

Calibration Verification

Purpose

This technical bulletin is intended to provide a basic protocol for performing calibration verification of the SureStepPro Professional Blood Glucose Management System and the SureStep Blood Glucose Monitoring System using spiked whole blood samples.

Overview

The College of American Pathologists (CAP) defines calibration verification as “the assaying of appropriate matrix materials with known values in the same manner as patient samples to confirm that calibration of the test system has remained stable.”¹ The frequency of testing is determined by the institution; calibration verification may be necessitated by changes in reagent lots, shifts in control solution data, or the compliance requirements of certain voluntary accrediting agency standards.

The purpose of calibration verification testing with blood glucose monitoring systems is to substantiate the initial and continued accuracy of the method across the reportable range of glucose concentrations. This may be achieved by testing the same blood samples with both the SureStepPro bedside unit/SureStep meter and a reference instrument; the extent to which these test results agree is a measure of the calibration status of the monitoring system. A minimum of three glucose concentrations is recommended, one each at or near the lower limit, midpoint, and upper limit of the monitoring system’s operating range.

Factors to Consider in Conducting Blood Glucose Tests

Defining Your Needs

The most important factors in performing calibration verification are the needs of the testing institution, in terms of both a) levels of performance and b) regulatory requirements. The range of glucose concentrations in a study should reflect the operating range of the monitoring system and the levels of glucose seen in day-to-day monitoring. Interpretation of regulatory requirements will vary from institution to institution, and each laboratory is ultimately responsible for its own compliance. Criteria for acceptable performance should be established by the institution prior to initiating the calibration verification procedure.

¹ Commission on Laboratory Accreditation Inspection Checklist, Point-of-Care Testing, Section: 30. College of American Pathologists, 1997.

Blood Samples

- Observe universal precautions for handling blood samples.
- The SureStepPro and SureStep Systems are calibrated to report plasma glucose concentrations. Use whole blood for bedside unit/meter tests; use plasma from the same samples for comparison tests (with a laboratory reference instrument).
- Use blood samples whose hematocrits fall between 25% and 60%. Avoid the use of blood samples from dehydrated subjects, whose elevated hematocrits may cause inaccurate results.
- Collect blood samples in heparin tubes. Do not use fluoride collection tubes, as this additive interferes with bedside unit and meter tests.
- Do not combine blood samples from different donors.
- Test blood samples within 24 hours of drawing.

Effects of Glycolysis

The concentration of glucose in a whole blood sample will diminish with time due to glycolysis. If not properly managed, this effect can have a negative influence on calibration verification testing.

To assure that changes in the sample's glucose concentration do not substantially affect the outcome of a calibration verification study, plasma samples should be prepared (by centrifugation) within 10 minutes of testing with the bedside unit or meter. (Removal of red blood cells from the sample effectively eliminates the glycolysis effect.) Comparison testing of plasma samples may then be performed at a later time, preferably within 30 minutes of the bedside unit or meter tests of the sample.

Reference Instrument

Comparison testing with a reference instrument is a primary element of the calibration verification procedure described in this technical bulletin. Since the SureStepPro bedside unit and the SureStep meter report plasma-calibrated results, the reference instrument must be capable of testing plasma samples. Handheld meters or bedside units should not be used as reference instruments.

Quality Control

All instruments must be operated in accordance with manufacturer's instructions. Before performing a calibration verification test, thoroughly familiarize yourself with the correct operation of the SureStepPro or SureStep System and perform all quality control procedures specified in the SureStepPro Bedside Unit Operator's Guide or the SureStep Owner's Booklet.

Spiking

A procedure called “spiking” (described below) allows the adjustment of the glucose concentration of a whole blood sample to desired levels. Spiking gives the experimenter a high degree of control over glucose concentration but requires careful management of time and materials. If whole blood samples with wide-ranging glucose concentrations are readily available, spiking may not be necessary.

Spiking is performed by adding a small volume of concentrated glucose solution to a whole blood sample and mixing thoroughly; a simple calculation (below) is used to determine the volume of glucose solution required to achieve the target concentration.

The initial concentration of the whole blood sample must be known and must be lower than (or equal to) the target concentration. Using a whole blood sample whose endogenous glucose has been depleted by glycolysis (see below) will simplify the spiking procedure, especially when testing at low glucose concentrations. To ensure that depletion is complete (or nearly so), start with a blood sample whose glucose concentration is less than 100 mg/dL.

To spike a whole blood sample to a target glucose level:

1. Incubate the sample at room temperature for at least 16 hours to deplete the glucose in the sample (if necessary).
2. Obtain a standard D10W solution (or prepare a 10% glucose solution by dissolving 10 grams of anhydrous D-glucose in 100 ml of distilled or deionized water).

Note: The 10% glucose solution must be prepared at least 24 hours before use, or erroneous results will be obtained.

