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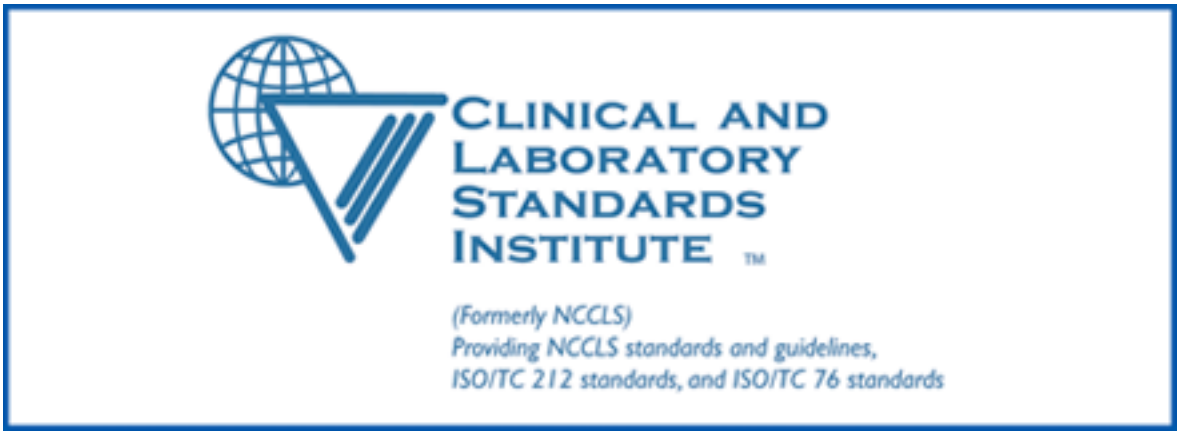
Harmonization of Glycohemoglobin Measurements; Approved Guideline



This document describes an established program to harmonize glycohemoglobin (GHB) testing results among laboratories to a common, outcomes-based reference system and includes recommendations for the clinical application of harmonized GHB testing results.

A guideline for national application developed through the NCCLS consensus process.

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- the revision of documents in response to comments by users
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Healthcare professionals in all specialties are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

Harmonization of Glycohemoglobin Measurements; Approved Guideline

Abstract

NCCLS document C44-A—*Harmonization of Glycohemoglobin Measurements; Approved Guideline* provides a scheme for harmonization of glycohemoglobin testing results among laboratories. The document approach describes an established program, the National Glycohemoglobin Standardization Program (NGSP). The guideline is intended for manufacturers of glycohemoglobin testing products, laboratorians, clinicians, and others interested in glycohemoglobin testing. It includes information on the rationale for harmonization of glycohemoglobin testing results, the process (including the administrative structure of the NGSP), and the clinical application of harmonized glycohemoglobin measurements in the management of patients with diabetes mellitus. The appendix contains an outline of the NGSP website which includes the current NGSP protocol and sample data collection forms for manufacturers of glycohemoglobin assay methods, as well as for clinical laboratories for certification testing.

While this document will serve as a useful resource for a wider audience, it is intended for use primarily in the United States.

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Harmonization of Glycohemoglobin Measurements; Approved Guideline

Volume 22 Number 25

David E. Goldstein, M.D., Chairholder
V. Michelle Chenault, Ph.D., M.T.(ASCP)
W. Jeff Durban
John H. Eckfeldt, M.D., Ph.D.
J. Gilbert Hill, M.D., Ph.D.
Lowry J. Messenger, Ph.D.
Gary L. Myers, Ph.D.
Ewald Vorberg, Ph.D.

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John H. Eckfeldt, M.D., Ph.D.	Fairview-University Medical Center Minneapolis, Minnesota
J. Gilbert Hill, M.D., Ph.D.	The Hospital for Sick Children Toronto, Canada
Lowry J. Messenger, Ph.D.	LXN Corporation San Diego, California
Gary L. Myers, Ph.D.	Centers for Disease Control and Prevention Atlanta, Georgia
Ewald Vorberg, Ph.D.	Roche Diagnostics Germany

Advisors

Cynthia R. Blessum	Beckman Coulter, Inc. Brea, California
Nina M. Chace, M.S.	Food and Drug Administration Center for Devices/Rad. Health Rockville, Maryland
Rita Ellerbrook, Ph.D.	Helena Laboratories Beaumont, Texas

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Ralph K. Ito, Ph.D., MCLS	Olympus America, Inc. Milville, New York
Randie R. Little, Ph.D.	University of Missouri School of Medicine Columbia, Missouri
Richard Mack, D.O.	New Jersey Medical School Newark, New Jersey
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Kory M. Ward-Cook, Ph.D., M.T.(ASCP), FACB	ASCP Board of Registry Chicago, Illinois
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Contents

Foreword

Background

Diabetes mellitus is a major public health problem worldwide, affecting more than 100 million people.[1] Recent National Health and Nutrition Examination Survey (NHANES) data show that in the U.S. alone, the prevalence of diabetes is estimated at 5.1% of the population, or 10.2 million people.[2] In addition, the prevalence is increasing dramatically: the prevalence of diabetes in people 40 to 74 years of age increased from 8.9% in the years 1976 to 1980 to 12.3% in the years 1988 to 1994. Complications from the disease are serious, accounting for a large share of vision loss, kidney failure, nontraumatic limb amputation, and cardiovascular disease. The disease is also economically devastating, accounting for more than 100 billion U.S. dollars in actual expenditures in 1992[3]; it is estimated that one in every seven healthcare dollars in the U.S. is spent on diabetes, with the majority of the expense for treating the chronic complications of diabetes rather than for primary prevention.[4]

The cause of diabetes complications, and in particular, the relationship between the level of glycemia and complications, had been debated vigorously for more than 50 years, until 1993 when the results of the landmark Diabetes Control and Complications Trial (DCCT) were published.[5] The DCCT was a nine-year clinical trial in patients with Type 1 diabetes. Study volunteers were randomly assigned to either intensive therapy, designed to bring blood glucose levels as close as possible to those in people without diabetes, or to standard therapy, designed to approximate conventional diabetes therapy. Study results showed dramatic reductions in the development and progression of microvascular and neuropathic complications with intensive therapy compared to standard therapy. Risks were directly related to the level of glycemic control, regardless of treatment group. Glycemic control was assessed by serial glycohemoglobin (GHB) determinations performed in a central laboratory.

Based on the DCCT results, the National Institutes of Health (NIH), the American Diabetes Association (ADA), and other expert groups recommended that all patients with diabetes be treated to bring blood glucose levels as close to normal as possible to decrease risks of complications.[6,7] For the first time, there was a firm scientific basis for recommending specific glycemic goals, and these were based on DCCT GHB values. For example, the ADA recommended that most patients with diabetes should aim for GHB levels of 7% or less (nondiabetic reference range of 4 to 6%); levels of 8% or greater were considered to require "additional action."[8]

Unfortunately, DCCT GHB numbers were not readily available to patients and their healthcare providers. The state of GHB testing was, in fact, in considerable disarray.[9] GHB testing was first performed in routine clinical laboratories in the late 1970s. By the time the DCCT results were published in 1993, there were many different types of GHB assay methods, and no harmonization of test results among laboratories. Thus, two different assay methods would likely give very different numerical results for the same blood specimen.[10] Data from the College of American Pathologists proficiency testing program (CAP Survey) for GHB demonstrated that even among similar assay methods, GHB results varied considerably, and interlaboratory coefficients of variation for proficiency testing specimens were large.[11]

A number of previous studies in both the U.S. and Europe had demonstrated the feasibility of harmonizing GHB test results against a common reference, but no organized approach to actually harmonize test results had been developed.[12-15] Thus, in 1993, even before the DCCT results were announced, the American Association for Clinical Chemistry (AACC) authorized formation of a Subcommittee on Glycohemoglobin Standardization.[16,17] In this document, the term "standardization," as it applies to GHB testing, is synonymous with the term "harmonization," the process by which GHB test results among laboratories are made comparable to a common reference.)

