UnityPoint Health Proctor
5409 N Knoxville Avenue

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Hemoglobin A1C
Glycohemoglobin
High Performance Liquid Chromatography
Test Code Glyco

I. **PRINCIPLE:**

The TOSOH G8 uses non-porous ion exchange, high performance liquid chromatography (HPLC) and microcomputer technology to measure the HbA1c as a percentage of the total amount of hemoglobin present in the sample. A dilute specimen of whole blood is injected into the G8 column. The hemoglobin fractions attach to the resin surface. These fractions are removed from the column by performing elutions using differing Concentrations of the three buffers. The separated hemoglobin components pass through the LED photometer flow cell where the analyzer measures the changes in absorbance at 415 nm. The analyzer calculates the percentages of each hemoglobin fraction. The Tosoh Automated Glycohemoglobin Analyzer is certified by the National Glycohemoglobin Standardization Program (NGSP). The final reportable result is traceable to both the Internationational Federation of Clinical Chemistry (IFCC) and the Diabetes Control and Complications Trial (DCCT).

II. CLINICAL SIGNIFICANCE: Although fasting blood glucose gives the clinician information about the patient's status over the last twelve hours, the stable HbA1c offers a more accurate indication of the patient's long-term diabetic control over the last two to three months. The results from this assay should be used in conjunction with other data (symptoms, results of other tests, clinical impression, degree of adherence to therapy, etc.). This test should not be used to diagnose diabetes mellitus.

III. **SPECIMEN:**

Collect whole blood specimens in K3EDTA collection tube and mix thoroughly. The specimen may be stored up to 24 hours at room temperature or 14 days at 2-8 degrees.
Minimum volume required for direct tube sampling is 1 ml.
Minimum volume in a sample cup is 50ul of whole blood.
Minimum volume of a diluted sample in a sample cup is 150 ul
Specimens with a low hematocrit should be spun down and a 1:250 dilution made with the packed red blood cells using Hemolysis & Wash Solution. Specimen Dilutions should be made just prior to testing in a micro sample cup and labeled with the patient last name and accession number. The holder for the sample cup should be labeled with a duplicate barcode label from Sunquest. Sample aliquots should be labeled with an aliquot label from

the Sunquest computer and stored as listed above.

IV. REAGENTS

- Elution Buffer 1-open expiration date for buffers is 3 months Store at Room Temperature
- Elution Buffer 2
- Elution Buffer 3
- Hemolysis & Wash Solution- open expiration date is 3 months
- TOSOH Calibrator Set- stable 7 days @ 2-8 degrees. Use Calibration material that is verified for the TOSOH G8 instrument
- Linearity Kit- stable until the date printed on the label @ 2-8 degrees.Use AMR validation material that is verified for the TOSOH G8 instrument
- Control – Normal and Abnormal- Store at – 10 to -70 degrees until the expiration date or 6 months at 2 to 8 degrees or until the expiration date on the bottle.
  Once open the control is good for 14 days stored at 2 to 8 degrees C.

V. INSTRUMENTATION/EQUIPMENT

1. EQUIPMENT
   - Tosoh G8 analyzer
   - G8 Variant Column- Keep refrigerated and use before the expiration date
2. SUPPLIES
   - Filter
   - Thermal Paper
   - Sample Cups
   - Racks
   - 10 ul and 500 ul MLA pipette
   - Pipette tips
   - Plastic Pipette

VI. Daily Maintenance:

The following procedures should be performed on a daily basis before patient samples are assayed. Document the maintenance on the G8 maintenance log.

1. Check flow during warm up and look for leaks. Tighten fittings as required.
2. Record the pump pressure during warming up. Replace the filter element if the pressure is above the pressure range listed on the column package insert.
3. Check and Record the filter counter (replace at 400 injections) and column counter.
4. Check the volume and open expiration dates of the reagents.
   A. Replace the buffers when the alarm sounds. There will be 5% reagent left, so there should be enough reagent to finish the run. <u>All Buffers</u> must match with the column letter.

