



Harmonizing Hemoglobin A1c Testing

A better A1C Test means better diabetes care.

Protocol

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Overview

The NGSP consists of a Steering Committee and a network of reference laboratories including the Central Primary Reference Laboratory (CPRL), backup Primary Reference Laboratories (PRLs) and Secondary Reference Laboratories. The CPRL and PRLs analyze HbA1c using the Bio-Rex 70 cation-exchange assay method used in the Diabetes Control and Complications Trial (DCCT). The SRLs work directly with manufacturers and laboratories to assist them in calibrating their methods, and provide data for certification of traceability to the DCCT. The SRLs are monitored monthly against the CPRL monthly, and the NGSP network is monitored against the IFCC Laboratory Network two times per year, via sample comparisons. The effectiveness of the NGSP program in harmonizing HbA1c results is assessed via the College of American Pathologists (CAP) GH-2 whole blood survey performed twice a year.

A: Steering Committee

The Steering Committee works with the Laboratory Network to implement the GHB Standardization Program according to the protocol. The committee is responsible for reviewing policy/protocol changes and certification data submitted by the Laboratory Network.

B. Laboratory Network

The Network consists of an Administrative Core (NETCORE), a Central Primary Reference Laboratory (CPRL), three Primary Reference Laboratories (PRLs), and seven Secondary Reference Laboratories (SRLs).

1. Administrative Core (NETCORE)

The Administrative Core coordinates the certification process and communicates directly with the Steering Committee. The Core analyzes all certification and network monitoring data, sends reports to the Steering Committee, and issues certificates to laboratories and manufacturers.

2. CPRL

The CPRL analyzes GHB (HbA1c) by HPLC using Bio-Rex 70 resin following the existing CPRL method protocol and sets the initial calibration for the standardization program based on the "set-point" used in the DCCT. The CPRL must document an accepted level of precision (total imprecision <3%) following NCCLS EP5-A2 guidelines (1). Back-up Primary Reference Laboratories (PRLs) and Secondary Reference Laboratories (SRLs) calibrate their assays so that results from fresh blood specimens agree with the CPRL (see section 2. and 3. below). The CPRL administers a monitoring program for all certified PRLs, SRLs, and Level 1 Laboratories (outlined below in section 5.).

3. Primary Reference Laboratories (PRLs)

PRLs serve as back-up laboratories for the CPRL to ensure that the CPRL function continues without interruption in the event that the CPRL cannot meet the program needs as outlined in section 2. All PRLs

analyze GHB (HbA1c) using the primary reference method according to the CPRL method protocol.

- a. **Primary Reference Method Calibration:** A single hemolysate calibrator is prepared by the CPRL; the target value is established by the CPRL based on the mean of at least 50 runs. All PRLs use this calibrator with assigned value to calibrate their instruments with each run according to the CPRL method protocol.
- b. **Primary Reference Method Quality Control:** The CPRL prepares hemolysate QC specimens (two levels, a non-diabetic level and a diabetic level of at least 10% GHB) following the same protocol as for the calibrator preparation. Each PRL must establish their own QC mean and range criteria and accept or reject assays following the CPRL QC guidelines.
- c. **Certification of Traceability:** A proposed PRL must perform precision testing following NCCLS EP5-A2 guidelines (1). The proposed PRL must also participate in a fresh blood sample comparison (n=100) for estimation of bias from the CPRL following modified NCCLS EP9-A2 guidelines (2). For bias estimation, fresh specimens are collected by either the proposed PRL or the CPRL and shipped to the other party. All data for calculation of precision as well as estimation of bias are submitted to the CPRL for analysis.

In order to be considered traceable to the CPRL: 1) total imprecision (CV) must not be statistically significantly >3%, 2) one within-method outlier is acceptable; one between-method outlier is acceptable, 3) 95% confidence interval for predicted bias should overlap the + 3% range of the CPRL at 2 GHB levels (6 & 9% HbA1c). All data analysis is performed by the NETCORE following NCCLS EP5-A2 and EP9-A2 guidelines. If criteria are not met, the proposed PRL may make changes as necessary, re-analyze the same set of samples from frozen aliquots and send the new set of data to the NETCORE for reevaluation. Final certification of traceability is issued by the NETCORE after review of the data by the Steering Committee.

