



## TECO DIAGNOSTICS

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## GLYCOHEMOGLOBIN REAGENT SET

### INTENDED USE

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<sup>1</sup> 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 for diabetic, since glycohemoglobin levels approach normal values for diabetics in metabolic control.<sup>2,3,4</sup>

Glycohemoglobin has been defined operationally as the "fast fraction" hemoglobins (HbA<sub>1a</sub>, A<sub>1c</sub>), which elute first during column chromatography with cation-exchange resins. The nonglycosylated hemoglobin, which consists of the bulk of the hemoglobin, has been designated HbA<sub>0</sub>. The present glycohemoglobin procedure employs a weak binding cation-exchange resin for the rapid separation of glycohemoglobin (fast fraction) from non-glycosylated hemoglobin.

### PRINCIPLES

A hemolyzed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, HbA<sub>0</sub> 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 415 nm of the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent glycohemoglobin.

### MATERIALS PROVIDED

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.

### PREPARATION OF REAGENTS AND STANDARD

1. *Glycohemoglobin Lysing Reagent*: Bring contents to room temperature.
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.
3. *Glycohemoglobin Standard*: Reconstitute with volume of distilled water indicated on the vial label. Swirl to dissolve.

### STORAGE AND STABILITY

Reagents are stable until the expiration date stated on the labels. Store Resin and Lysing reagents at 2-30°C. Store standard at 2-8°C.

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

### INTERFERING SUBSTANCES

Samples that 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 HbA<sub>1</sub> 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 × 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

#### 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 × 100 mm glass tube\* labeled: Standard, Control, Sample 1, etc.

*\*Do not use plastic tubes.*

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

### 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 415 nm 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.

### CALCULATIONS

Results for the unknowns should be determined in the following manner:

$$\% \text{ Glyco. (unknown)} = \frac{R(\text{unknown})}{R(\text{standard})} \times \text{Standard conc}$$

where:

$$R(\text{unknown}) = \text{Ratio (unknown)} = \frac{\text{Abs. of Glyco (unknown)}}{\text{Abs. of Total Hb (unknown)}}$$

$$R(\text{standard}) = \text{Ratio (standard)} = \frac{\text{Abs. of Glyco (standard)}}{\text{Abs. of Total Hb (standard)}}$$

Example:

A standard containing 8.0% glycohemoglobin had Abs. = 0.480 for the glycohemoglobin and Abs. = 0.575 for the total hemoglobin. An unknown sample had glycohemoglobin Abs. = 0.962 and total hemoglobin Abs. = 0.746. The glycohemoglobin concentration of the unknown is calculated as follows:

$$R(\text{unknown}) = \frac{0.962}{0.746} = 1.29$$

$$R(\text{standard}) = \frac{0.480}{0.575} = 0.835$$

$$\% \text{ Glyco. (unknown)} = \frac{1.29}{0.835} \times 8.0 = 12.4\%$$

### LIMITATIONS OF PROCEDURE

Sample from patients with hemoglobinopathies 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 diabetes. 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 equal to 0.84.

### PERFORMANCE CHARACTERISTICS

#### 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 × 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

<u>Level</u>	<u>Mean</u>	<u>Std. Dev.</u>	<u>% CV</u>
Normal	7.8	0.21	2.7
Elevated	13.4	0.23	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.

<u>Level</u>	<u>Mean</u>	<u>Std. Dev.</u>	<u>% CV</u>
Normal	7.6	0.31	4.1
Elevated	13.0	0.60	4.6

### CORRELATION

A comparative study of the Teco 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

1. Trivelli, L.A., Ranney, P.H., and Lai, H.T., *New Eng. J. Med.* 284, 353 (1971).
2. Gonen, B., and Rubenstein, A.H., *Diabetologia* 15, 1 (1978).
3. Gabbay, K.H. Hasty, K., Breslow, J. L., Ellison, R.C. Bunn. H.F., and Gallop, P.M.J. *Clin. Endocrinol. Metab*, 44, 859 (1977).
4. Bates, H.M., *Lab Manag.*, Vol. 16 (Jan. 1978).

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Manufactured by:

