**NHANES 2001-2002 Data Release**
**May 2004**
**Documentation for Laboratory Results**

# Laboratory 10 – Glycohemoglobin

**(1) Documentation File Date – September 3, 2003**

**(2) Documentation File Name-Laboratory 10 – Glycohemoglobin**

**(3) Survey Years Included in this File Release-2001-2002**

**(4) Component Description**

**Diabetes mellitus will be assessed by measures of blood glycohemoglobin, plasma glucose, serum insulin, and serum c-peptide in participants aged 12 years and over.**

**Glycohemoglobin measures are available for a full sample. Measures of blood glycohemoglobin, plasma glucose, serum insulin, and serum c-peptide in the morning examination session only can be found in the Lab10AM data file.**

**Diabetes is a leading cause of disease and death in the United States. Eight million Americans are known to have diabetes, and an equal number have undiagnosed diabetes. In 1993, nearly 18 percent of all deaths for persons over the age of 25 were among people with diabetes. The prevalence of diabetes and overweight (one of the major risk factors for diabetes) continue to increase. Substantial new efforts to prevent or control diabetes have begun, including the Diabetes Prevention Trial and the National Diabetes Education Program.**

**Information on the prevalence of diabetes disease, especially in its early stages, and associated risk factors will be used to help develop early intervention and prevention programs for the disabling consequences of this condition. Specifically, the diabetes disease examination will provide population data to: 1) determine a national estimate of diabetes disease prevalence (diagnosed and undiagnosed), including those at high risk for the late complications of the disease (i.e., ulceration and amputation); 2) identify the risk factors of diabetes disease; 3) permit a national cohort to be established for follow-up studies of this condition; and 4) provide critical information to clinicians and public health officials for the development of preventive care and community-based interventions.**

**(5) Sample Description:**

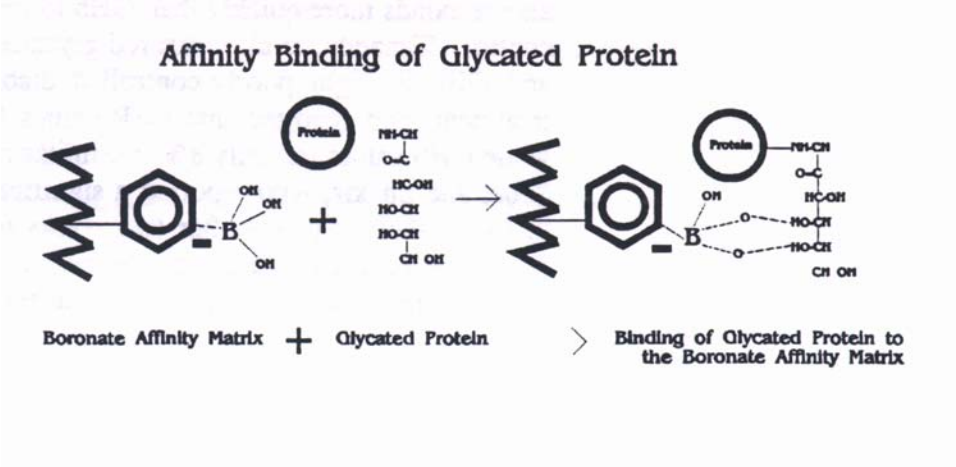
**5.1 Eligible Sample**

**Participants aged 12 years and older were tested.**

**(6) Description of the Laboratory Methodology**

**6.1 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 [11a,11b,11c]. 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.**




**The Primus instrument is a fully automated glycohemoglobin analyzer, which utilizes the principle of boronate affinity high performance liquid chromatography (HPLC) [11d]. 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. The report is then printed with the sample information, raw Glycohemoglobin and standardized Glycohemoglobin results.**

**(7) Laboratory Quality Control and Monitoring**

**The NHANES quality control and quality assurance protocols (QA/QC) meet the 1988 Clinical Laboratory Improvement Act mandates. Detailed quality control and quality assurance instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed QA/QC protocols.**

**(8) Data Processing and Editing**

**Blood specimens were processed, stored and shipped to University of Missouri-Columbia, Columbia, Missouri for analysis. Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Read the LABDOC file for detailed data processing and editing protocols. The analytical methods are described in the Description of the Laboratory Methodology section.**

**(9) Data Access:**

**All data are publicly available**

**(10) Analytic Notes for Data Users:**

**The analysis of NHANES 2001-2002 laboratory data must be conducted with the key survey design and basic demographic variables. The NHANES 2001-2002 Household Questionnaire Data Files contain demographic data,**

**health indicators, and other related information collected during household interviews. They also contain all survey design variables and sample weights for these age groups. The phlebotomy file includes auxiliary information such as the conditions precluding venipuncture. The household questionnaire and phlebotomy files may be linked to the laboratory data file using the unique survey participant identifier SEQN.**

**LBXGH: Glycohemoglobin**

**Glycohemoglobin measurements for NHANES 2001-2002 were performed by the Diabetes Diagnostic Laboratory at the University of Missouri-Columbia using Primus CLC330 and Primus CLC 385 (Primus Corporation, Kansas City, MO). 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.**

**(11) References**

**a. Fluckiger R, et al. Quantitation of glycohemoglobin by boronate affinity chromatography. Diabetes 1984;33:73-6.**

**b. Gould BJ, et al. A sensitive method for the measurement of glycosylated plasma proteins using affinity chromatography. Ann Clin Biochem 1984;21:16-21.**

**c. 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.**

**d. Primus Corporation Glycated Hemoglobin and Plasma Protein Analyzer Operator's Manual for the Diabetes Care Test Package of the CLC330$^{TM}$ and CLC385$^{TM}$ (Primus Corporation, Kansas City, MO 64110).**