The Helena GLYCOTek Affinity Column Method is an affinity chromatographic methodology for the quantitation of glycated hemoglobin (HbA1c) in whole blood to indicate a 120-day time-averaged blood glucose level. An algorithm is provided for calculating the % HbA1c, from the % HbG.

**SUMMARY**

Accurate assessment of chronic (time averaged) glucose control is one of the major difficulties in managing diabetic patients. Even patients with mild disease may show large fluctuations in blood glucose, and single glucose determinations may correlate poorly with mean blood glucose levels. Measurement of glycated hemoglobin (HbG) has gained acceptance as a good assessment of diabetic control.17 Glycated hemoglobins are all hemoglobins binding a sugar or other carbohydrate at any one of many sites on the globin molecule. The term "glycated hemoglobin" has been applied in the past to certain hemoglobin fractions (HbA) separated on the basis of molecular charge, but these fractions do not wholly represent the glycated hemoglobins. Glycation does not occur during biosynthesis but is a non-enzymatic, two-stage condensation of glucose with various amino groups of the hemoglobin molecule. Glycation occurs slowly throughout the life span of the mature red blood cell and is dependent on the circulating level of blood glucose. Therefore, it represents the time-averaged (chronic) blood glucose level. Two advantages are evident in measuring glycated hemoglobins: (1) a simple replacement can determine multiple glucose determinations performed at timed intervals and (2) glycated hemoglobin levels, determined by affinity chromatography, are not affected by the presence of uremic, cyanate-derived cyanate with the N-terminal aminogroups on the beta chains of HbA. This urea-bound hemoglobin elutes with HbA carbamylated hemoglobin (in uremic patients).11,12 The labile globins.10 A significant advantage of affinity chromatography is the accuracy, specificity and linearity of glycated hemoglobin determinations obtained on samples from 44 non-diabetic and 11 diabetic donors were compared to HbA1c values obtained using the Helena methodology for quantitating glycated hemoglobin (GHb) to determine if any signs of fungal or bacterial growth are present.

### 3. GLYCOTek Developer A

**Ingredients:** The reagent contains 0.05 M magnesium chloride, 2% Triton X-100, 0.1 M glycine and sodium azide as a preservative.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. DO NOT PIPETTE BY MOUTH. For the Diabetes Control and Complications Trial (DCCT).13

### REAGENTS

#### 1. GLYCOTek Affinity Column

**Ingredients:** Each column contains cellulose resin covalently bonded to dihydroxyboryl glyoxyl groups, in a low ionic strength preservative solution containing 0.1% sodium azide.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. Refer to the Sodium Azide Warning. For DIABETES: Use: Store the columns in the dark at room temperature. Instructions for preparation of the columns for testing are provided in the Step-by-Step Method.

**Storage and Stability:** The columns should be stored in the dark at 15 to 30°C and are stable until the expiration date on the label.

**Signs of Deterioration:** The columns should contain a dense off-white resin with a clear supernatant.

#### 2. GLYCOTek Hemolysate Reagent

**Ingredients:** The reagent contains 0.05 M magnesium chloride, 2% Triton X-100, 0.1 M glycine and sodium azide as a preservative.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. Refer to the Sodium Azide Warning. Preparation for Use: The reagent is ready for use as pack-aged. Allow to equilibrate overnight to room temperature prior to use. A water bath or running tap water may be used to equilibrate the reagent for a shorter length of time. Proper temperature should be maintained for one hour before use.

**Storage:** The reagent should be stored at 2 to 6°C and is stable until the expiration date on the label.

**Signs of Deterioration:** Discard the reagent if any signs of fungal or bacterial growth are present.

### Cat. No. 5351

**GLYCOTek Affinity Column Kit**

**Cat. No. 5351**

**GLYCOTek Affinity Columns (50)**

**GLYCOTek Developer A (2 x 250 mL)**

**GLYCOTek Developer B (1 x 200 mL)**

**GLYCOTek Hemolysate Reagent (1 x 50 mL)**

**Other Supplies and Equipment**

For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.