3. Determine the initial glucose concentration of the sample with the reference instrument, bedside unit, or meter.
4. Calculate (to the nearest microliter) the volume of 10% glucose solution needed to attain the target glucose concentration, using the formula:

$$V_s = \frac{V_b (\text{Target Concentration} - \text{Initial Concentration})}{10}$$

where V_s is the volume of 10% D-glucose solution required (in μL) and V_b is the volume of blood being spiked (in mL). For instance, if you wish to spike a 1 mL aliquot of blood (that has an initial glucose concentration of 10 mg/dL) to a target concentration of 50 mg/mL:

$$V_s = \frac{1 \text{ mL} (50 \text{ mg/dL} - 10 \text{ mg/dL})}{10} = 4 \mu\text{L} \text{ 10\% glucose solution}$$

5. Combine the blood and 10% glucose solution in a capped test tube; mix the sample gently but thoroughly (e.g. 10 minutes on a test tube rocker).

Note: The calculations presented above are approximate; attaining an exact target concentration is generally not important, since the “true” glucose concentration of the spiked sample is measured with the reference instrument. If the calculated volume (V_s) is too small to be pipetted accurately, dilute the spiking solution with distilled or deionized water and adjust the spiking volume accordingly.

Materials

- SureStepPro bedside unit(s) or SureStep meter(s)
- SureStepPro Test Strips - Approximately 15 test strips per bedside unit or meter, from the same lot
- Venous whole blood - Two or three samples (approximately 7 mL each), collected in heparin or EDTA tubes within the previous 24 hours from healthy donors
- Transfer pipettes
- *10% Glucose solution or standard D10W solution
- *Test tubes with stoppers (approximately 5)
- *Adjustable pipettors, 0 to 1000 μ L
- Test tube rocker
- Centrifuge
- Laboratory reference glucose analyzer
- Calibration Verification Record(s) - one for every two bedside units or meters

* Not necessary if you are able to obtain fresh whole blood samples whose glucose concentrations cover the desired concentration range.

Procedure

1. Ensure that the SureStepPro or SureStep System(s) have been prepared for testing and that all quality control procedures have been successfully performed, in accordance with the SureStepPro Bedside Unit Operator's Guide or the SureStep Owner's Booklet.
2. Record bedside unit/meter and test strip information on the Calibration Verification Record. Confirm that test strips have not reached their expiration date.
3. With the first donor sample, spike an aliquot (1 to 3 mL) at or near the lower limit of the monitoring system's operating range. Mix sample gently but thoroughly to ensure uniform distribution of red blood cells.
4. Perform three (3) separate bedside unit or meter tests on the spiked sample; record results on the Calibration Verification Record.

Note: Refer to the SureStepPro Test Strips package insert ("Blood Application") for an explanation and illustration of correct sample application. Examine the bedside unit or meter results for aberrant values which may unduly influence the outcome of the analysis; retest as necessary.

5. Centrifuge an aliquot of the spiked whole blood to obtain a plasma sample.
6. Perform duplicate comparison tests (with reference instrument) on the plasma portion of the spiked sample; record results on the Calibration Verification Record.
7. Repeat the testing procedure with spiked samples whose glucose concentrations are at or near the midpoint and upper limit of the monitoring system's operating range.
8. Repeat the procedure with the other donor samples(s).

Data Analysis

1. Calculate the mean comparison result at each level and record on the Calibration Verification Record.
2. Calculate the mean bedside unit or meter result for each spiked sample and record on the Calibration Verification Record.
3. Calculate the “bias“ of each bedside unit or meter at each glucose level. Record the results on the Calibration Verification Record.

- a. For glucose values **greater than or equal to 100 mg/dL**, calculate the **percent difference**:

$$\% \text{ Difference} = \frac{\text{Mean Bedside Unit/Meter Result} - \text{Mean Comparison Result}}{\text{Mean Comparison Result}} \times 100$$

- b. For glucose values **less than 100 mg/dL**, calculate the **difference**:

$$\text{Difference (mg/dL)} = \text{Mean Bedside Unit/Meter Result} - \text{Mean Comparison Result}$$

Note: Absolute difference (rather than percent difference) is used for values below 100 mg/dL to avoid exaggerated biases resulting from low glucose values.

4. Determine the expected bias range for each sample based on institutional requirements and record on the Calibration Verification Record. Compare to the bedside unit/meter results.

Calibration Verification Record

Bedside Unit/Meter Name & Serial # _____

Test Strip Lot # _____ Reference Instrument _____

Results	Lower Limit	Spiked Blood Samples Midpoint	Upper Limit
Comparison Results (mg/dL):			
Dupl. 1			
Dupl. 2			
Mean			
Bedside Unit/Meter Results (mg/dL):			
Sample 1			
Sample 2			
Sample 3			
Mean			
Absolute Difference (mg/dL)			
Percent Difference			
Expected Bias Range			

Performed by _____ Reviewed by _____

Signature/Date

Signature/Date

Bedside Unit/Meter Name & Serial # _____

Test Strip Lot # _____ Reference Instrument _____

Results	Lower Limit	Spiked Blood Samples Midpoint	Upper Limit
Comparison Results (mg/dL):			
Dupl. 1			
Dupl. 2			
Mean			
Bedside Unit/Meter Results (mg/dL):			
Sample 1			
Sample 2			
Sample 3			
Mean			
Absolute Difference (mg/dL)			
Percent Difference			
Expected Bias Range			

Performed by _____ Reviewed by _____

Signature/Date

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