Foreword (Continued)

The subcommittee's charge was to develop a glycohemoglobin harmonization program. Although the subcommittee's first priority was to develop a harmonization program for U.S. laboratories, the importance of international harmonization of GHB testing was recognized; the subcommittee included members from Europe who had extensive experience in GHB harmonization and proficiency testing.

The AACC subcommittee first determined that a suitable definitive/reference method for GHB determinations and purified GHB reference standards were not available, but ultimately would be important components of a universal harmonization program. The subcommittee recommended that while investigations proceeded to develop purified reference standards and definitive/reference methods, a harmonization program similar in design to the Cholesterol Reference Method Laboratory Network could be initiated relatively quickly using the DCCT reference laboratories already in place, as interim anchors for harmonization.[18] Thus, routine clinical laboratories using harmonized GHB assay methods would be able to report GHB test results that were comparable to DCCT values; this would give patients with diabetes and their healthcare providers a laboratory test result that quantified both mean glycemia and complication risks.[10]

When the DCCT ended in 1993, it was succeeded by another long-term study called the Epidemiology of Diabetes Interventions and Complications (EDIC).[19] The EDIC required serial measurements of GHB in the former DCCT study volunteers for up to ten years. Thus, the DCCT reference laboratories remained operational when the DCCT ended to provide glycohemoglobin measurements for the EDIC.

The DCCT reference system consisted of two laboratories—one at the University of Missouri and the other at the University of Minnesota. The Missouri laboratory had a modular ion-exchange high performance liquid chromatography (HPLC) system, a designated comparison method that served as the reference anchor for the DCCT.[20,21] The Minnesota laboratory had an automated ion-exchange HPLC system dedicated to GHB determinations and was the site where routine study specimens were analyzed.[5]

The AACC subcommittee thus developed a harmonization protocol around the DCCT/EDIC reference laboratories, which was approved in 1995 after a consensus conference with U.S. manufacturers. In 1996, the AACC subcommittee was disbanded and replaced by the National Glycohemoglobin Standardization Program (NGSP), which began operation in July 1996.[22,23] The primary focus of the NGSP was to assist manufacturers in establishing proper calibration and then to document comparability of their assay results to the DCCT database. An important adjunct to the NGSP program was a new CAP GHB proficiency testing program, initiated in May 1996, which used fresh blood specimens.[24] By December 1996, the first group of GHB assay methods had been formally tested and certified as having results comparable to those obtained with the DCCT reference method. In 1997, the ADA endorsed the NGSP.[25]

In 1998 the results of the UK Prospective Diabetes Study (UKPDS) were published.[26] This ten-year study in patients with Type 2 diabetes replicated the findings in the DCCT with respect to reduction in complication risk associated with a reduction in GHB. The laboratory that performed GHB determinations for the UKPDS used an NGSP-certified assay method and, in addition, participated in careful comparison procedures with the NGSP reference network to assure comparability of test results between the DCCT and UKPDS.[27] Thus, GHB values in both the DCCT and UKPDS could be used in patients with either Type 1 or Type 2 diabetes to estimate a person's risk of developing complications.[28]

Foreword (Continued)

The Role of the IFCC Reference Method in the Harmonization Process

During the same time period that the AACC was forming its strategy to harmonize GHB results in the U.S., the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) formed a Working Group on HbA_{1c} Standardization (IFCC-WG) in 1995 to achieve uniform international standardization of HbA_{1c} measurements. Two candidate reference methods and reference material were developed and validated to serve as the analytical base for global harmonization. In the first step hemoglobin is cleaved into peptides by a proteolytic enzyme, and thereafter the specific glycated and nonglycated N-terminal peptides of the B-chains are measured by HPLC and either mass spectrometry or capillary electrophoresis. The IFCC-WG created an international network of reference laboratories incorporating these two reference methods.[29-31] These reference methods could potentially provide a better analytical anchor for the NGSP than the current designated comparison method. However, studies show that the IFCC reference methods give different results for the same blood samples compared to the current NGSP anchor. Consequently the IFCC results are numerically different from the clinically validated DCCT-, EDIC- and UKPDS-based results. Studies are currently underway to 1) evaluate the stability of the IFCC reference methods over time, and 2) establish the relationships between the IFCC reference methods and the current NGSP anchor. At such time as the IFCC method is fully validated, the NGSP and other structured harmonization schemes can use it as the reference method to provide a stable anchor for harmonization.

Current evidence-based medical practice in several countries is based on the DCCT-derived numerical values.[26-28] In these countries, adoption of the IFCC reference method as harmonization anchor will require development of a statistically robust algorithm to define the numeric relationship to the DCCT- based results. Alternatively, the clinical interpretive guidelines can be modified to the IFCC numeric values with appropriate clinical trial validation.

The Role of NCCLS and C44 in the Harmonization Process

One of NCCLS's overriding organizational goals is the achievement of worldwide harmonization in its standards and guidelines wherever possible. NCCLS defines harmonization as a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity; and recognizes that harmonization is an evolutionary and educational process that begins with new projects and revisions of existing documents.

Because current international studies indicate that the IFCC reference methods for glycohemoglobin measurements are better analytical anchors for NGSP than the current designated comparison method, NCCLS and its Area Committee on Clinical Chemistry and Toxicology and the Subcommittee on Glycohemoglobin Measurements is committed to revising the C44 guideline when the IFCC methodology studies are complete and the NGSB anchor has been changed.

A Note on Terminology

NCCLS recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in NCCLS, ISO, and CEN documents; and that legally required use of terms, regional usage, and different consensus timelines are all obstacles to harmonization. In light of this, NCCLS recognizes that harmonization of terms facilitates the global application of standards and is an area of immediate attention.

In keeping with NCCLS's commitment to align terminology with that of ISO, the following terms are used in C44:

The term “trueness” is used when referring to the closeness of the agreement between the average value from a large series of measurements and to an accepted reference value. The term “accuracy,” in its metrological sense, refers to the closeness of the agreement between the result of a (single) measurement and a true value of a measurand, thus comprising both random and systematic effects.

NCCLS consensus documents are developed through an open process that ensures wide review and broad application. This unique approach leads to standards and guidelines for medical testing and healthcare services that address identified needs of both its global and national constituents. Most NCCLS consensus documents are intended for global application. Under certain circumstances, however, an NCCLS standard or guideline may be intended for primary use in a specific country or region.

NCCLS document C44-A—*Harmonization of Glycohemoglobin Measurements; Approved Guideline* is one such consensus document. While this document will serve as a useful resource for a wider audience, it is intended for use primarily in the United States.

The imprint of the flag and the unique tagline on the cover call attention to its national focus, and differentiate C44-A from our global consensus documents.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to “standard precautions.” Standard precautions are new guidelines that combine the major features of “universal precautions and body substance isolation” practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals*. Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80), (MMWR 1987;36[suppl 2S]:2S-18S), and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

Key Words

Diabetes mellitus, glycohemoglobin, harmonization, hemoglobin A_{1c}, proficiency testing

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents through a gap analysis. The approach is based on the model presented in the most current edition of NCCLS HS1—*A Quality System Model for Health Care.* The quality system approach applies a core set of "quality system essentials (QSEs)," basic to any organization, to all operations in any healthcare service's path of workflow. The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

C44-A addresses the following quality system essentials (QSEs):

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
								X			

Adapted from NCCLS document HS1— *A Quality System Model for Health Care*.