B. Make sure the instrument is in STAND-BY
C. Remove the old Elution Buffer container. REAGENTS MUST BE AT ROOM TEMPERATURE. DO NOT SAVE AND MIX OLD AND NEW REAGENTS
D. Place the color-coded tubing into the new bag. Ensure that the end of the tubing is touching the bottom of the container.
E. Mark the Open Date and Expiration on the new Buffer
F. Highlight the "Reagent Change" key.
G. Highlight the key corresponding to the replaced reagent(s).
H. Press the "CHANGE" key and the confirmation message will appear.
I. If reagents have been changed, press the "OK" key.
J. The reagent(s) will automatically be pulled through the analyzer.
K. Confirm that the graph for the reagents returns to 100%.
L. Run QC material after Reagent Changes.
M. Document the Lot Number and Reagent Changed on the G8 Maintenance Log.
N.Replace the Hemolysis & Wash Solution when the remaining volume is low using the same procedure E-M.

VII. QUALITY CONTROL:

Two levels of quality control material will be assayed once every 24 hours of use, after Reagent change, after calibration and after column replacement/calibration. Quality Control Material once opened is good for 14 days at 2-8 degrees.

1. Allow the vials to come to room temperature. Swirl to mix thoroughly.
2. Remove 10 ul of Level 1 and Level 2 QC material and place into a labelled cup.
3. Add 1,500 ul of Hemolysis & Wash Solution to the 10 ul Aliquot to make a 1:150 working dilution.
4. Swirl to mix.
5. Place the labelled Level 1 cup onto a rack with a cup adapter labelled Level 1 and the labelled Level 2 cup into the adapter labelled Level 2.
6. Turn on the G8 and allow the analyzer to go through the Warm-Up
7. The results from the G8 auto verify. Run the QC through first without patients to ensure the analyzer is working properly.
8. Press "START".
9. The ID number for Level 1 will be 0001-01 and Level 2 will be 0001-02 (if this is the first rack)

10. Enter the results in Sunquest

a. Function: Result Entry
b. Interfaced = Configuration YG8
   or Manual = Configuration YTOSHO PRP A1C
c. Acc. no.: C-YGLYC1 for Level 1 and C-YGLCY2 for Level 2
d. Label the tape.
e. Record the results from the tape. If the results are not in range, repeat and fill out a corrective action sheet.
f. Do not run the analyzer if the QC is not in range
g. To observe the Levy-Jennings graphs for shifts and trends when running QC
h. Function: RP
i. Printer: 0 for on screen viewing or designate a printer in the lab
j. Select 7- QC reports
k. Select (L) Levy-Jennings
l. Start Date: Enter date in the Past
m. End Date: T (for today)
n. Test: GLYA1C
o. Method: Return Worksheet: Return
p. Control: YGLYC1
   YGLYC2
q. Return at shift, Tech
r. Select A(accept) at the following prompts- test, worksheet, method, controls, shifts, techs
s. Select S (plotted singly)
t. If quality control results do not fall into range on repeat, then recalibrate the test and rerun control material. Do not report patient results if the controls are not in range.

11. Check to make sure that the chromatographs for QC and patients are well defined and have a sharp peak. Changes in the chromatographs are an indicator of a need to reverse/change the column.
12. Quality Control Data and QC statistics are evaluated monthly. Level 1 CV% should be less than or equal to 2% and Level 2 CV% should be less than or equal to 2%. We also participate in a peer comparison program. Investigate all SDI > 2.0.
13. When a new lot of QC material is received,enter the new lot into Sunquest but do not make the Lot Active. Run the QC in parallel with the old lot to make sure the material is

running within the manufacturers range. Insert the manufacturers assayed range in Sunquest. Enter the QC values as C-GLYC1,0 and C-GLYC2,0

