POINTE SCIENTIFIC, INC.
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Glycohemoglobin Reagent Set

Intended Use
For the quantitative determination of Glycohemoglobin (HbA_1) in blood by cation exchange resin. The test is to be used to monitor long-term glucose control in diabetes mellitus.

Summary and Explanation of Test
Throughout the circulatory life of the red cell, glycohemoglobin is formed continuously by the adduction of glucose to the N-terminal of the hemoglobin beta chain. This process, which is non-enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. In a classical study, Trivelli et al[1] showed glycohemoglobin in diabetic subjects to be elevated 2-3 fold over the levels found in normal individuals. Several investigators have recommended that glycohemoglobin serve as an indicator of metabolic control of the diabetic, since glycohemoglobin levels approach normal values for diabetics in metabolic control.[2,3,4]
Glycohemoglobin has been defined operationally as the "fast fraction" hemoglobins (HbA_{1a}, A_{1b}, A_{1c}) that elute first during column chromatography with cation-exchange resins. The non-glycosylated hemoglobin, which consists of the bulk of the hemoglobin has been designated HbA_0. The present glycohemoglobin procedure employs a weak binding cation-exchange resin for the rapid separation of glycohemoglobin (fast fraction) from non-glycosylated hemoglobin. Over 80% of the labile fraction of glycohemoglobin is removed during the separation step in this procedure due to the inclusion of the borate buffer system.[5]

Principle
A hemolyzed preparation of the whole blood is mixed continuously for five minutes with a weak binding cation-exchange resin. During this time, HbA_0 binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohemoglobin from the resin. (Note: This binding is temperature dependent. Therefore, a standard should be included in each run.) The percent glycohemoglobin is determined by measuring the absorbance at 415 nm (405-420 nm acceptable) of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent glycohemoglobin.

Reagents
40 Test Kit containing:
1 x 120ml Bottle 8mg/ml Cation-exchange Resin in a borate buffer, pH 6.9.
1 x 30ml Bottle Glycohemoglobin Lysing Resin, 10mM Potassium Cyanide, surfactant added.
40 serum separators.

Reagent Storage
Store reagent at room temperature (21-26°C).

Expiration Dating
All reagents are stable to expiration date stated on the labels. Do not use the reagents past their expiration date.

Reagent Deterioration
Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

Instruments
Use a spectrophotometer able to read at 415nm with linearity to at least 1.5 O.D. units. (405-420 nm is acceptable.)

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Not for internal or external use in humans or animals.
3. Lysing reagent contains cyanide (poison). Do not ingest. Do not mix with acid or HCN gas may be released.

Specimen Collection and Preparation
Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than anticoagulants are required. Collect venous blood with EDTA using aseptic technique. All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.).

Storage
Glycohemoglobin in whole blood collected with EDTA is stable for one week at 2-8°C.

Interferences
Samples that are severely lipemic may cause elevated results. It has been reported that bilirubinemia may interfere with ion-exchange methods.[6]
Fetal Hemoglobin (HbF) may interfere in this assay. Blood samples with total hemoglobin greater than 18 g/dl should be diluted x 2 with deionized water before assay.

Materials Provided
Refer to "Reagents"

Materials Required but not Provided
1. 20 ul and 100 ul Micropipettors
2. 500 ul, 3 ml and 5 ml Pipettes or Dispensers
3. 13x100 mm Glass Tubes
4. Glass or plastic Test Tubes to hold 0.6 ml and 5 ml
5. Rocker or Rotator (e.g. Miles Inc., Diagnostics Division 4651)
6. Glycohemoglobin standard (Cat. No. G7540-STD)
7. Glycohemoglobin controls (Cat. No. G7540-2)
8. Deionized water

Procedure
A. Hemolysate Preparation
   1. Dispense 500ul Lysing Reagent into tubes labeled: Standard, Control, etc. Note: Plastic or glass tubes of appropriate size are acceptable.
   2. Place 100ul of the well-mixed blood sample, standard or control into the appropriately labeled lysing reagent tube. Mix.
   3. Allow to stand for 5 minutes or until complete lysis is evident.