Harmonization of Glycohemoglobin Measurements; Approved Guideline

1 Introduction

Harmonization of glycohemoglobin (GHB) testing results is essential to maximize the clinical utility of GHB testing. This guideline describes an established program, the National Glycohemoglobin Standardization Program (NGSP), the purpose of which is to harmonize GHB test results among laboratories to a common, outcome-based reference system. This guideline has two main sections. The first describes the administrative/organizational structure and procedures employed by the NGSP, with an emphasis on the harmonization process rather than on specific NGSP protocol details (an outline of the NGSP website is included in the appendix). The second section provides information on the clinical application of GHB testing, including recommendations for the clinical application of harmonized GHB testing results.

2 Scope

This document presents information on the rationale for harmonization of glycohemoglobin testing among clinical laboratories. The process is described by which the NGSP has established, on a large scale, comparability of GHB test results among laboratories to a common reference. The reference values are, in turn, indexed to clinical outcomes data. The document also includes information about the structure of the laboratory network which is the backbone of the harmonization program, as well as procedures for monitoring the network, for testing and certifying manufacturers' assay methods and laboratories, and for monitoring the effectiveness of the program by proficiency testing of routine clinical laboratories. The information is designed to facilitate participation in the NGSP by manufacturers of GHB testing methods and materials, as well as routine clinical laboratories. This document also includes information on clinical application of GHB testing results and should be useful to both laboratorians and healthcare providers involved in the care of patients with diabetes mellitus, as well as to individuals and organizations involved in quality assurance programs for patients with diabetes mellitus. In addition, this document is a starting point for discussion regarding development of a universal harmonization program for GHB.

While C44-A may serve as a useful resource for a wider audience, it is intended for use primarily in the United States.

3 Definitions[a]

The definitions of method type used in this document conform to the National Reference System for the Clinical Laboratory (NRSCL) guidelines. (See NCCLS document NRSCL13—*The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results.*)

Accuracy - Closeness of the agreement between the result of a measurement and an accepted reference value of the measurand/analyte; **NOTE**: See *Note on Terminology* in the Foreword.

Bias - 1) The systematic, signed deviation of the test results from the accepted reference value; **NOTES:** a) Defined in ***(ISO3534-1/93-3.13)*** as "the difference between the expectation of the test results and an accepted reference value"; b) In general, the deviation/difference is based on replicate measurement using an accepted reference comparison method and the method being tested, and expressed in the units of the

[a] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents.* For complete definitions and detailed source information, please refer to the most current edition of that document.

measurement or as a percentage; **2) Interinstrument/intermethod or Interlaboratory bias** - The difference observed by comparing two specified instruments' or laboratories' methods under specified conditions of analysis, concentration range, method, etc.

Designated comparison method, DCM – A fully specified method(s), which, in the absence of an NRSCL-credentialed reference method, serves as the common basis for the comparison of "field" reference materials and methods, and for the development of principal assigned values (PAVs) or principal assigned characteristics (PACs) for an analyte or process; **NOTES:** 1) This is the third-highest ranking assay method in the NRSCL hierarchy; 2) This is comparable to a Secondary Reference Measurement procedure.

Glycohemoglobin, GHB - The generic term for a family of compounds arising from the nonenzymatic reaction between the free aldehyde group of glucose or other sugars and the unprotonated form of the free amino groups of hemoglobin; **NOTE:** The terms "glycated hemoglobin" and "glycohemoglobin" are synonymous.

Harmonization – *In GHB testing*, the process by which GHB test results among laboratories are made comparable to a common reference.

Hemoglobin A_{1c}, HbA_{1c} - Hemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta-chains and is a major component of glycohemoglobin.

Netcore – The administrative unit in the NGSP responsible for overall coordination of laboratory network activities.

Primary reference laboratory, PRL – A laboratory in the NGSP laboratory network using a DCM; **NOTES:** a) There is a Central Primary Reference Laboratory (CPRL) and several backup laboratories (PRLs); b) This type of laboratory is referred to as a "reference measurement laboratory" in ISO terminology.

Reference method, RM – A thoroughly investigated method, in which exact and clear descriptions of the necessary conditions and procedures are given for the accurate determination of one or more property values, and in which the documented trueness and precision of the method are commensurate with the method's use for assessing the trueness of other methods for measuring the same property values or for assigning reference method values to reference materials; **NOTES:** a) This is the second-highest ranking method in the NRSCL hierarchy; b) This definition is analogous to a reference measurement procedure in ISO terminology. A reference measurement procedure is a thoroughly investigated measurement procedure shown to have an uncertainty of measurement commensurate with the intended use, especially in assessing the trueness of other measurement procedures for the same quantity and in characterizing reference materials (adopted from ISO/DIS 15193, EN 12286).

Secondary reference laboratory, SRL – A laboratory in the NGSP laboratory network using an assay method that is certified as traceable to a PRL using a DCM; thus the SRL provides a link between routine clinical laboratory methods and the DCM.

Trueness - The closeness of agreement between the average value obtained from a large series of test results and an accepted reference value; **NOTES:** a) See also **Accuracy, Bias;** b) See *Note on Terminology* in the Foreword.

4 Rationale for Harmonization of Glycohemoglobin Measurements

For many analytes used routinely in clinical medical practice, test results are not harmonized among laboratories, yet patient care does not appear to suffer. Harmonization of test results is a high priority only if the clinical information value of the test is thought to be enhanced by harmonization. For example, however desirable harmonization of serum thyroxine determinations might seem to laboratorians, optimal use of the test, assuming it is performed with high precision, requires only that the test result and the reference range for the test be reported. The clinician who orders the test is likely to be interested in whether the test result is within, below, or above the reference range (and perhaps, roughly how far below or above the reference range the result is) in order to assess clinical significance of the test result. Harmonization of serum thyroxine testing among laboratories would not be likely to improve patient care appreciably, at least based on current medical practice.

In contrast, when the results of the DCCT were published in 1993 showing a direct relationship between GHB levels and risks for the development of diabetes complications, the need to harmonize GHB test results among laboratories and to DCCT values became obvious. It was not a question of determining only if a GHB value was within or outside the reference range for the laboratory, but rather, precisely what the test result was and what DCCT value that number could be related to, since it was only DCCT values which were directly related to both mean blood glucose and outcome risks. The DCCT results were considered so important for patient care that within months of the publication of the DCCT results, the American Diabetes Association (ADA) published a position statement defining diabetes treatment goals based on specific DCCT GHB test results.[6,8] Thus, it was clinical outcomes data that made harmonization of GHB testing a high priority.

