

NHANES 1999-2000 Public Data Release File (June 2002)

Laboratory 10 – Glycohemoglobin

Description

Glycohemoglobin

The glycohemoglobin measure is available for a full sample 12+ years of age.

Eligible Sample

Participants 12 years and older

Data Collection Methods

Blood specimens were processed, stored and shipped to the University of Missouri-Columbia for analysis.

Examination Protocol

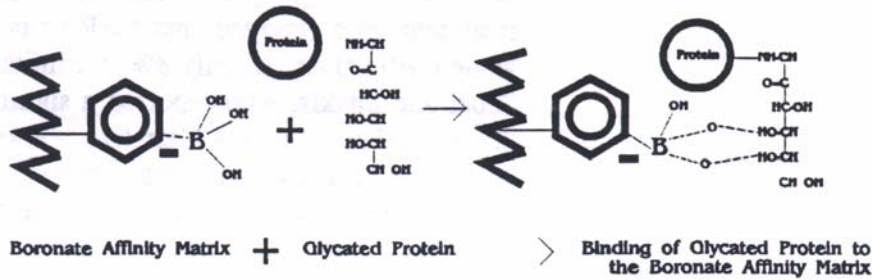
Detailed specimen collection and processing instructions are discussed in the [NHANES Laboratory/Medical Technologists Procedures Manual \(LPM\)](#). Vials are stored under appropriate refrigerated (4-8 degrees Centigrade) and frozen (- 20 degrees Centigrade) conditions until they are shipped to University of Missouri-Columbia for testing. The analytical methods are described in the Analytic methodology section.

Analytic Methodology

Glycohemoglobin

Glycated proteins differ from non-glycated proteins by the attachment of a sugar moiety(s) at various binding sites by means of a ketoamine bond. Glycohemoglobin (GHb) thus contains 1,2-cis-diol groups not found in non-glycated proteins. These diol groups provide the basis for separation of glycated and non-glycated components by boronate affinity chromatography^{1,2,3}. In this analytical technique, a boronate such as phenylboronic acid is bonded to the surface of the column support. When a solution of proteins (e.g. hemolysate) is passed through the column, the glycated component is retained by the complexing of its diol groups with the boronate. After the unretained non-glycated component elutes from the column, the glycated component is eluted from the column with a reagent that displaces it from the boronate.

Affinity Binding of Glycated Protein



The Primus instrument is a fully automated glycohemoglobin analyzer, which utilizes the principle of boronate affinity high performance liquid chromatography (HPLC)⁴. The analytical column contains aminophenylboronic acid bonded to a porous polymer support (gel). The low- and high-pressure pumps transfer reagents through the analytical column, with reagent selection executed by a switching valve. Hemolyzed samples are automatically injected onto the column during the flow of A-Elution Reagent #1. The glycated component binds to the boronate, while the non-glycated component passes through the column to the spectrophotometric detector, where it is detected at wavelength of 413±2 nm. After the elution of non-glycated component, the Primus instrument pumps B-Elution Reagent #2, which displaces the glycated component from the column. The glycated component then passes through the detector. In the final stage of each sample cycle, the column is re-equilibrated with Elution A-Reagent #1. All reagent selection occurs in a timed sequence designed to allow complete elution of non-glycated and glycated components.

Microprocessors (Model CLC330) or the PC computer (Model CLC385) control all functions in the liquid chromatograph and computing integrator. The signal from the spectrophotometric detector is processed and the concentration of glycohemoglobin is calculated as a percentage of the total detected. Integration is by peak area in millivolt-seconds. The chromatogram is plotted first as the signal is received by the detector. The raw % glycohemoglobin is calculated when glycated hemoglobin peak area is divided by the total hemoglobin peak area. Primus HPLC uses two point calibrators with HbA1c assigned values to obtain a final standardized glycohemoglobin. The Schiff base does not interfere with boronate affinity method..

Analytic Notes

LBXGH: Glycohemoglobin

Glycohemoglobin measurements for NHANES 1999 are performed by the Diabetes Diagnostic Laboratory at the University of Missouri-Columbia using Primus CLC330 and Primus CLC 385⁴. The Boronate Affinity High Performance Liquid Chromatography (HPLC) system determines total glycohemoglobin by measuring 1,2-cis diol group found in glycated hemoglobin. The system has been standardized to the reference method used for the Diabetes Control and Complications Trial (DCCT). The affinity chromatographic method has demonstrated excellent, long-term precision (interassay CV's <3.0%) and is not affected by the presence of hemoglobin variants S, C, D and elevated HbF. The method is also less sensitive to hemoglobin degradation due to improper sample handling.

References

1. Fluckiger R, et al. Quantitation of glycosylated hemoglobin by boronate affinity chromatography. *Diabetes* 1984;33:73-6.
2. Gould BJ, et al. A sensitive method for the measurement of glycosylated plasma proteins using affinity chromatography. *Ann Clin Biochem* 1984;21:16-21.
3. Mallia AK, et al. Preparation and use of a boronic acid affinity support for separation and quantitation of glycosylated hemoglobins. *Anal Lett* 1981;14:649-61.
4. Primus Corporation Glycated Hemoglobin and Plasma Protein Analyzer Operator's Manual for the Diabetes Care Test Package of the CLC330TM and CLC385TM (Primus Corporation, Kansas City, MO 64110).