VIII. PROCEDURE:

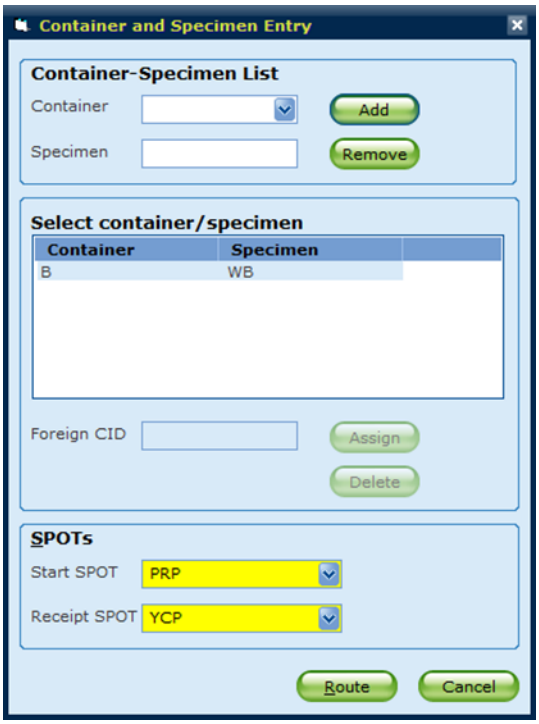
1. Load the QC first in the QC sample holder cups without any patient samples. Make sure the QC results are acceptable before starting the patient samples.
2. Mix the whole blood samples by inversion and place in the sample rack with the barcode label facing toward the instrument
3. Load the rack (s) onto the sample loader.
4. Place an empty rack at the end.
5. Press Start.
6. Add samples to a run by removing the end of run rack and adding a new sample rack. Replace the end of run rack when finished.
7. Results will print on the tape. The accession number is the Sample NO. and the rack and position is the Sample ID.
8. Look at the chromatograph to be sure that there are distinct peaks.
9. Make sure the total area is between 500 and 4000.
10. Look for error messages below the chromatogram.
11. Repeat any unusual samples. If the repeated sample is the same, refer to the Interpretation guide. The Hematology Coordinator or DPIC will review all abnormal chromatographs.

IX. REPORTING RESULTS

1. Function: Result Entry
2. Interfaced = Configuration YG8
   or Manual = Configuration YTOSHO PRP A1C
3. Result
4. The samples autoverify if there are no flags or interpretive messages. Review the analyzer tape for any sample with an error message or flag. These results will be in Result entry.
5. Repeat samples that have POO peaks prior to the AO . Samples with Low area will need to have more sample and less Hemolysis Wash. Samples with High area will need more Hemolysis Wash with the sample dilution.
6. Review the sample for validity.
   A. Hemoglobin Variant peaks after the AO peak can be turned out if the A1C is less 9.0%. Hemoglobin Variant peaks with an A1C of 9.0 or greater CAN NOT be resulted and must be sent to Methodist Lab to run on the BioRad instrument.
   B. POO peak (s) that are after the AO peak the result can be turned out.
7. When the result is valid, click on the check box to Release the result and accept.
8. Results greater than 15.5% or lower than 3.4% should be repeated to verify results and

consider limitations. Report results outside the reportable range as less than 3.6% or greater than 18.7%.

9. Place the tape in the top drawer with the under the box of new rolls of paper. Tapes are stored for 2 years in the box with the XT printouts.
10. Results with peaks prior to the AO peak or Hbg Variant on samples with an A1C greater than 8.9% will be sent Methodist Campus for confirmation.
    a. Cancel the GLYCO accession number as duplicate
    b. Reorder the test code GLYCO on a new accession number with all of the correct collect and receive information.
        1. Do NOT Go PAST THIS Screen



        2. It is necessary to change the start spot and Receipt spot. Start Spot PRM Receipt VCP
        3. Route the specimen. The label that is produced when you route this test will have the Methodist instrument start spot of VROB
        4. Label the specimen with the new label.
        5. Put the specimen on a batch list to Methodist