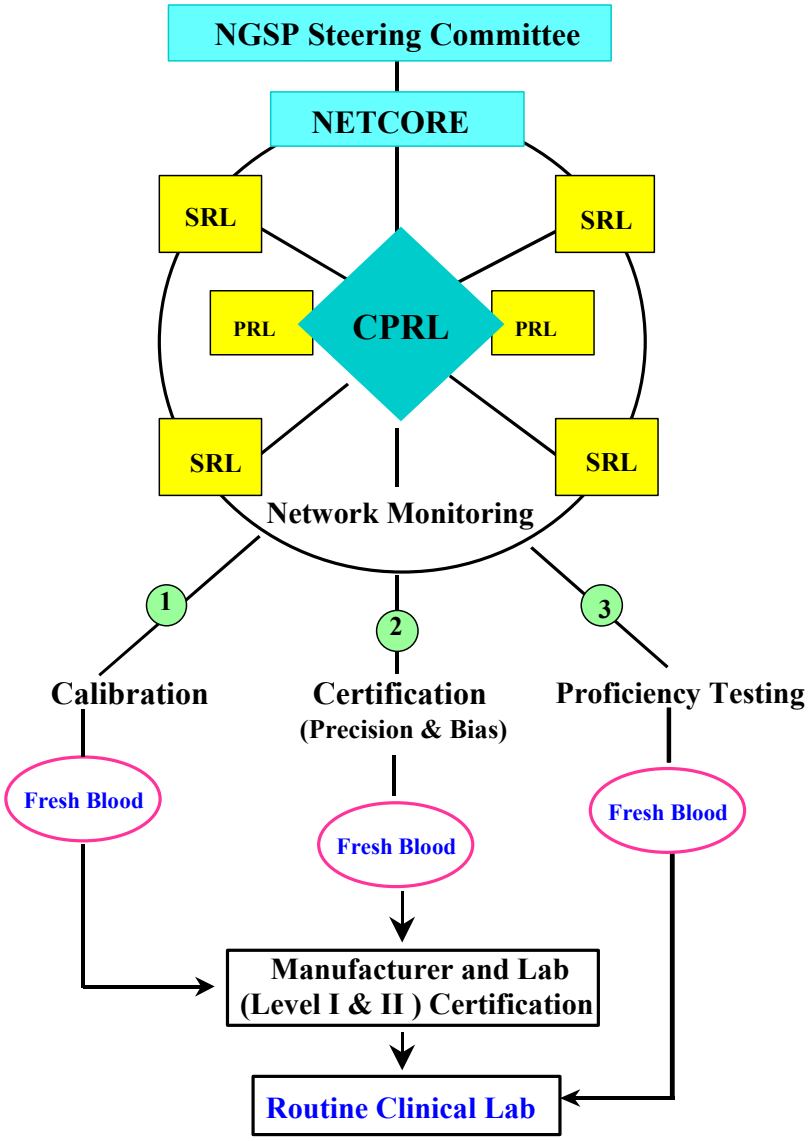

Figure 1. Structure of the NGSP. (From the National Glycohemoglobin Standardization Program website [www.missouri.edu/~diabetes/ngsp.html]. Reprinted with permission.)

Key: Netcore = NGSP Administrative Unit
CPRL = Central Primary Reference Laboratory
PRL = Primary Reference Laboratory
SRL = Secondary Reference Laboratory

5 Description of the Harmonization Scheme

5.1 Overview of the NGSP

In 1996 the NGSP, a working program for GHB harmonization, was started. The NGSP was modeled closely on the Cholesterol Reference Method Laboratory Network Program.[32] In the cholesterol program, manufacturers of cholesterol assay methods document traceability to the National Reference System for Cholesterol by performing split-sample comparisons with the cholesterol reference method. For GHB harmonization, the NGSP maintains a network of reference laboratories that are calibrated to DCCT reference values. A central laboratory sets the initial calibration and is responsible for monitoring network laboratories. The network laboratories work directly with manufacturers of GHB assay methods, first to calibrate their methods to provide DCCT-equivalent values, and then to provide comparison data for method certification according to protocol. Assay methods that show high precision and the desired comparability of fresh sample results with one of the network laboratories are awarded certificates by the NGSP. Proficiency testing (PT) data using fresh sample surveys in routine clinical laboratories are used to assess the overall effectiveness of the certification process (see Figure 1).

In summary, the harmonization process has three major steps: the first is calibration of an assay method by a manufacturer to achieve GHB values comparable to those obtained by an NGSP network laboratory; the second is certification testing by the manufacturer to document the protocol-specified comparability using split fresh blood samples; and the third is participation by individual laboratories in a proficiency testing program using fresh blood with target values set by the NGSP laboratory network.

5.2 Steering Committee

The steering committee has overall responsibility for the harmonization program. The committee works closely with the laboratory network to assure implementation of the program in accordance with the program protocol, which was prepared by the steering committee. The committee is responsible for reviewing policy/protocol changes and certification data submitted by the laboratory network. The committee membership has broad representation from private and public organizations with interests in GHB testing and/or diabetes patient care. The steering committee, along with the laboratory network, is responsible for assuring widespread dissemination of information about the harmonization program among private and public agencies, laboratorians, clinicians, and manufacturers of GHB assay methods and controls. The NGSP maintains a website with up-to-date information about certification (criteria, certified methods, laboratories, etc.) which greatly facilitates information exchange. The NGSP has also found regularly scheduled manufacturer forums and presentations at national and international meetings to be useful methods of information exchange.

5.3 Laboratory Network

The NGSP Laboratory Network consists of an administrative unit (NETCORE) and several reference laboratories.

5.3.1 Administrative Unit

The administrative unit (NETCORE) was established by the steering committee. NETCORE is responsible for overall coordination of laboratory network activities. NETCORE communicates with manufacturers of GHB assay methods who request certification; provides information regarding the program; and arranges manufacturer/network laboratory interactions. NETCORE analyzes all certification and network monitoring data; sends summary reports to the steering committee; issues certificates to manufacturers and laboratories after review of the data by the steering committee; and reviews results of

proficiency testing surveys. NETCORE is responsible for maintaining a website (see the appendix) with up-to-date information about the program.

5.3.2 Central Laboratory (CPRL)

The CPRL was responsible for setting the initial calibration for the harmonization program and for ensuring that network results remain consistent over time. This laboratory provides the direct link to clinical outcomes data from the DCCT. Under the direction of NETCORE, the CPRL administers the certification process and the ongoing monitoring program for all network laboratories.

5.3.3 Other Network Laboratories

There are backup primary reference laboratories (PRLs) included in the network using the same assay method and calibrated to the CPRL. The other network laboratories, called "secondary reference laboratories (SRLs)," use a variety of assay methods, but all are calibrated to the CPRL method. Any of the SRLs can work directly with manufacturers of GHB testing methods to calibrate their assay methods and to participate in the certification testing according to the program protocol. All network laboratories are certified as having test results that are traceable to the CPRL prior to any interaction with manufacturers.

5.3.4 Performance Monitoring of the Network Laboratories

Monthly monitoring using fresh blood samples is performed among all network laboratories. Specific criteria are used to set limits on acceptability. (See the NGSP website for details—http://www.missouri.edu/~diabetes/ngsp.html.)

5.4 Implementation of the Harmonization Program

The NGSP process includes three major components: calibration of assay methods, certification of assay methods (and individual laboratories), and proficiency testing of routine clinical laboratories.

5.4.1 Calibration of Methods by Manufacturers

Prior to certification of assay methods, the manufacturers should ensure that their assay results are comparable to laboratory network results. The manufacturers may request assistance from a network laboratory. The processes by which manufacturers calibrate their assay methods will differ depending on the specific assay method. For example, some GHB manufacturers have calibrated their assay methods by assignment of calibrator values, while others have used conversion equations; different assay methods may or may not include calibration by the end user. Network laboratories may assist manufacturers to determine the best approach to calibration of the assay; to evaluate different calibrator materials; to assign preliminary values to calibrators with the understanding that an adjustment in the values might have to be made based on results of fresh sample comparisons; and to perform analyses of fresh samples to provide a basis for calibrator value assignment.

