23. Select Finish, Print, and Close.
24. In the Primus data base select A1c Lab Result
25. Enter A1c Result and select the Premier method.

26. Enter results.

27. Print Batch number and tech initials.

iii. Calculations:

1. GHB to HbA1c: $GHB = [1.701 * NGSP] - 2.901$

2. NGSP to IFCC: $NGSP = [0.09148 * IFCC] + 2.152$

3. HbA1c to eAG: $eAG = [28.7 * HbA1c] - 46.7$

iv. Reporting Format – Results are expressed as % HbA1c aligned to NGSP units and are rounded to one decimal place

1. Visual verify for each chromatogram

a. Total area is within allowable bounds

b. Pressure fluctuations are not extreme

c. Baseline drops below 5 mm between non-glycated and glycated peaks

d. HbA1c result on instrument printout matches result reported in database printout.

2. Verify control values meet acceptance criteria

3. Once the results are acceptable, clear these results in the Primus database.

4. Pathology results are cleared in Pathnet by selecting the “UH HbA1c Tosoh” worklist and by selecting the corresponding worklist ID number on the located on the top left of the laboratory worksheet.

V. Reporting Results Comments

a. HbA1c results that exhibit presumptive variant peaks (on the G8 HPLC method) should be reported with a 138 comment code “An abnormal peak consistent with hemoglobin was detected. This individual should be advised that some other HbA1c testing methods may show interference from certain hemoglobin variants. A boronate affinity method was used to determine this interference –free HbA1c result.”

b. Presumptive variants identified on the G8 HPLC method are further analyzed on the Sebia Capillarys CE platform. Refer to the

NHANES Sebia Capillarys 2 Flex-Piercing Hemoglobin Electrophoresis Manual.

9. REPORTABLE RANGE OF RESULTS

3.1% – 19.5%

Results below 4.0% or above 14.0% are reanalyzed for verification prior to results being reported. Results outside of current linearity values are reported as < (low linearity value) or > (high linearity value) as appropriate.

- i. It is the responsibility of the tech to check the acceptability of each chromatograph and HbA1c result prior to submitting all relevant paper work to the supervisor (or delegate). A verification check should include acceptability of QC values, peak areas/valley heights, stable baseline, etc. Refer to the operator's manual Chapter 7 for additional details.
- ii. A Batch Summary Report summarizes information from the individual reports in convenient form for review. A Batch Summary Report is automatically generated at the finish of each batch run.
- iii. The chromatogram is a plot of the signal output of the spectrophotometric detector. The first peak is non-glycated (hemoglobin not bound to the boronate resin); the second contains the glycated components (hemoglobin that binds to the boronate).
- iv. Submit all instrument printouts, Premier HPLC Diary Sheet, and all relevant paperwork to the lab supervisor (or delegate) for the results to be verified and released.

10. QUALITY CONTROL (QC) PROCEDURE

Preparation and stability – Aliquots from Liquid Nitrogen to -70 °C.

1. Removed one box of controls from the liquid nitrogen phase to the vapor nitrogen phase for at least 2 hours. After 2 hours, transfer box into the -70°C freezer for at least 2 hours to allow materials to acclimate. If box is already in the vapor nitrogen phase, move box into

the -70°C freezer and allow materials to acclimate.

2. After the acclimation period, transfer 50 tubes (while working in the freezer) into a box labeled the material's name, date removed from liquid nitrogen, and with an expiration date 60 days into the future (studies have shown whole blood stability for up to two years at - 70°C).
 3. Return the box with the remaining control aliquots to the vapor nitrogen phase of the liquid nitrogen. Controls are stable for up to five years or longer in nitrogen.
 4. Low and high control aliquots are pulled from freezer daily and placed in the refrigerator (2-8 °C) at start of the day. Prior to use, thawed materials at room temperature, and mixed by inversion gently.
 5. QC specimens are tested in the same manner as patient specimens and by the same personnel performing patient testing.
- iii. The lab supervisor (or delegate) verifies the acceptability of the run.
- iv. Frequency
1. Controls are run at the beginning and at the end of a run (per batch).
 1. Immediately after calibration.
 2. After a PM