Helena Laboratories warrants its products to meet our published specifications and to be free from material defects and workmanship. Helena Laboratories shall be the sole arbiter of any question of replacement or refund of any amount not to exceed the purchase price attributable to the lot as to which such claim is made. These alternatives as to the remedy in the event of breach are in lieu of any warranties implied, whether of merchantability or fitness for any purpose.

In order to maintain satisfactory performance of the products, it is necessary to observe the conditions of use as specified in the product information. In the event that the product does not perform to specifications, the customer is asked to return a portion of the unused material for evaluation. Helena Laboratories will evaluate the material and determine any course of action to be taken.

### 1. BACKGROUND

**BIBLIOGRAPHY**


### 2. HEME SPEC PLUS

**Ingredients:** Cellulose resin covalently bonded to dihydroxyboryl groups, in a low ionic strength preservative solution containing 0.1% sodium azide.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. Refer to the Sodium Azide Warning.

**Preparation for Use:** Store the columns in the dark at room temperature. Instructions for preparation of the columns for testing are provided in the Step-by-Step Method.

**Storage and Stability:** The columns should be stored in the dark at 15 to 30°C and are stable until the expiration date on the label.

**Signs of Deterioration:** The columns should contain a dense off-white resin with a clear supernatant.

### 3. GLYCOTek Developer A

**Ingredients:** The reagent contains 0.05 M magnesium chloride, 2% Triton X-100, 0.1 M glycine and sodium azide as a preservative.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. Refer to the Sodium Azide Warning.

**Preparation for Use:** Use: The reagent is ready for use as pack-aged. Allow to equilibrate overnight to room temperature prior to use. A water bath or running tap water may be used to equilibrate the reagent for a shorter length of time. Proper temperature should be maintained for one hour before use.

**Storage:** The reagent should be stored at 2 to 6°C and is stable until the expiration date on the label.

**Signs of Deterioration:** Discard the reagent if any signs of fungal or bacterial growth are present.

### 4. GLYCOTek Hemolysate Reagent

**Ingredients:** The reagent contains 0.05 M magnesium chloride, 2% Triton X-100, 0.1 M glycine and sodium azide as a preservative.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. Refer to the Sodium Azide Warning.

**Preparation for Use:** Use: The reagent is ready for use as pack-aged. Allow to equilibrate overnight to room temperature prior to use. A water bath or running tap water may be used to equilibrate the reagent for a shorter length of time. Proper temperature should be maintained for one hour before use.

**Storage:** The reagent should be stored at 2 to 6°C and is stable until the expiration date on the label.

**Signs of Deterioration:** Discard the reagent if any signs of fungal or bacterial growth are present.
for a shorter length of time. Proper temperature should be maintained for one hour before use.

Storage and Stability: The developer should be stored in the dark at 2 to 6°C and is stable until the expiration date indicated on the label.

Signs of Deterioration: Do not use developer if discolored or if fungal or bacterial growth is present.

4. GLYCO-Tek Developer

Ingredients: The reagent contains sorbitol, buffer and 2-1% sodium azide.

WARNING: FOR IN-IVITRO DIAGNOSTIC USE. DO NOT INGEST. DO NOT PIPEETTE BY MOUTH. Refer to the Sodium Azide Warnings in the user guides that accompany the reagents.

Preparation for Use: Allow the reagent to equilibrate overnight to room temperature prior to use. A water bath or warming tap water may be used to speed the equilibration process. Allow the reagent to equilibrate to room temperature for a shorter length of time. Proper temperature should be maintained for one hour before use.

Storage and Stability: The reagent should be stored in the dark at 2 to 6°C and is stable until the expiration date indicated on the label.

Signs of Deterioration: Do not use developer if discolored, or if fungal or bacterial growth is present.

SODIUM AZIDE WARNING

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of toxic sodium azides which, when highly concentrated in metal plumbing, are highly explosive. In addition to purging pipes with water, plumbing should be decontaminated with 10% NaOH.