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X. LIMITATIONS OF THE PROCEDURE

1. Patients who possess no Hgb A will not have HbA1c.
2. Levels of up to 15% fetal hemoglobin do not interfere with HbA1c.
3. Patients with low hematocrits may have a total area less than 500. Make a 1:250 dilution from the packed cells
4. Patients with hemoglobinopathies, which elute prior to the AO peak, will not have reliable results.
5. Hemoglobin Variant peaks for HbA1c values over 8.9% show a negative bias and cannot be turned out. These samples must be sent to a lab using an instrument other than the TOSOH G8. (heterozygous AS, AD and AC)
5. Poo Peaks eluting after the AO peak will not be included in the calculations and do not interfere with the HbA1c result.
6. Glycemic monitoring of patients with homozygous Hgb S, S or SC cannot be accomplished with this method .
7. Falsely elevated results can occur if hemoglobin attaches to other substances besides sugars. Literature cites examples of individuals who may have adducts that may co-migrate with stable HbA1c. These include individuals with opiate addiction, lead poisoning, uremia alcoholism and those on large doses of aspirin.
8. The life span of the red blood cells is shortened in patients with hemolytic anemias, depending on the severity. As a result, specimens from these patients will exhibit decreased glycohemoglobin levels compared to patients with the expected rbc life span.
9. The life span of red cells is lengthened in polycythemia or post-splenectomy patients. Specimens from such patients exhibit increased glycohemoglobin levels. Reference range values will not apply.
10. A1C fractions less than normal range should be repeated/reviewed for a homozygous variant, possible hgb S/C or a shortened life span of the red blood cells.

XI. REFERENCE RANGE:

Glycohemoglobin Male and Female = 4.3-6.1 %
Estimated Average Glucose is turned out with each glycohemoglobin result.
(28.7 X Glycohemoglobin result) – 46.7 = EAG

TECHNICAL RANGE (REPORTABLE RANGE):

Changes with each AMR verification (every 6 months). Send the results to Streck and they will return the report with the new AMR. Have the System Manager enter the new range into Sunquest. Change the information in this procedure.

XII. CALIBRATION

Calibration frequency should be based upon the following criteria:
A. After a new column is installed

B. QC results that will not come into range.
C. Poor Chromatogram quality. Peaks should be sharp and defined. Calibration is needed if the peeks are broad, not separated, jagged or broken into two.
D.After plunger seal replacement or major maintenance
E. When reagents and column change letters (lots)
D.Once every 6 months

1. Reconstitute Calibrators 1 and 2 with Reagent Grade water using a volumetric pipette to add 4.0 ml.
2. Replace the caps and mix thoroughly by inversion.
3. Select "CALIB" from the main screen
4. Select "MENU"
5. Select "PARAMETER"
6. Press CALIB-1 and enter the assigned value for this calibrator. Printed on the bottle.
7. Press CALIB-2 and enter the assigned value for this calibrator. Printed on the bottle.
8. Pipette at least 800 ul of each calibrator into a labeled sample cup.
9. Place calibrator 1 in position 1 of the rack and calibrator 2 in the next position.
10. Place the rack on the loader and press the start key.
11. When the calibration is complete, the analyzer automatically accepts or rejects the calibration results. If the calibration is unsuccessful, an error message appears and the run aborts. Fill out a Corrective Action Report and Call the Hotline to resolve the problem of failed Calibration. Do not use the analyzer until there is a successful calibration is successful and the QC and patient samples validate recalibration.
12. The Sample number of all calibrators begin with "9000". The Calibration Report will print on the tape. The old and new calibration values will print**.**
13. Fill out the Calibration Worksheet on the next page. Calibration is acceptable when :
    A. The Difference between sample 2 and 3 is < 0.3
    B. The Difference between sample 4 and 5 is < 0.3
    C. The percent difference from the assigned value of samples 2, 3, 4 and 5 is <20%
    D. Run the Calibrator as a patient. The result should be plus or minus 0.4 from the assigned value
2. Run QC and 2 patients from the previous run to validate successful calibration. Patient results should match within +/- 10%.
3. Staple the print outs to a blank piece of paper and place in the Supervisors box for approval. Store the documentation in the Glycohemoglobin Maintenance Book.