4. Secondary Reference Laboratories (SRLs)

SRLs work directly with manufacturers to assist them in standardizing their methods and in providing comparison data for certification of traceability as outlined in section C. Each SRL analyzes HbA1c by a precise method (documented total imprecision $\leq 2\%$) which can be calibrated to the Primary Reference Method. A proposed SRL must demonstrate traceability to the CPRL method.

Secondary Reference Method Calibration: Calibrator may be obtained from a manufacturer or prepared by the proposed SRL. Calibrator values for SRLs are either assigned by the CPRL (by direct analysis or based on fresh sample comparisons with the CPRL) or by the SRL method while calibrated with the initial or previous lot of calibrator.

Secondary Reference Method Quality Control: Each SRL prepares or purchases their own QC specimens (two levels; a non-diabetic level and a diabetic level of approximately 10% HbA1c) and sets their own limits and acceptance criteria using appropriate QC guidelines.

Certification of Traceability: A proposed SRL must perform precision testing following CLSI EP5 guidelines (1). The proposed SRL must have also successfully participated in at least four consecutive NGSP quarterly monitoring comparisons (10 samples each) as a certified Level 1 laboratory. All data for calculation of precision and monitoring are submitted to the NETCORE for analysis.

Secondary Reference Method Certification Criteria:

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- 20 day precision evaluation following EP5 using two fresh-frozen single use aliquots of WB, one in the normal non-diabetic range (HbA1c ~5%; 31 mmol/mol) and one in the diabetic range (HbA1c: ~9-10%; 75 – 86 mmol/mol). Total imprecision (CV) must not be statistically significantly >2%.
- Comparison to NGSP network SRLs: 40 of the most recent previous quarterly monitoring (MMON) samples using single results (first of each duplicate) compared to the mean of SRL results.
- 38 of 40 results must be within $\pm 6\%$ (relative) of the SRL mean.
- For each quarterly monitoring comparison, candidate method results must also fall within a defined acceptance ellipse based on the slopes and intercepts of the differences between the individual SRLs results and the medians of all SRLs.

All data analysis is performed by NETCORE. If the criteria are not met, the proposed SRL may make changes to their method as necessary and provide documentation of these changes to NETCORE for approval to repeat the certification process. Final certification of traceability is issued by the NETCORE.

Requirements for new SRLs

Candidate SRLs that have not previously participated in the NGSP network must be able to fulfill the following requirements:

- Must be able to participate in monthly monitoring (10 samples/month) by CPRL.
- Have experience with and be able to communicate with interested manufacturers and laboratories without conflicts of interest.
- Must be able to collect and analyze certification samples according to the NGSP protocol, which includes screening for Hb variants and elevated HbF (>2%), and provide them to manufacturers and laboratories upon request.
- Be able to send invoices to manufacturers and laboratories participating in the certification process following NGSP fee schedule and pay NGSP the data analysis portion upon receipt of NGSP (University of Missouri) invoice. Rates for payment to NGSP are \$350 for each manufacturer and level II laboratory certification, \$1850 for each level I laboratory certification.
- International (non-U.S.) laboratories must provide NGSP with documentation that they have the support of the professional clinical community society in their country.
- Depending upon shipping logistics/costs, international laboratories may need to provide some or all of the costs of shipping monthly monitoring specimens to their laboratory.
- Consideration for Network membership will be given to the laboratory's geographic location and the need for an additional network laboratory. **The NGSP Steering Committee reserves final authority in determining which laboratories are eligible to participate in the Network.**

5. Performance Monitoring of Primary and Secondary Reference Laboratories

Monthly surveys are administered by the CPRL to monitor performance of the network Primary and Secondary Reference Laboratories. Each month, the CPRL ships 10 specimens (fresh or fresh/frozen whole blood) over the desired clinical range (4-10% HbA1c) to each network laboratory within 2 days of collection. The CPRL and/or each PRL prepares hemolysates following the CPRL sample preparation procedure (aliquots of each hemolysate may be frozen at -70oC or colder prior to assay). Each SRL prepares samples following their method protocol. The CPRL, PRLs and SRLs analyze the specimens in two separate runs on two separate days. All data is sent to the NETCORE for analysis. Each Level I-certified laboratory receives monitoring specimens quarterly.