INSTRUMENTS

A spectrophotometer capable of reading absorbance accurately at 415 nm with a range of absorbance (ABS) must be used. The HemeSpec Plus (Cat. No. 1103) is recommended for reading and automatically determining the % GHb.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh, whole blood samples are collected into sterile venipuncture tubes containing EDTA, heparin or citrate as an anticoagulant, or packed cells are the specimens of choice. Do not use specimens collected from an anticoagulant.

Packed Cell Preparation: Centrifuge whole blood specimen at 2000 g for 5-10 minutes. Carefully remove the stopper and aspirate all plasma and buffy coat from the cells. Thoroughly mix the red blood cells before use. Make hemolysates according to instructions in the STEP-BY-STEP section.

Interfering Substances: Hemolyzed, lipemic or icteric specimens are not recommended for use in this procedure.

Specimen Storage: The use of fresh blood specimens is recommended. If necessary, specimens may be stored up to 15 days at 2 to 6°C. Storage at room temperature for more than 12 hours will cause erroneous results and prolonged storage at 2-6°C.

Specimen Preparation: Detailed instructions for specimen preparation are included in the STEP-BY-STEP METHOD.

PROCEDURE

Materials Provided: The following materials needed for the method are provided in the GLYCO-Tek Affinity Column Kit (Cat. No. 5335).

10 Small Collection Tubes (3 mL)
10 Large Collection Tubes (15 mL)
Ou Cuillum® Rack
HemeSpec® (Cat. No. 1103)

Materials Needed but not provided:
- Deionized Water
- Disposable Piscot pipettes
- Disposable serological pipets (5 mL)
- Disposable glass test tubes

STEP-BY-STEP METHOD

IMPORTANT: Always use the GLYCO-Tek Affinity reagents to equilibrate overnight to room temperature (15 to 30°C) before testing. If the reagents are used with incomplete temperature equilibration, slow reagent flow will be observed. Perform all steps of the procedure at this temperature.

1. Obtain a GLYCO-Tek Collection Tube and a Large Collection Tube and a GLYCO-Tek Affinity Column for each patient and control to be tested.

2. Preparation of the Patient Sample(s)
   a. Place 50 μL of packed cells or whole blood in a small disposable glass test tube.
   b. Add 460 μL GLYCO-Tek Hemolyte Reagent to the test tube.
   c. Shake or vortex the tube to ensure complete hemolysis of the sample. Excessive hemolysis should be avoided.
   d. Allow the sample(s) to stand at least 5 minutes but not more than 45 minutes prior to use.

3. Prepare each GLYCO-Tek Affinity Column
   a. Up-end each column twice to remove resin adhering to the top cap closure. Place the column in the GLYCO-Tek Hemolyte Reagent and centrifuge at 2000 g for 5-10 minutes.
   b. Remove the top cap closure and resuspend the resin completely using a disposable Pasteur pipette. While squeezing the bulb of the pipette, position the tip slightly above the top of the resin bed. Suck the resin in and out of the pipette as you move it down to the top of the filter in the column. Be careful not to let any of the slurry spill from the top of the column. Do not introduce air bubbles into the column at this point as it may slow the flow of the eluent.
   c. Place the columns over the sink or a container and remove the bottom tip closure. Solution will escape to elute and the resin to pack.
   d. When the resin has settled to a level of 1.1 cm, or the level of supernatant has dropped below the shoulder of the column, remove the liquid to the top of the resin bed with a transfer pipette.

4. Add 1.0 mL of Sample to A and B to each column, and complete elution of the developer into the sink or a container. Complete elution of Developer A from the column in this step is critical. Failure to elute the developer completely will result in falsely low patient GHb values.

NOTES: The technique used to add the developers to the columns is critical to accurate, reproducible results. Using a disposable serological pipette, allow about five drips of Developer A to drop down the wall of the column providing a shield to prevent the disturbance of the top of the resin bed. Allow the remainder of the developer to drain from the column. Fill the column to the top of the resin bed with Developer B. This will ensure that the resin is equilibrated to room temperature (15 to 30°C) before testing. A water bath or warming tap water may be used to speed the equilibration process. Allow the resin to equilibrate to room temperature for a shorter length of time.