XIII. AMR VALIDATION

1. Validation of the analytical measurement range (known as AMR) of the glycohemoglobin test will be done when one or more of the following criteria are met.

- Every 6 months
- When a new method is put into place
- When a new company's reagents are used

2. Remove the linearity kit from the refrigerator and allow to come to room temperature for 15 minutes.
3. Hold the vials horizontally between your palms and roll back and forth for 20-30 seconds.
4. Mix by rapid inversion 8 to 10 times to ensure complete cell suspension immediately before sampling.
5. Analyze all levels from lowest to highest vial number in the patient mode.
6. Run with the daily run of quality control material.
7. Run each vial 3 times, mixing before each sampling.
8. Make sure the QC results are within range.
9. To evaluate the Validation of the AMR we will calculate the r value.
   A. Open the Glycohemoglobin Calibration Verification Worksheet found in Excel in the Hematology folder.
B. Record each mean from the package insert on the AMR Worksheet
C. Record the mean of each of the 5 results obtained
D. Graph the points.
E. The program will calculate the slope, intercept and correlation.
F. Your correlation should be a straight line and r value greater than 0.90, R square greater than 0.95. Slope between 0.90 and 1.10
G. Send the report to the company for the Validation of the AMR. When the report returns have the System Manager insert the validated range into Sunquest.

XIV. REFERENCES:

Operator's Manual, Tosoh G8 version 3.0, April 2014.
TOSOH Calibrator Package Insert P0300301, July 2013
TOSOH Hemoglobin A1c Controls, April 2014
Bio-RSF HbA1c Calibration Verification/Linearity Package Insert, October 2014

Glycohemoglobin Calibration Worksheet

Calibrator lot # _____ Expiration Date _____

Calibrator 1 assigned value _____
Calibrator 2 assigned value _____

Difference between SA1c of the 2nd and 3rd sample _____ Acceptable 0-0.3

Difference between SA1c of the 4th and 5th sample _____ Acceptable 0-0.3

Result #2 % difference from assigned value _____
Assigned value – Result X 100 = % difference
Assigned value

Acceptable % difference is < ± 30%

Result #3 % difference from assigned value _____

Result #4 % difference from assigned value _____

Result #5 % difference from assigned value _____

Run calibrator with the patient run and 2 Previous Samples
Calibrator 1 value ____ Acceptable ± 0.4 from the assigned value

Calibrator 2 value ____ Acceptable ± 0.4 from the assigned value

Patient 1 new value _____ old value _____Acceptable ± 10%

Patient 2 new value _____ old value _____Acceptable ± 10%

Is Calibration acceptable? Yes No
Attach documentation to this sheet.

Technologist: Date:

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Unitypoint Health Laboratory is a CAP accredited facility, the responsibility of new and/or substantially revised policies and procedures will be restricted the Laboratory Director whose name appears on the CLIA certificate, whose signature appears below.

Policy Created by: Cindy Schroeder Date: May 26, 2015

Medical Director Approval: _____ Date: _____

Change of Medical Director: _____ Date: _____

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
0	Initial Release	Cindy Schroeder	5/26/2015
1	Hemoglobin Variant Protocol for A1C > 8.9%	Cindy Schroeder	4/21/2017
2	Updated Reportable Range	Sheanea LaCock	06/20/2017

Reviewed by

Lead	**Date**	**Coordinator/ Manager**	**Date**	Medical Director	**Date**
Sheanea LaCock	05/01/ 2017				
Sheanea LaCock	6/21/ 17				