To maintain certification, a network (PRL and SRL) laboratory must fulfill the following bias and precision

requirements: 1) the mean of the differences (n=10) between the network laboratory and the CPRL must not exceed 0.35% HbA1c and 2) the estimate of the standard deviation of the difference in sample replicates must not exceed 0.229 (99th percentile of the sampling distribution around a target SD of 0.15). SRL results must also fall within a defined acceptance ellipse based on the slope and intercept of the differences between the individual SRLs results and the medians of all SRLs. In addition, results for individual specimens where the within-SRL replicates differ by >0.4% HbA1c or the mean result differs from the median of the SRLs by >0.5% HbA1c will be excluded from the final SRL mean calculations. All results outside these limits are investigated. All monitoring results are shared among network laboratories.

6. Monitoring of the relationship between the IFCC and NGSP Networks

Twice each year five frozen whole blood specimens are analyzed by both the NGSP and IFCC networks. Each year the relationship between the networks is compared to the master equation which is based on eight comparison studies over a four-year period. A detailed statistical analysis is performed to insure that the relationship has not significantly deviated from the master equation.

References:

1. NCCLS. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline; Second Edition. NCCLS document EP5-A2 (ISBN 1-56238-542-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
2. NCCLS. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline; Second Edition. NCCLS document EP9-A2 (ISBN 1-56238-472-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2002.

C. Manufacturer Certification

1. **Standardization of Methods Prior to Certification of Traceability:** The process by which a method is standardized to the Reference will depend on the specific method and is determined by the manufacturer, e.g. standardization may be accomplished by re-assignment of calibrator values or by conversion equation since the method may or may not include calibration by the end user. Manufacturers may request assistance from the any network laboratory to 1) help determine the best approach to standardization, 2) recommend or to evaluate different calibrator materials 3) assign preliminary values to calibrators with the understanding that an adjustment in the values may have to be made based on results of fresh sample comparisons, and/ or 4) perform analyses of fresh samples to provide the manufacturer with data on the new method's values compared to reference values in order to provide a basis for calibrator value assignments.
2. **Certification of Traceability to the Reference Method:** Manufacturers desiring certification of their method must contact the NETCORE. The NETCORE will then assign a SRL based on manufacturers preference, method type, and/or potential conflicts of interest. A method comparison must be performed for certification of a method as traceable to the DCCT Reference. Before pursuing certification through the SRL, manufacturers should establish that their analytical instrument systems / methods:

have had all required preventive maintenance procedures performed

be in peak operating condition

be operated with the same parameters in all runs of the comparison and precision studies (e.g. instrument, reagent lot, calibrator lot, calibrator assigned values)

be operated in the same manner as they would by a customer

Manufacturers must participate in a fresh blood sample comparison (n=40, no hemoglobin variants or HbF>2%) for estimation of bias from the SRL. Manufacturers may perform the comparison analyses at the manufacturing site or they may choose to have a laboratory (that is NOT an NGSP network laboratory) using their system perform the testing. For the sample comparison, one set of fresh samples may be split and used to evaluate several applications or methods, thus necessitating only one evaluation by the SRL. 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 the NETCORE.

In order for a commercial method to be considered traceable to the CPRL, 37/40 of the individual results must be within 6% of the SRL means.

All data analysis will be performed by the NETCORE. Outliers will be analyzed for informational purposes only; an outlier is defined as $> \text{mean} + 3\text{SD}$ of the absolute differences between pairs. All outliers will be investigated by the NETCORE to determine if the discrepancy could be due to characteristics of the specimen rather than the assay method. If results show that a discrepancy could be due to characteristics of the specimen, then the manufacturer will be asked to submit a new specimen and the data will be reanalyzed.

Manufacturers are awarded Certificates of Traceability for successfully completing fresh sample comparisons for the specific reagent lots, calibrator lots and instrumentation. Traceability to the DCCT applies only to results from fresh or fresh frozen blood samples. Final certification of traceability is issued by the NETCORE. Certificates of Traceability are valid for one year. Certified methods are listed on the NGSP web site. Methods are required to be re-certified annually to remain listed on the NGSP web site. A new certification is also required in the event of significant changes, such as those that would require a new 510(k) form to be filed with FDA.