5. After the developer has drained from the column, place the column over a Large Collection Tube (non-GHb). Do not allow the column to set dry for more than 10 minutes.

6. Apply 50 μL of sample hemolysate prepared from packed cells, or 100 μL of hemolysate prepared from whole blood to the top of the resin bed.

7. Allow the sample to set on the resin for 8 minutes (minimum) (recommended 10 minutes). Begin the timing of the sample incubation after the addition of sample to the first column.

8. After 8 minutes incubation, add 0.5 mL GLYCO-Tek Developer A to each column, washing any hemolyte adhering to the sides of the column into the resin. Allow to elute.

9. Apply an additional 0.4 mL GLYCO-Tek Developer A to the column carefully avoiding any disturbance of the resin bed. Allow the column to develop for approximately 9 to 10 minutes.

10. Place the column over a Small Collection Tube (GHb). After 2000 g for 5-10 minutes. Carefully remove the stopper and aspirate all plasma and buffy coat from the cells. Thoroughly mix the red blood cells before use. Make hemolysates according to instructions in the STEP-BY-STEP section.

11. Transfer collected fraction to cuvette and read cuvette immediately after invasion.

12. Determine % Ghb for the appropriate range 6-10 minutes). Begin the timing of the sample incubation after the addition of sample to the first column.

To determine % GHb from the absorbance results, use the following calculation and displays the GHb percentage.

\[
\text{Abs} \frac{\text{GHb Tube}}{\text{non-GHb Tube}} = 0.410 \times 100\% = 6.4\% 
\]

Calculation of % GHb:

After calculation of the % GHb, the following equation can be used to determine % Hgb in small collection tubes.

\[
\text{Hgb} = 0.864 \times \text{GHb} + 0.973256 
\]

LIMITATIONS

The results of the GLYCO-Tek Affinity Column Method may be affected by the following conditions:

1. Hemolytic Anemia: Erythrocytes of patients who have hemolytic anemia may spew off hemoglobin in a paroxysmal life span. Because the erythrocytes are being destroyed prematurely, normal or low values for GHb may be obtained, although the time averaged blood hemoglobin level will be falsely low. Hemoglobin electrophoresis levels can still be used to monitor the hemolytic anemia patient, but this type of patient must be monitored against himself - usually to assess red cell mass.

2. Temperature: For optimum results, kit components must be equilibrated to room temperature (15-30°C) prior to use. Lower temperatures cause slower flow times. Unlike the reagents used in this method, column equilibration rates are not affected by variations in room temperature. The following chart shows actual % GHb variation with temperature variation.

3. Sample Absorption: Failure to allow the sample to sit on the column the appropriate length of time will result in erroneous values.

4. Drug Therapy: At present, no data exist to indicate that drug therapy interferes with glycated hemoglobin measurements using the GLYCO-Tek Affinity Column Method.
Calculation of the % GHb:

RESULTS

Materials Provided:

PROCEDURE

The GLYCO-Tek Normal (Cat. No. 5352) and Quality Control:

Stability of End Product:

Lipemic specimens and those with therapy interferes with glycated hemoglobin measurements using the GLYCO-Tek Affinity Column methodology.

4) Determine the GHb% as per instructions in the

At present, no data exist to indicate that drug preparation are included in the STEP-BY-STEP METHOD.

Specimen Preparation:

Hemolyzed, lipemic or icteric specimens are recommended. If necessary, specimens may be stored up to 15 days at 2 to 6°C. Storage at room temperature for more than 12 hours will cause erratic results, as will prolonged storage at 2-6°C.

Specimen Storage:

The reagent should be stored in the dark at 2 to 6°C and is stable until the expiration date indicated on the label.

Storage and Stability:

IMPORTANT: Allow the GLYCO-Tek Affinity reagents to equilibrate overnight to room temperature (15 to 30°C) before testing. If the reagents are not equilibrated at room temperature, equilibration, slow heat flow will be performed. All steps of the procedure at this temperature.

