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GLYCOHEMOGLOBIN REAGENT SET

GLYCOHEMOGLOBIN
For the Determination of Glycohemoglobin in Blood.

SUMMARY AND EXPLANATION OF TEST
Throughout the circulatory life of the red cell, glycohemoglobin is formed continuously by the addition 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 serves 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 (HbAla, Alc) which elute first during column chromatography with cation-exchange resins. The nonglycosylated hemoglobin, which consists of the bulk of the hemoglobin, has been designated HbAo. The present glycohemoglobin procedure employs a weak binding cation-exchange resin for the rapid separation of glycohemoglobin (fast fraction) from non-glycosylated hemoglobin.

PRINCIPLES OF TESTS
A hemolyzed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbAo binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The percent glycohemoglobin is determined by measuring the absorbance at 415nm of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent glycohemoglobin.

REAGENTS (MATERIALS PROVIDED)
40 Test Kit containing:
1. Resin reagent : 8 mg/ml Cation-exchange Resin buffered at pH 6.9.
2. Lysing reagent : 10 mM Potassium Cyanide, surfactant added.
3. Glycohemoglobin Standard : 10 % glycohemoglobin.
4. Serum separators.

PREPARATIONS OF REAGENTS
1. Glycohemoglobin Lysing Reagent: Bring contents to room perature.
2. Glycohemoglobin Cation-Exchange Resin: Bring contents to room temperature. Swirl and gently invert a minimum of 10 times, swirl the bottle after addition to each 5 tubes.

STORAGE
Store reagents at 2 - 8 °C.

EXPIRATION DATING
All reagents are stable to expiration date stated on the labels.

PHYSICAL OR CHEMICAL INDICATIONS OF INSTABILITY
Alterations in the physical appearance of the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

INSTRUMENTS
Use a spectrophotometer or colorimeter set at 415 nm.

SPECIMEN COLLECTION AND PREPARATION
Special preparation of the patient is unnecessary. Fasting specimens are not required. No special additives or preservatives other than the anticoagulants are required. Collect venous blood with EDTA using aseptic technique.

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

INTERFERING SUBSTANCES
Sample which are severely lipemic may cause elevated results. Fetal hemoglobin (HbF) has resin binding characteristics similar to glycohemoglobin value if present. Glycosylated HbS and HbC bind more tightly than HbA1 and produce lower values. Other hemoglobinopathies (e.g., betathalassemia and hemolytic anemia) also produce lowered results.

MATERIALS REQUIRED BUT NOT PROVIDED
1. 20 µl and 100 µl micropipettes.
2. 500 µl, 3 ml and 5 ml pipettes or dispensers.
3. 13 x 100 mm glass tubes.
4. Glass or plastic test tubes to hold 0.6 ml and 5 ml.
5. Rocker or rotator.
6. Glycohemoglobin controls: Normal Level & Elevated Level

PROCEDURAL OUTLINE
Details of Procedure:

A. Hemolysate Preparation
1. Dispense 500 µl Lysing Reagent into tubes* labeled: Standard, Control, Sample 1, etc.
2. Place 100 µl of the well-mixed blood sample, standard or control into the appropriately labeled tube. Mix well.
3. Allow to stand for 5 minutes.
   *Plastic or glass tubes of appropriate size are acceptable.

B. Glycohemoglobin Preparation
1. Dispense 3.0 ml of Glycohemoglobin Cation-exchange Resin into 13 x 100 mm glass tube* labeled: Standard, Control, Sample 1, 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 µl of the hemolysate (from Step A3).
3. Position the Filter Separators in the tubes so that the rubber sleeve is approximately 1 cm 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 Separator into the tubes until the resin is firmly packed.
7. he supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.
8. Adjust the instrument to zero absorbance at 415 nm with deionized water as the blank.(Wavelength range:390-420).

9. Read and record the absorbance values for Standard, Control, Sample 1, etc. These readings are for glycohemoglobin.

   *Do not use plastic tubes.

C. Total Hemoglobin Fraction
   1. Dispense 5.0 ml deionized water into tubes* labeled: Standard, Control, Sample 1, etc.
   2. Place 20 µl of the hemolysate (from Step A3) into the appropriately labeled tube. Mix.
   3. Adjust the instrument to zero absorbance at 415nm with deionized water as the blank.
   4. Read and record the absorbance values for Standard, Control, Sample 1, etc. These readings are for total hemoglobin.

*Plastic or glass tubes of appropriate size are acceptable.

QUALITY CONTROL

The reliability of test results should be monitored routinely using stable quality control materials and analyzed in the same manner employed for the unknowns. We suggest the use of Glycohemoglobin Controls: Normal, Elevated.

Note: This glycohemoglobin assay should be performed at room temperature, 21 - 24°C. When performing the assay outside this range, results may be corrected with a factor. The factor is obtained by the use of a standard. 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.

CALCULATIONS

Results for the unknowns and controls are calculated as follows:

$$\frac{\text{Absorbance of Glycohemoglobin}}{\text{Absorbance of Total Hemoglobin}} \times 10^{*} = \text{Glycohemoglobin \%}$$

***** = Dilution Factor

Example: An unknown sample had an absorbance of 0.662 for glycohemoglobin and an absorbance for total hemoglobin of 0.737. The glycohemoglobin concentration of the unknown is:

$$\frac{0{,}662}{0.737} \times 10 = 9.0\%$$

Note: When using a standard or when a test is performed outside temperature range, (see section "Quality Control:"), the results may be corrected by multiplying with a factor (F).

$$F = \frac{\text{Conc. of Std}}{10} \times \frac{\text{Absorbance of Total Hemoglobin Std.}}{\text{Absorbance of Glycohemoglobin Std.}}$$

e.g the concentration of a standard is 8%. Absorbance of total hemoglobin is 0.590, absorbance of glycohemoglobin is 0.484. The factor is:

$$F = \frac{8}{10} \times \frac{0.590}{0.484} = 0.975$$

Correlated Result: 9.0% x 0.975 = 8.8%

LIMITATIONS OF PROCEDURE

Sample from patients with hemolglobinopathies or decreased erythrocytes survival times may show incorrect results. See section on "Specimen Collection."

EXPECTED VALUES: 6.6 - 8.6%

This range represents the 95% confidence interval for 100 outpatient Subjects with normal glucose values and no history of diabeted. A Study of 31 diabetic subjects showed glycohemoglobin values from 8.4 % to 16.0% . For the diabetic population, a comparison of the fasting plasma glucose with the glycohemoglobin level gave a correlation coefficient equel to 0.84.

PERFORMANCE CHARACTERISTCS

LINEARITY:

The glycohemoglobin assay shows linearity for glycohemoglobin level in the range of 4.0 - 20.0%. Blood samples with total hemoglobin greater than 18 g/dl should be diluted x 2 with deionized water before assay.

PRECISION:

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

Level	Mean	Std. Dev.	% CV
Normal	7.8	0. 21	2.7
Elevated	13.4	0. 2 3	1.7

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	% CV
Normal	7.6	0. 31	4.1
Elevated	13.0	0. 60	4.6

CORRELATION

A comparative study of the glycohemoglobin procedure and another widely used commercial method showed correlation (r) of 0.96.

SENSITIVITY

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

REFERENCES

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