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

1. The values obtained for controls must fall within the established ranges approved for each control level.
2. Controls are reviewed and results must be considered acceptable prior to reporting patient results.
3. If results are outside of the acceptable limits, **patient results should NOT be reported**. Patient samples are to be re-analyzed once corrective actions has been taken and QC results are acceptable.
4. Corrective actions could include repeating QC with a new set of QC aliquots, or pulling new QC material from liquid Nitrogen, or adjusting the flow rate, changing the column, etc.

5. QC results must be acceptable prior to releasing patient results. If QC results are still unacceptable after performing corrective actions immediately, call the Tosoh technical line at 1-800-2486764 for additional technical trouble-shooting advice and alert the supervisor or delegate immediately

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

- i. For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (for example, signs and symptoms, duration of diabetes, results of other test, age of patient, clinical impressions, degree of adherence to therapy, etc.).
- ii. The life span of red blood cells is shortened in patients with hemolytic anemias, depending upon the severity of the anemia. Consequently, specimens from such patients may exhibit decreased HbA1c levels.
- iii. The life span of red blood cells is lengthened in polycythemia or postsplenectomy patients. Specimens from such patients may exhibit increased HbA1c levels.
- iv. Levels of Fetal Hemoglobin (HbF, $\alpha_2\gamma_2$) are greater than 10% of the total area on an IE-HPLC method such as Tosoh G8⁴.
- v. Refer to the Premier Hb9210™ Operator's Manual, Chapter 3 for additional details.

13. REFERENCE RANGES (NORMAL VALUES)

Range: 4.0 to 6.0 % (equivalent of mean blood glucose of 60 – 120 mg/dL). The normal range for the HbA1c test was established at the Diabetes Diagnostic Laboratory in February 2000 based on 181 non-diabetic subjects collected from the continental United States. Subjects were confirmed to have fasting blood glucose less than 110 mg/dL (2000 standard for non-diabetic classification). The mean HbA1c was 5.0%, with a 99% Confidence Interval of 4 to 6%. Reference Range studies were repeated in 2004, 2009 and 2012 in the same manner except that subjects were included only if their fasting glucose was less than 100 mg/dL (Current

ADA criteria for non-diabetic classification). In all studies, the original range of 4-6% was confirmed.

Interpretation: The level of HbA1c is an index of average blood/plasma glucose during the preceding 3-4 months and has strong predictive value for diabetes complications. HbA1c \geq 6.5% is diagnostic for diabetes. HbA1c 5.7-6.4% indicates increased risk for diabetes Note: Any condition that shortens erythrocyte survival or decreases means erythrocyte age (e.g. hemolytic anemia) will falsely lower HbA1c results regardless of the assay method.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable for this procedure

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

1. Samples are to be kept refrigerated at 2-8°C immediately after collection.
2. Transport under refrigerated conditions.
3. Once received and prepared for analysis, specimens are to be immediately returned to 2-8°C storage. Specimens may be frozen and stored at -70°C (DO NOT FREEZE SAMPLES AT -20°C).

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Not applicable for this Procedure