1. Obtain a GLYCO-Tek Collection Tube and a Large Collection Tube and a GLYCO-Tek Affinity Column for each patient and control to be tested.

2. Preparation of the Patient Sample(s)

a. Place 50 µL of packed cells or whole blood in a small disposable glass test tube.

b. Add 400 µL GLYCO-Tek Hemolysate Reagent to the test tube. Shake or vortex the tube to ensure complete hemolysis of the sample. Excessive clearing should be avoided.

c. Allow the sample(s) to stand at least 5 minutes but not more than 45 minutes prior to use.

3. Prepare the GLYCO-Tek Affinity Column

a. Up-end each column twice to remove resin adhering to the top cap closure. Place the column in the GLYCO-Tek HemeSpec Plus.

b. Remove the top cap closure and resuspend the resin completely using a disposable Pasteur pipette. While squeezing the bulb of the pipette, position the tip slightly above the top of the resin bed. Suck the resin in and out of the pipette as you move it down to the top of the filter in the column. Be careful not to let any of the slurry spill from the top of the column. Do not introduce air bubbles into the column at this point as it may slow the flow of the eluant.

c. Place the columns over the sink or a container and remove the bottom tip closure. Allow complete buffer elution (requires approximately 5-10 minutes). The eluent in the Large Collection Tube contains the non-glycated hemoglobins.

4. Drug Therapy:

Patient 6.7% 6.9% 6.7%

Normal 6.1% 6.4% 6.3%

Abnormal

EXAMPLE: Abs of GHb tube = 0.140;
Abs of non-GHb tube = 0.411

Calculation of the % GHb:

After calculation of the % GHb, the following equation can be used to determine the % HbA1c:

HbA1c = 0.6846 x % GHb + 0.973258

RESULTS

The results of the GLYCO-Tek Affinity Column Method may be affected by the following conditions:

1. Hemolytic Anemia: Erythrocytes of patients who have hemolytic anemia may be destroyed in the specimen collection and handling process.

2. Temperature: For optimum results, let components be maintained to room temperature (15-30°C) prior to use. Lower temperatures cause slower flow times. Unlike the ion exchange column method, the affinity column methodology is not significantly affected by variations in room temperature. The following results show actual % GHb variation with temperature variation.

Donor 15°C 23°C 30°C
Abnormal Control 16.7% 16.6% 16.6%
Abnormal Patient 15.4% 15.1% 15.1%
Normal Control 6.1% 6.4% 6.3%
Normal Patient 6.7% 6.9% 6.7%

3. Sample Absorption: Failure to allow the sample to sit on the column the appropriate length of time will result in erroneous values.

4. Drug Therapy: At present, no data exist to indicate that drug therapy interferes with glycated hemoglobin measurements using the GLYCO-Tek Affinity Column methodology.

5. Abnormal Hemolysate: Hemolysate down the wall and into the column. Those with elevated bilirubins can be used with this method. It is suggested that packed cells be used instead of whole blood since the nature of the specimen will cause retardation flow rates.

for a shorter length of time. Proper temperature should be maintained for one hour before use.

Storage and Stability: The developer should be stored in the dark at 2 to 6°C and is stable until the expiration date indicated on the label.

Signs of Deterioration: Do not use developer if discolored or if fungal or bacterial growth is present.

4. GLYCO-Tek Developer B

Ingredients: The reagent contains sorbitol, buffer and 0.1% sodium azide as a preservative; pH 6.0.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST. DO NOT PIPETTE BY MOUTH. Refer to the Sodium Azide Warning.

Preparation for Use: Allow the reagent to equilibrate overnight over night to room temperature prior to use. A water bath or running tap water may be used to equilibrate the reagent for a shorter length of time. Proper temperature should be maintained for one hour before use.

Storage and Stability: The reagent should be stored in the dark at 2 to 6°C and is stable until the expiration date indicated on the label.

Signs of Deterioration: Do not use reagent if discolored, or if fungal or bacterial growth is present.

SODIUM AZIDE WARNING

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of explosive azides which, when highly concentrated in metal plumbing, are highly explosive. In addition to pouring pipes with water, plumbing should be decontaminated with 10% NaOH.