5.4.2 Certification

5.4.2.1 Manufacturer

Manufacturers desiring certification of assay methods should contact NETCORE. NETCORE will then assign an SRL based on the manufacturer's preference, method type, and/or potential conflicts of interest. Precision testing (as well as method comparison with fresh blood samples) should be performed according to the established certification protocol. (See the NGSP website for details.)

Manufacturers may perform the precision and comparison analyses at the manufacturing site, or they may choose to have a clinical laboratory perform the testing using its system/assay. Collection of patient samples may be done either by the manufacturer (or designated laboratory) or by the SRL, as long as sample stability requirements for both the SRL and the manufacturer's method can be met. All data should be sent from the manufacturer and the SRL directly to NETCORE.

All data analyses are performed by NETCORE following the established protocol with evaluation of precision and bias. Manufacturers are awarded certificates for successfully completing precision testing and fresh sample comparisons for the specific reagent lots, calibrator lots, and instrumentation used. NGSP certification applies only to results from fresh blood samples. Analyses of processed (e.g., lyophilized) material may be subject to matrix effects, and any comparisons to the DCCT using results from processed specimens are not recommended at this time.

5.4.2.2 Laboratory

Although the primary goal of the NGSP is to certify manufacturers' methods, certification of individual laboratories is also offered. The NGSP offers two types of laboratory certification. For a "level one" certification, the laboratory follows the same certification procedure as for manufacturers. In addition, the laboratory participates in quarterly proficiency testing administered by the network. This type of certification is recommended for laboratories that are involved in large-scale research studies, such as clinical trials where long-term, high precision of GHB measurements is critical, i.e., where a small shift in GHB results could affect the final outcome of the study.

For "level two" certification, the laboratory follows the same procedure as for level one, except that quarterly proficiency testing by the laboratory network is not performed. This type of certification testing is highly recommended if the laboratory uses an assay method that is not NGSP-certified.

5.4.3 Proficiency Testing to Document Success of the GHB Harmonization Program (See Section 6.3.)

To receive maximal benefit from the certification program, clinical laboratories should participate in a proficiency testing (PT) program that uses fresh whole blood samples with target values assigned by the network. Proficiency testing data are used to assess the effectiveness of the harmonization program by: 1) estimation of bias from the target (by laboratory, by method, by method type, and including all GHB methods); and 2) interlaboratory comparability within and between methods.

6 Clinical Application

6.1 Rationale for Using an NGSP-Certified Method

As discussed in the Foreword of this document, the DCCT showed the relationship of GHB measurements to glycemic control and to risks for the development of microvascular and neuropathic complications of diabetes.[5] The DCCT provided the scientific basis for current recommendations for specific GHB and blood glucose targets to lower the risks of complications in patients with diabetes mellitus.

In a recent position statement, the ADA recommended that physician-coordinated healthcare teams take into account the results of clinical trials such as the DCCT for setting individual glycemic targets in the treatment goals for patients with Type 1 diabetes.[8] The target values of GHB recommended for glycemic control in current use are those based on the DCCT guidelines. Physicians and healthcare teams seeking to apply the knowledge gained from the DCCT should have the assurance that the GHB test values reported by their local laboratories' methods are comparable with those of the DCCT.

Use of methods that are NGSP-certified provide the best guarantee that the GHB results from one clinical laboratory are numerically equivalent to the results of the DCCT and are also interchangeable with the results from other clinical laboratories using NGSP-certified methods.[31] An NGSP-certified method thus provides optimal use of GHB testing.

6.2 Consideration for Selecting a GHB Test Method

To assure that results from a laboratory are numerically equivalent to the results of the DCCT and are interchangeable with results from other laboratories, the ADA has recommended that laboratories use only GHB assay methods that have been NGSP-certified.[25] In addition to providing comparable results to the values and ranges established during the DCCT, an NGSP-certified method has good reproducibility; the total imprecision (CV) of an NGSP-certified method has been demonstrated to be less than 5% during certification testing performed by the manufacturer.

Commercially available GHB assay methods employ many different principles of operation. When a laboratory considers the purchase of a GHB assay method, it should be noted that the various assay methods for glycohemoglobin (GHB) have different technical advantages and disadvantages and may be affected by different interferences due to technical, environmental, analytical, or clinical factors. Thus, results obtained in the presence of assay interference may not be a true reflection of the patient's time-averaged blood glucose.

Laboratories serving populations with a high prevalence of hemoglobinopathies, (e.g., HbS, HbC, HbE, HbG, HbH, Wayne, thalassemias) should be aware that some GHB assay methods may show falsely increased or decreased GHB test results with some hemoglobin variants and/or high levels of HbF.[33-35]

Some assays may produce falsely elevated GHB test results caused by an acutely generated, reversible, nonenzymatically linked glucose intermediary product, called “labile GHB” or “pre-A_{1c},” or Schiff base, which is present after a heavy meal or glucose-tolerance test. The higher the prevailing ambient level of blood glucose, the higher the level of labile GHB. If an assay is affected by labile GHB, blood samples should be treated to remove this fraction before assay.[36-45]

The presence of elevated levels of lipids (triglycerides) or bilirubin also may interfere with some GHB test methods.[46] Results of ion-exchange resin column assays may vary with temperature, pH, elution rate, column size, sample load, and ionic strength.[46] These factors are more difficult to control with open-column (nonautomated) assay systems.

Some methods measuring GHB cannot separate the HA_1 subfraction: $HbA_{1a\text{-}c}$. These methods thus quantify HbA_1. GHB assay methods that determine HbA_1 have many limitations and interferences not seen with other technologies. Methods based on HbA_1 determination generally have less accuracy, due to the presence of the HbA_{1a} and A_{1b} fractions, which do not reflect mean blood glucose levels as reliably as does the HbA_{1c} fraction.[47, 48] In addition, if blood specimens are stored at temperatures above 4 ^{o}C, the $HbA_{1a\text{ and }b}$ fractions show steady increases that are time- and temperature-dependent. [46]

Some methodologies also may have falsely elevated results caused by various substances other than sugars that can form adducts with hemoglobin, thereby altering the charge characteristics. Examples include individuals with opiate addiction,[49] lead poisoning, uremia, and alcoholism,[46] and persons receiving large doses of aspirin.[50] Vitamins C and E are reported to decrease GHB levels, possibly by inhibiting the glycation process.[51,52] Demonstration of these effects, however, is method dependent. The clinical significance of these effects on GHB is not well understood. Iron-deficiency anemia is reported to falsely increase GHB results.[53]

A laboratory should consider a number of technical issues when selecting a test method. Some GHB test methods allow batching of samples. Some methods require a hemoglobin separation or hemolysis step

and/or labile removal. Some methods demonstrate better precision than others. Some methods have high start-up costs, require expensive specialized equipment, or have technically demanding operating or maintenance requirements. Some assay methods employ small bench-top analyzers that are mobile. Some have faster turnaround times than others. Some allow primary tube sampling without cap removal or sample transfer.

Other considerations for choosing a GHB assay method are space constraints, compatibility of the method with the laboratory's data management system; how often the manufacturer requires controls and calibrators to be run; whether there is a statistically significant number of users listed on the proficiency testing report; what the cost is per reportable result; extent of technical support; and whether there are additional service charges.

Laboratorians should consult product labeling or contact the manufacturer directly with questions. GHB test limitations should be found in the product labeling under the **"Summary and Explanation,"** "**Specimen Collection and Handling,"** or the **"Limitations"** section of the package insert.