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable for this Procedure

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

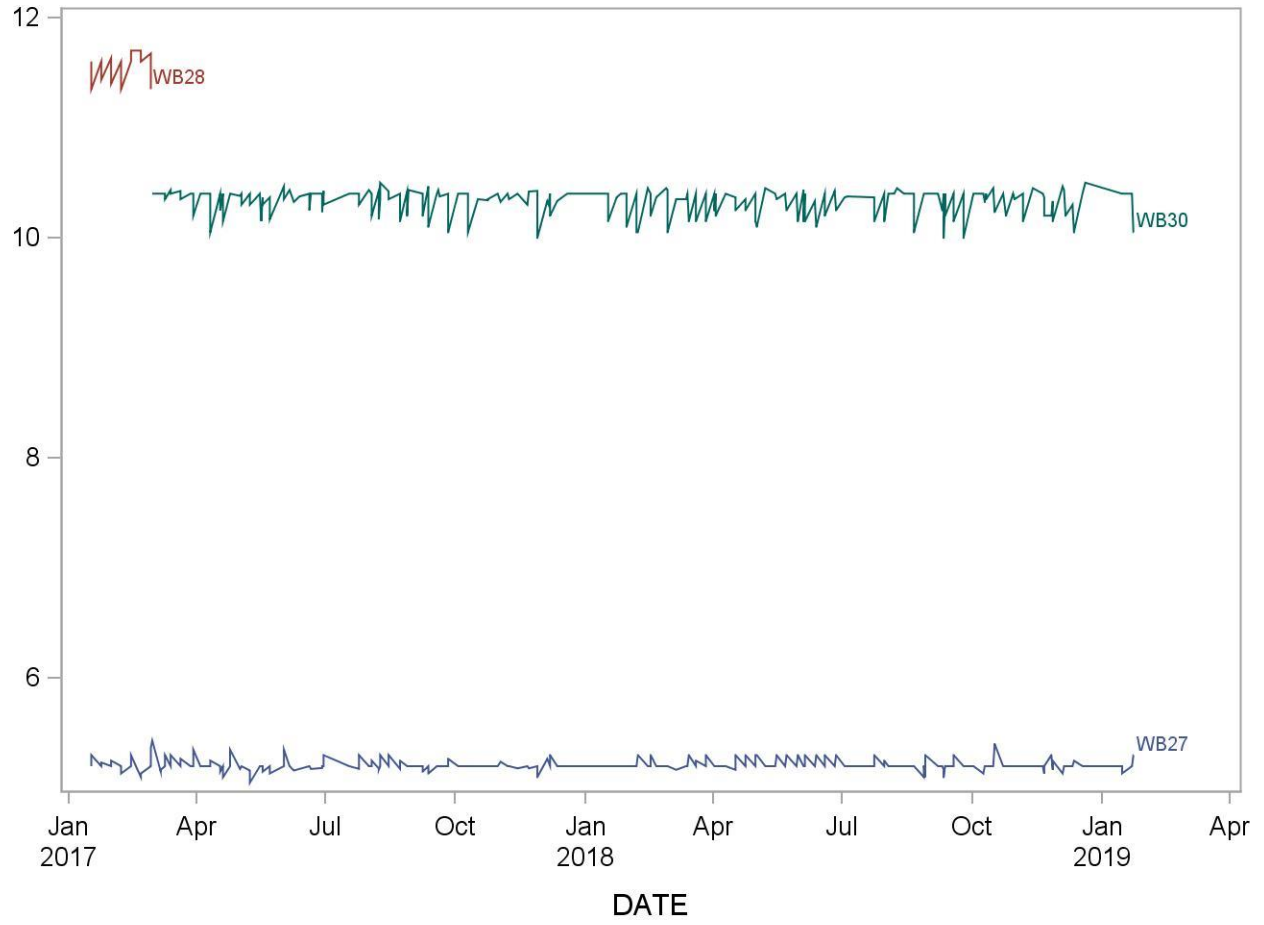
Not applicable for this Procedure

19. SUMMARY STATISTICS AND QC GRAPHS

See following pages.

2017-2018 Summary Statistics and QC Chart for Glycohemoglobin (%)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
WB27	269	17JAN17	23JAN19	5.22	0.06	1.1
WB28	14	17JAN17	28FEB17	11.54	0.13	1.2
WB30	257	01MAR17	23JAN19	10.29	0.13	1.3



20. REFERENCES

1. Trinity Biotech Premier Hb9210 Operator's Manual.
2. Stability Study (see Premier validation binder)
3. Aalto Package Insert
4. Little RR, Rohlfing CL, Hanson SE, Schmidt RL, Lin C-N, Madsen RW, et al. The effect of increased fetal hemoglobin on 7 common Hb A1c assay methods. Clin Chem. 2012;58: 945–947.