INSTRUMENTS

A spectrophotometer capable of reading absorbance accurately at 415 nm with a range of 0 to 2.0 (Abs) must be used. The HemeSpec Plus (Cat. No. 1103) is recommended for reading and automatically determining the % GHb.

SPECIMEN COLLECTION AND HANDLING

Fresh, whole blood should be removed into sterile venipuncture tubes containing EDTA, heparin or citrate as an anticoagulant, or packed cells are the specimens of choice. Do not use specimens collected as an anticoagulant.

Packed Cell Preparation: Centrifuge whole blood specimen at 2000 g for 5-10 minutes. Carefully remove the stopper and aspirate all plasma and Buffy coat from the cells. Thoroughly mix the red blood cells before use. Make hemolysates according to instructions in the STEP-BY-STEP section.

Interfering Substances: Hemolysed, lipemic or icteric specimens are not recommended for use in this procedure.

Specimen Storage: The use of fresh blood samples is recommended. If necessary, specimens may be stored up to 15 days at 2 to 6°C. Storage at room temperature for more than 12 hours will cause erratic results and prolonged storage at 2-4°C.

Specimen Preparation: Detailed instructions for specimen preparation are included in the STEP-BY-STEP METHOD.

PROCEDURE

Materials Provided: The following materials needed for the method are provided in the GLYCO-Tek Affinity Column Kit (Cat. No. 5351).

GLYCO-Tek Affinity Columns (50)
GLYCO-Tek Developer A (2 x 250 mL)
GLYCO-Tek Developer B (1 x 200 mL)
GLYCO-Tek Hemolysate Reagent (2 x 150 mL)

Additional materials provided by Helena Laboratories but not contained in the kit:

GLYCO-Tek Normal Control (Cat. No. 5352)
GLYCO-Tek Abnormal Control (Cat. No. 5363)
Guin Kolumn Equipment Kit (Cat. No. 5336)

10 Small Collection Tubes (3 mL)
10 Large Collection Tubes (15 mL)
Guin Kolumn® Rack
HemeSpecTM a disposable Pasteur pipettes
Disposable syringes (5 mL)
Disposable glass test tubes

HemeSpecTM a standard laboratory spectrophotometer, GHb results are calculated by the following formula:

Abs. of GHb Tube x 100% = % GHb
(Abs. of GHb Tube) + 5.0 (Abs. of non-GHb Tube)

%GHb = percentage of glycated hemoglobin in the sample
Abs. of GHb tube = absorbance of the contents of the Small Collection Tube (415 nm)
Abs. of non-GHb tube = absorbance of the contents of the Large Collection Tube at a wavelength of 415 nm

5.0 = dilution factor (15 mL or non-GHb tube) x 3/15 mL = percentage conversion factor

EXAMPLE: Abs of non-GHb tube = 0.411
0.140 x 100% = 6.4%
0.140 + 5.0 (0.411)

Calculation of the % GHb:

After calculation of the % GHb, the following equation can be used to determine the % HbA1c:

HbA1c = 0.6846 x % GHb + 0.973258

LIMITATIONS

The results of the GLYCO-Tek Affinity Column Method may be affected by the following conditions:

1. Hemolytic Anemia: Erythrocytes of patients who have hemolytic anemia may be destroyed in the specimen collection and handling process. Because the erythrocytes are being destroyed prematurely, normal or low values for GHb may be obtained, although the true average value of the unaltered hemoglobin levels can still be used to monitor the hemolytic anemia patient, but this type of patient must be monitored himself - he is not a MAP normal healthy adult.

2. Temperature: For optimum results, let components be maintained to room temperature (15-30°C) prior to use. Lower temperatures cause slower flow times. Unlike the ion exchange column method, the affinity column methodology is not significantly affected by variations in room temperature. The following results show actual % GHb variation with temperature variation.

Donor 15°C 23°C 30°C
Abnormal Control 16.7% 16.6% 16.6%
Abnormal Patient 15.4% 15.1% 15.1%
Normal Control 6.1% 6.4% 6.3%
Normal Patient 6.7% 6.9% 6.7%

3. Sample Absorption: Failure to allow the sample to sit on the column the appropriate length of time will result in erroneous values.