6.3 Proficiency Testing

A critical part of any effort to harmonize results of a clinical laboratory test among laboratories is development of a program to test clinical laboratories' comparability of test results on patient specimens. Such programs are usually called "proficiency testing (PT) programs" in North America or "external quality assessment schemes (EQAS)" in other countries. While design of such PT/EQAS programs would appear to be simple (i.e., send out aliquots of a large pool of a material that exactly mimics clinical specimens, and compare results of all laboratories regardless of the analytical method they use), in reality things are not quite so straightforward. PT/EQAS materials must be stable across time and place. Consequently, they are generally processed and/or have preservatives added to enhance stability. However, processing and stabilizers often subtly alter the responsiveness of a clinical analyzer for the analyte(s) of interest. While such clinical methods could be called nonrobust, they have been optimized to give accurate results of fresh human specimens, not stabilized PT/EQAS specimens. Regardless of whether this altered responsiveness is an analyzer's or a PT/EQAS material's shortcoming, this altered responsiveness leads to what has been termed "matrix biases" which often make direct laboratory-to-laboratory and method-to-method comparisons very difficult.[54,55] For GHB, lyophilization has been clearly demonstrated to cause biases across methods that are greater than biases seen with fresh materials.[31,56] One approach to avoid such matrix biases is to obtain samples from healthy individuals and diabetics that are collected, aliquotted, and circulated to the laboratories in the PT/EQAS program for testing within a few days of phlebotomy. This is the approach taken by the College of American Pathologists' GHB GH2 Survey that was introduced in 1996.[55] From 1996 through 1998, individual single-donor units were aliquotted and mailed to participating clinical laboratories for direct comparison of results from all analytical methods. More recently, pooled specimens are being used.

One potentially serious shortcoming of fresh whole blood PT/EQAS programs is that aging artifacts may develop between the time of blood collection and the time of GHB measurement. Several analytical methods are very sensitive to such aging effects. Thus, even when "fresh" PT/EQAS specimens are used, aging effects may introduce unexpected artificial method-to-method biases that would not be seen in typical fresh clinical blood specimens. Thus, the logistics for collection, vialing, and shipping of blood specimens become very demanding and make intercontinental PT/EQAS programs very difficult to perform.

An alternate approach for PT/EQAS specimens is use of stabilized (e.g., lyophilized) pools with mathematical correction of any analyzer's result for the method-specific matrix biases that the stabilization process may have introduced.[57] For this approach to assess a method's trueness, the method-specific matrix biases in the material should be stable with time. Furthermore, there should be some assurance that both the clinical method and any new lots of stabilized PT/EQAS materials have not

changed in any way that significantly impact the magnitude of the historically derived mathematical matrix bias correction factor. Critical to this approach is the initial generation of data to define the exact nature of the bias, prior to establishment of a correction factor. Experience in the Dutch PT/EQAS program provides limited data that lyophilized material can be made consistently, and that the method-specific matrix biases are quite reproducible from batch-to-batch of lyophilized material.[58] One major advantage of lyophilized vs. fresh whole blood PT/EQAS specimens is that lyophilized specimens can be used for as long as several years, thus enabling a PT/EQAS program to test laboratory precision over years, rather than at a single point in time. Furthermore, when individual PT/EQAS samples are made from mixtures of low and high master pools that have been combined in a quantitative manner, the resulting sample set can then be used to test analytical linearity. Another potential advantage is that planned excess vials from PT/EQAS samples can be used later as well-characterized quality control materials. However, there are some concerns that subtle changes in production of new lots of PT/EQAS material or in the clinical method's reagents or instruments themselves will change the exact magnitude of the matrix bias correction factor. Should this happen, false conclusions as to a given method's trueness could then be reached.

The frequency that PT/EQAS programs challenge a laboratory's performance varies widely from monthly to semiannually. It would seem that twice a year is the very minimum frequency for external assessment of laboratories' performance. Many international programs challenge laboratories as frequently as monthly. The less frequently PT/EQAS challenges occur, the stronger a laboratory's intralaboratory quality control program should be in detecting any assay drift.

At present the NGSP recommends the use of only fresh whole blood specimens for PT/EQAS. Laboratories that use GHB assay methods certified by the NGSP are encouraged to participate in a PT/EQAS program that uses fresh whole blood specimens with target values set by the NGSP laboratory network. This is an important "final check" of the certification process; the laboratory should achieve results consistently close to the target values.

6.4 Clinical Interpretation

GHB measurements are now an essential component of the clinical management of patients with diabetes mellitus. The level of GHB is a measure of glycemic control during the preceding 120 days, the mean erythrocyte lifespan.[10] Fructosamine and other glycated serum proteins are also analytes that reflect glycemic control, but over a much shorter time span (days to weeks) than GHB. The role of glycated serum proteins in clinical management of patients with diabetes mellitus is less clearly defined than GHB and is not covered in this document.[10]

Elevated levels of GHB suggest the need for more aggressive treatment of glycemia. The American Diabetes Association recommends that a primary goal of therapy should be a GHB value <7%, and that physicians should reevaluate the treatment regimen in patients with GHB values consistently >8%.[8] These GHB values apply only to assay methods that are NGSP-certified. Regression analysis of the DCCT data showed that a GHB value of 7% correlates to a mean blood glucose result of 8.58 mmol/L (156 mg/dL), and each 1% change (e.g., change in GHB from 7 to 8%) in GHB reflects a change in mean blood glucose of approximately 1.66 mmol/L (30 mg/dL).[59]

Although GHB is useful as a surrogate measure of mean glycemia, complementing the results of patients' daily blood glucose testing, it is perhaps more important as a measure of outcome risk. Both the DCCT and the UKPDS showed that risks for the development and/or progression of microvascular and neuropathic complications of diabetes were directly related to glycemic exposure as quantified by serial GHB determinations.[5,26-28] For example, in the DCCT a reduction in the GHB result (as HbA_{1c}) from approximately 9 to 7% was associated with a risk reduction of 76% for development of clinically significant diabetic retinopathy. Each 10% lowering of the GHB result (e.g., 12 vs. 10.8% or 8 vs. 7.2%) was associated with approximately 45% lower risk for the progression of retinopathy.[60] Virtually identical

risk reductions for progression of retinopathy were found in the DCCT and UKPDS in relation to the same absolute decrements in GHB.[5,26-28]

6.4.1 Factors Other Than Glycemia That May Affect Test Results

Certain clinical situations may alter GHB values independent of mean blood glucose. Any situation that increases erythrocyte turnover or enriches the erythrocyte pool with younger cells (such as hemolytic anemia or acute and chronic blood loss) will lower the level of GHB for any given level of mean blood glucose.[10] Vitamins C and E are reported to lower GHB values, possibly by inhibition of the glycation process.[51,52] Demonstration of these effects, however, are method dependent. Iron deficiency anemia is reported to raise GHB levels, although the mechanism is not known.[53] Other *in vivo* conditions may alter GHB values by interference with some GHB assay methods (see Section 6.2).

Many different hemoglobinopathies have been reported to interfere with GHB assays, some falsely increasing and others falsely decreasing results.[34,61,62] Interferences are assay method specific, and may differ even among assay methods of the same general type, e.g., ion-exchange chromatography. It is important to separate hemoglobinopathy-related assay interferences and effects due to shortened erythrocyte survival, as found with some hemoglobinopathies.