B. Glycohemoglobin Preparation
   1. Dispense 3.0ml of Glycohemoglobin Cation-exchange Resin into 13 x 100 mm glass tubes labeled: Standard, Control, etc. Note: Before use, mix the resin by inverting at least 10 times. Swirl the bottle after addition to each 5 tubes.
   2. Add 100 ul of the hemolysate (from step A3) to resin reagent.
   3. Position the filter separators in the tubes so that the rubber sleeve is approximately 1cm above the liquid level.
   4. Place the tubes on the rocker or rotator and mix continuously for 5 minutes.
   5. Remove the tubes from the rocker or rotator.
   6. Push the filter separators into the tubes until the resin is firmly packed.
   7. The supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.

8. Zero the instrument at 415 nm (405-420nm acceptable) with deionized water as the blank.
9. Read and record the absorbance values for Standard, Control, etc. These readings are for glycohemoglobin.

C. Total Hemoglobin Fraction
1. Dispense 5.0ml deionized water into plastic, or glass tubes labeled: Standard, Control, etc.
2. Place 20 ul of the hemolysate (from step A3) into the appropriately labeled tube of total hemoglobin diluent. Mix.
3. Adjust the instrument to zero absorbance at 415nm (405-420nm acceptable) with deionized water as the blank.
4. Read and record the absorbance values for Standard, Control etc. These readings are for total hemoglobin.

NOTE: This glycohemoglobin assay should be performed at room temperature, 21-26°C. The final reaction products for glycohemoglobin and total hemoglobin appear quite stable. However, the test samples should be read within an hour before evaporation becomes significant.

Limitations

1. This assay should not be used for the diagnosis of diabetes mellitus.
2. This method can be influenced by temperature. Patient specimens should always be assayed with a calibrator included in the run to eliminate temperature influences.
3. Glycosylated HbS and HbC bind more tightly than HbA, and produce lower values. Other hemoglobinopathies (e.g., betathalassemia and hemolytic anemia also produce lowered results.)
4. Results may be inconsistent in patients who have the following conditions: opiate addiction, lead-poisoning, uremia (carbamylated Hb), alcoholism, ingest large doses of aspirin.[7,8,9,10]

Quality Control

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available glycohemoglobin controls with an assayed range. If controls do not fall into the assayed range patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed.

Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

Calculations

Results for the unknowns and controls are calculated as follows:

For each sample, calculate the ratio (R) of the glycohemoglobin absorbance to the total hemoglobin absorbance. Use the following equation to determine unknown concentrations:

$$\text{Unknown (\%)} = \frac{\text{R (Unk)}}{\text{R (Std)}} \times \text{Std Conc. (\%)}$$

Example: A standard containing 10.0% glycohemoglobin had Abs. = 0.490 for the glycohemoglobin fraction and Abs. = 0.560 for the total hemoglobin fraction. An unknown sample had glycohemoglobin Abs. = 0.750 and total hemoglobin Abs. = 0.625. The glycohemoglobin concentration of the unknown is:

$$\text{Standard R} = \frac{0.490}{0.560} = 0.875$$

$$\text{Unknown R} = \frac{0.750}{0.625} = 1.200$$

$$\text{Unknown \%} = \frac{1.200}{0.875} \times 10.0\% = 13.7\%$$

Expected Values

Normal: 6.0 – 8.3%

The normal range represents the 95% confidence interval for 75 subjects with normal glucose values and no history of diabetes. Each laboratory should establish its own expected values. In using glycated Hb to monitor diabetic patients, results should be interpreted individually. That is, the patient should be monitored against him or herself. There is a 3-4 week time lag before % glycohemoglobin reflects changes in blood glucose level.

Performance

1. Linearity: The glycohemoglobin assay shows linearity for glycohemoglobin levels in the range of 4.0%-20%.
2. Comparison: A study on normal and abnormal human specimens between this glycohemoglobin procedure and a widely used column procedure yielded a correlation coefficient of 0.970 and a linear regression equation of y=1.10x-3.00. (n=36, range=5.9-14.2%)
3. Precision:

Within Run: The intra assay precision was established by assaying blood with normal and elevated glycohemoglobin levels twenty times each.

Level	Mean	Std. Dev.	% C.V.
Normal	7.7	0.24	3.1
Elevated	12.8	0.21	1.6

Run To Run: The inter run precision was established by assaying blood with normal and elevated glycohemoglobin levels for ten runs conducted over a five day period.

Level	Mean	Std. Dev.	% C.V.
Normal	8.0	0.32	4.0
Elevated	14.8	0.45	3.0

4. Sensitivity: This glycohemoglobin procedure has a sensitivity of 0.02% glycohemoglobin per 0.001 units of absorbance.

References

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