4. Drug Therapy: At present, no data exist to indicate that drug therapy interferes with glycated hemoglobin measurements using the GLYCO-Tek Affinity Column methodology.

5. Abnormal Hemolysate: Hemolysate down the wall and into the column. Those with elevated bilirubins can be used with this method. It is suggested that packed cells be used instead of whole blood since the nature of the specimen will cause retardation flow rates.
The Helena GLYCO-Tek Affinity Column Method is an affinity microchromatographic methodology for the quantitation of glycated hemoglobin (GHb) in whole blood to indicate a 120-day time-averaged blood glucose level. An algorithm is provided for calculating the % HbA\textsubscript{1c} from the % GHb.

**SUMMARY**

Accurate assessment of chronic (time averaged) glucose control is one of the major difficulties in managing diabetic patients. Even patients with mild disease may show large fluctuations in blood glucose, and single glucose determinations may correlate poorly with mean blood glucose levels. Measurement of glycated hemoglobin (GHb) has gained acceptance as a good assessment of diabetic control.\textsuperscript{17} Glycated hemoglobins are all hemoglobins binding a glucose or other carbohydrate at any one of many sites on one or more of the globin molecules.\textsuperscript{10} The term “glycated hemoglobin” has been applied in the past to certain hemoglobin fractions (HbA\textsubscript{c}) separated on the basis of molecular charge, but these fractions do not wholly represent the glycated hemoglobins. Glycation does not occur during biosynthesis but is a non-enzymatic, two-stage condensation of glucose with various amino groups of the hemoglobin molecule. Glycation occurs slowly throughout the life span of the mature red blood cell and is dependent on the circulating level of blood glucose. Therefore, it represents the time-averaged (chronic) blood glucose level. Two advantages are evident in measuring glycated hemoglobins: (1) a single determination can replace multiple glucose determinations performed at timed intervals and (2) glycated hemoglobin levels, determined by affinity chromatography, are not affected by the fasting or physical exercise.\textsuperscript{11}

Affinity chromatography has several characteristics that increase the accuracy, specificity and linearity of glycated hemoglobin determinations.\textsuperscript{1} The method detects all glyated hemoglobins, not just HbA\textsubscript{c}\textsuperscript{1} and it is not affected by moderate fluctuations in temperature and pH.\textsuperscript{1, 9} or by the presence of abnormal hemo- globins.\textsuperscript{10} A significant advantage of affinity chromatography is the lack of interference by “labile” glycated hemoglobins and globins.\textsuperscript{10} A study was performed on 47 samples (13 normal and 34 abnormal) comparing the % HbA\textsubscript{1c} results obtained on samples from 44 non-diabetic and 11 diabetic donors to HbA\textsubscript{c} results obtained on Helena HbA\textsubscript{c}, Automated Analyzer (HPLC). The derived correlation equation and correlation coefficient was obtained.

\[ Y = 0.969X + 0.169 \]
\[ r = 0.997 \]
\[ Y \text{ = GLYCO-Tek} \]

The following line items, needed for performance of the GLYCO-Tek Affinity Column Kit, must be ordered individually.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>GLYCO-Tek Normal Control</th>
<th>GLYCO-Tek Abnormal Control</th>
<th>GLYCO-Tek Hemolysate Reagent</th>
<th>GLYCO-Tek Developer A</th>
<th>GLYCO-Tek Developer B</th>
</tr>
</thead>
<tbody>
<tr>
<td>5350</td>
<td></td>
<td></td>
<td></td>
<td>2 x 250 mL</td>
<td>1 x 200 mL</td>
</tr>
<tr>
<td>5352</td>
<td></td>
<td></td>
<td></td>
<td>50 mL</td>
<td>1 x 200 mL</td>
</tr>
</tbody>
</table>

For Sales, Technical and Order Information, and Service Assistance, call 800-231-5663 toll free.

---

**Résumé**

...