Other reported interferences include chronic alcohol ingestion, Vitamin C, salicylates, acetylated Hb, carbamylated Hb, hypertriglyceridemia, hyperbilirubinemia, lead poisoning, and opiate addiction.[46,49,50,52,63,64]

Physicians and laboratorians should refer to the manufacturer's technical literature to determine whether known potential interferences (e.g., medications, hemoglobin variants) affect that particular GHB assay method.

6.4.2 Evaluating a Discrepancy Between Clinical Imprecision and Test Result

Any large discrepancy between clinical impression of glycemic status and GHB test result warrants investigation. Typical situations would include a GHB result <4% or >14%, a change in GHB result of 2% (e.g., a change of GHB result from 8 to 10%) within 6 to 12 months with no major change in therapy, or a change in compliance with the prescribed medical care plan. Although the GHB test is generally a reliable measure of mean glycemia, the discrepancy could be the result of laboratory error or an *in vivo* condition, such as an alteration in red blood cell lifespan.[65]

7 Summary

The NGSP has as its primary goal harmonization of GHB test results among laboratories. Participation by manufacturers and laboratories is voluntary. Results from proficiency testing surveys that use fresh whole blood specimens have shown dramatic improvements in comparability of GHB test results among laboratories, particularly those using an NGSP-certified method. [66]

It is anticipated that this program and subsequently this document will undergo significant changes, as part of the consensus process, as new scientific data accumulate from the certification process and from proficiency testing programs, and as new clinical outcome data are available.

Although this guideline describes a harmonization program for one specific analyte, the general approach may be adaptable to other analytes, particularly in situations similar to GHB where clinical outcome data become available in relation to a set of laboratory test results, but with no pre-existing harmonization of test results among routine clinical laboratories. The approach described here provides a harmonization scheme that can be applied to an analyte whether or not a definitive/reference method and/or purified reference materials are available.

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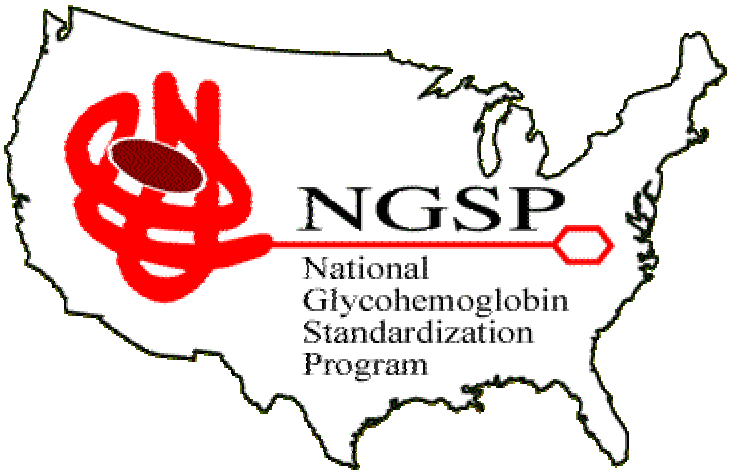
Appendix. The NGSP Protocol. (From the National Glycohemoglobin Standardization program website [http://www.missouri.edu/~diabetes/ngsp.html]. Reprinted with permission.)



NATIONAL GLYCOHEMOGLOBIN STANDARDIZATION PROGRAM (NGSP)

The purpose of the NGSP is to standardize glycohemoglobin test results so that clinical laboratory results are comparable to those reported in the Diabetes Control and Complications Trial (DCCT) where relationships to mean blood glucose and risk for vascular complications have been established. A key component of the program is the Reference Laboratory Network. The network interacts with manufacturers of GHB methods to assist them first in standardizing their methods and then in providing comparison data for NGSP certification.

- **Background**
- **Protocol**
- **How to Obtain Certification**
- **List of Certified Methods**
- **List of Certified Laboratories**
- **Steering Committee Members**
- **Laboratory Network Members**
- **ADA Recommendations**
- **CAP GH2 Data**
- **Announcements**

NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

C44-P: *Harmonization of Glycohemoglobin Measurements: Proposed Guideline*

General

1. The guideline should address what can be called "traceable to the NGSP" versus what can be called "traceable to the DCCT."

- **This issue has been addressed in Section 6.**

Section 6.2 and Section 6.4.1

2. Section 6.2 paragraph 7, and Section 6.4.1 paragraph 1, state that Vitamin C and B **lower** GHB values. In reality, this is method dependent. By electrophoresis, Vitamin C has been shown to significantly **increase** GHB (reference *Diabetes*. Feb. 1992;41:167-73).

- **A sentence has been added to both sections indicating that these effects are method dependent.**

References

3. Consider adding a recent reference reviewing hemoglobinopathy interferences in various GHB methods: Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem.* 2001;47:153-163.

- **The reference has been added.**

Summary of Delegate Comments and Subcommittee Responses

C44-A: *Harmonization of Glycohemoglobin Measurements; Approved Guideline*

Foreword

1. The document states that the IFCC method will be used as the anchor for harmonization for the NGSP. If we are to attain harmonization for worldwide measurements of glycated hemoglobin, a common reference procedure is preferable, since different calibrations for different pans of the world are not acceptable for manufacturers or clinicians. The document recognizes the position of the IFCC method as a higher order method but correctly calls out the fact that the clinical data collected in the United States has been based on the NGSP method, and clinical reference ranges would change or conversion tables would be required to accommodate the different reported results. The most important question to address is that of harmonization. If we are headed that way, then the efforts should be directed toward making the necessary transition in the clinical community to the new values and to begin to standardize glycated hemoglobin to the agreed upon reference methodology. There is little point in having the secondary method in a certified reference laboratory when the primary method is only marginally more difficult to execute. What is important for manufacturers to avoid is the selling of products that have different standardization protocols depending upon the geographical location. Costs to manufacture and consequently laboratories will increase even though the chemistry has not changed.

- **C44-A describes a highly successful active program for harmonization of glycated hemoglobin testing among laboratories. The guideline is based on the NGSP, which has been adopted in many countries since 1996 to help laboratories provide glycated hemoglobin assay results which are linked to clinical outcomes data, thereby optimizing the clinical utility of the test. The recently published five-year progress report of the NGSP (*Clin Chem*. 2001;47(11):1985-1992) documents the program's success.**

  The issue for this commenter seems to be the relationship between the NGSP and the newly developed IFCC network. The NGSP is presently evaluating the IFCC method(s) as a possible anchor for the NGSP network, replacing the Bio-Rex system that has been in place for many years. So far, the comparisons show excellent results with high correlation between the two networks, but the HbA_{1c} results are on a different scale. The current NGSP-certified methods report DCCT/UKPDS aligned values. There is a large amount of patient education material linking DCCT/UKPDS HbA_{1c} values with glycemic control and the risks for complications. At this time, the diabetes community has strongly expressed its desire not to change HbA_{1c} values, regardless of the anchor. The plan for the NGSP is to develop a master equation between the IFCC and the NGSP networks to allow "translation" of IFCC calibrated assay method values into DCCT-aligned values. An important aspect of the master equation would be ongoing network-to-network monitoring to document stability of the network-to-network relationship. If and when a master equation is accepted by the IFCC working group and the NGSP steering committee, the anchor of the NGSP may change, but the basic scheme of NGSP operation (calibration, certification, proficiency testing) will remain the same. Thus, changing the anchor should have little impact on the NGSP process and those changes will be included in a future revision of C44-A.

2. When this change from NGSP to IFCC occurs, it is imperative that the necessary stakeholders understand the conversion and the impact the conversion will have on the entire medical community. This must be well coordinated, and a sufficient amount of time to implement must be accommodated.