In the event that a method fails certification, the manufacturer must investigate the root cause(s) of the failure, document the reason(s) for the failure and describe the corrective measures implemented to prevent a failure in the future prior to a subsequent attempt to obtain certification. In addition, a description must be provided how these changes will be communicated to and applied to the methods in use by end-users. If the method is intended for use at the point of care, resubmission following a failure must include data for 3 different reagent lots. The passing criteria must be fulfilled separately for data from each reagent lot to be certified on a resubmission.

If a second attempt to certify is not successful, the manufacturer cannot resubmit for another attempt at certification until one year from the date of the notification of failure.

D. Level I Laboratory Certification

Level I Laboratory Certification includes both yearly certification of traceability to the DCCT and a quarterly monitoring component to insure stability throughout the year. This type of certification is recommended for large laboratories that are involved in research studies or clinical trials where long term precision of glycohemoglobin measurement is critical, i.e. where a small shift in glycohemoglobin results could affect the final outcome of a trial or research study. The certification protocol for Level I Laboratory Certification will be the same as followed for manufacturer method certification as outlined in section C. However, the certification criteria are more stringent; 38/40 of the individual results are within 6% of the SRL means.

Laboratories are awarded Certificates of Traceability for successfully completing fresh sample comparisons for the specific method, reagent lots, calibrator lots and instrumentation used. Traceability to

the DCCT applies only to results from fresh or fresh frozen blood samples. Analysis of processed (e.g. lyophilized) material may be subject to matrix effects and any comparisons to the DCCT using results from processed specimens should be made with caution.

Level 1-certified laboratories are also monitored on a quarterly basis using 10 fresh samples, analyzed in two separate runs on two separate days. To maintain certification a level I laboratory must fulfill the following bias and precision requirements: mean difference between laboratory and the mean of the SRLs $\leq 0.35\%$ HbA1c, SD of the difference in sample replicates < 0.229 . Level I Laboratories must submit quarterly monitoring data within 2 weeks of receipt of monitoring samples and pass the criteria in order to maintain their certification status. If a Level 1 laboratory either does not pass the monitoring criteria, or fails to send in data, for two consecutive quarters, the laboratory will be removed from the NGSP Certified Level 1 Laboratory list. If the laboratory then passes the NGSP monitoring criteria in a subsequent quarter, they will be placed back on the Certified Level 1 Laboratory list.

E. Level II Laboratory Certification

Laboratories can obtain documentation from the manufacturer of their system's performance and traceability to the DCCT, i.e. documentation of NGSP certification. However, a manufacturer's demonstration of a product's traceability (NGSP certification) does not in itself guarantee the accuracy and precision of that product in the hands of every user (clinical laboratory). Laboratories using methods that are not certified must take primary responsibility for establishing that system's traceability to the DCCT. Therefore, laboratories may also participate with a Network laboratory (SRL) to document traceability through a laboratory certification process. The certification process and the certification criteria for Level II laboratory certification are the same as that for manufacturers as outlined in section C. Laboratories are awarded Certificates of Traceability for successfully completing fresh sample comparisons for the specific method, reagent lots, calibrator lots and instrumentation used. Traceability to the DCCT applies only to results from fresh or fresh frozen blood samples. Analysis of processed (e.g. lyophilized) material may be subject to matrix effects and any comparisons to the DCCT using results from processed specimens should be made with caution.

F: Proficiency Testing to Document Success of the GHB Standardization Program

Laboratories are asked to participate in the GH2 proficiency testing (PT) program administered by the College of American Pathologists (CAP). The Survey began in 1996 as a pilot survey and now includes three fresh pooled whole blood specimens (at three GHB/HbA1c levels). Samples are shipped on cold packs using an overnight courier. Target values for each specimen are assigned by the mean result of all SRLs in the NGSP network. Proficiency testing data are used to assess the effectiveness of the standardization program by: 1) estimation of bias from the target (by laboratory, by method, by method type, and including all GHB methods), 2) interlaboratory comparability within and between methods, and 3) CV of all laboratory results. Beginning in 2007, NGSP target values are used for laboratory grading by the CAP.

Appendix A: NGSP Flowchart