- **The subcommittee agrees that it is imperative that the necessary stakeholders understand the conversion between the NGSP and IFCC anchor. It remains unclear what will happen with the**

HbA_{1c} numbers. Resolution of that issue must be dealt with by the IFCC, NGSP, and diabetes community and will be addressed in a future revision of C44.

Scope

3. If this document is to provide a mechanism for certification, then it should contain specific protocols and acceptance criteria within the document. These protocols and criteria should be developed only after being well publicized and brought forward and discussed by all stakeholders. Implications that arise due to changes should be well understood by the medical community before implementation begins. Criteria for the NGSP certification have changed three times in the last two years. This amount of change indicates a process not yet sufficiently mature. It is imperative to consider the impact of all changes prior to implementation. Therefore, a more detailed description of the requirements for making changes should be part of this document. Having protocols and criteria contained within the document would be a step toward meeting that objective.

- **In early drafts of C44, the specific NSGP protocols and certification criteria were included. Ultimately, there was consensus to remove this information from the main text of the document and incorporate it as an appendix. (Note: The information is also readily available online at the NGSP website [www.missouri.edu/~diabetes/ngsp.html].)**

Section 3

4. If the committee intends this document to be a model for other standardization programs then it is important to harmonize the terminology currently in use within international documents, such as ISO.

- **The nomenclature in the NGSP protocol has undergone considerable revision to be as consistent as possible with ISO nomenclature. In the definitions section of C44-A, the comparable ISO terms for NGSP terms are given.**

5. In the definition of "designated comparison method," add the following statement: "this is comparable to a Secondary Reference Measurement procedure in ISO terminology."

- **The definition has been modified as suggested.**

6. The “primary reference laboratory” must run the primary reference measurement procedure. If they are to continue running the DCCT method, then they cannot be considered primary.

- **It is generally accepted that the IFCC reference methods (mass spectroscopy and capillary electrophoreses) are more difficult to execute than the NGSP secondary reference laboratory methods. The NGSP secondary reference laboratory methods are "field" methods which are capable of both high precision (interassay CVs<3%) and the high throughput required to keep up with the increasing workload as more and more laboratories and manufacturers request certification. The NGSP designated comparison method used by the CPRL and PRLs is also considerably more complicated than the "field" methods used by the SRLs; this is why the NGSP laboratory network was originally set up as a "two-level" system.**

Section 4

7. The structure laid out in the flow chart does not align with the ISO 17511 terminology or hierarchy for glycohemoglobin that is being used today. (See prEN ISO 17511:2002.) It would be more helpful if the document better follows what is in the ISO document. This would make it more consistent and harmonized and easier to implement. When the IFCC method is used, then it can easily be dropped into the hierarchy.

- **As discussed in C44-A, much of the NGSP process has been patterned after the successful National Cholesterol Reference Network. C44-A "borrows" directly from the NGSP protocol, available online. For example, the document figure is taken directly from the NGSP protocol (with permission).**

8. The terms "primary laboratory" and "secondary laboratory" should be re-evaluated with respect to ISO 17511.

- **Note that the NGSP protocol (and C44-A) refers to the terms "primary reference laboratory" and "secondary reference laboratory." These terms refer to laboratory designations and not specific assay methods. Thus, it should not be inferred that the NGSP primary reference laboratory runs a primary reference method as defined by ISO.**

9. We note inconsistent wording in Section 4, first and second paragraphs. Please replace "normal range" with "reference range" in these paragraphs.

- **The phrase "normal range" has been replaced with the phrase "reference range" as suggested.**

Section 5.2

10. Mention should be made that the steering committee should be totally independent of the laboratories performing the testing. Since protocols and acceptance criteria are going to be developed by this committee, care should be taken to avoid any appearance of conflict of interest and allow for adequate review and comment by stakeholders.

- **The NGSP process does have broad representation on the steering committee, including two elected and rotating manufacturer representatives. The group has operated quite well over the years and a larger committee would be cumbersome. There is also a clinical advisory committee to NGSP representing patients and care providers.**

  To ensure the NGSP does not have conflicts of interest, results of certification analyses are "blinded" by the network coordinator prior to review by the remaining steering committee members. Thus only the network coordinator has knowledge of which manufacturers' data are being reviewed. Moreover, the criteria for acceptance or rejection of an application for certification are adopted from well-accepted statistical procedures. Grading is entirely objective and can be verified by participants.

Section 5.3.2

11. This laboratory, better called the “secondary reference measurement laboratory,” should also have a requirement to become certified by ISO. Please see ISO 15195 or ISO 17025. It is imperative to manufacturers and laboratories that use the resources of reference laboratories that a measure of quality assurance is provided. The best means to do this is by certification from an external organization; this should be required.

- **See response to Comment 10.**

Section 5.4.2

12. When using fresh blood, the reference laboratory should be aware of the requirements in the U.S. for IRB review and approval when human samples are used. The manufacturers will need this type of documentation when participating in the certification process, as well as laboratories that are becoming certified. A section in the document regarding this would be appropriate.

- **U.S. requirements for IRB review and approval when human subjects are used is a complicated subject. IRB regulations require IRB review if specimens are being obtained for research purposes. Activities that meet this definition are defined as "a systematic investigation, including research development, testing, and evaluation, designed to develop or contribute to generalizable knowledge." Certification testing materials are probably exempt. Each institution will need to consider this issue with its IRB.**

Section 5.4.2.1

13. The certification protocols should be developed by a more extensive list of stakeholders than the steering committee that is now in place. These protocols are important, and they should not be changed unless all stakeholders have time to consider the changes and provide input on the impact to the medical community.

- **Only one NGSP certification criteria change has been implemented since the program began in 1996. This change was discussed in detail with manufacturers and other interested parties prior to implementation. On a regular basis, the NGSP has manufacturer forums to discuss all aspects of the NGSP, including proposed protocol changes. All of these forums have been well attended. There is already very broad stakeholder involvement in the NGSP process.**

14. As part of review of new criteria a systematic approach should be taken in understanding the medical communities requirements. ISO 15196, *Determination of analytical performance goals for laboratory procedures based on medical requirements,* may be a useful tool in setting future performance goals or determining a process that would be followed when changing criteria.

- **The question of how to set performance goals for assay methods is difficult. The NGSP has had the benefit of reviewing proficiency testing results in addition to certification results. Almost without exception, assay methods that fail NGSP certification, or pass marginally, show poor performance on proficiency testing surveys. NGSP criteria for certification have already taken into account the medical community's requirements.**

Related NCCLS Publications*

EP5-A **Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1999).** This document provides guidance for designing an experiment to evaluate the precision performance of clinical chemistry devices; recommendations on comparing the resulting precision estimates with manufacturer's precision performance claims and determining when such comparisons are valid; as well as manufacturer's guidelines for establishing claims.

EP9-A2 **Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (2002).** This document addresses procedures for determining the bias between two clinical methods or devices and design of a method comparison experiment using split patient samples and data analysis.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

NRSCL13-A **The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results; Approved Guideline (2000).** This document contains procedures for developing and evaluating definitive methods, reference methods, designated comparison methods, and reference materials to provide a harmonized clinical measurement system.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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NCCLS ▾ 940 West Valley Road ▾ Suite 1400 ▾ Wayne, PA 19087 ▾ USA ▾ PHONE 610.688.0100
FAX 610.688.0700 ▾ E-MAIL: exoffice@nccls.org ▾ WEBSITE: www.nccls.org ▾ ISBN 1-56238-478-3