External Quality Assessment in Primary Health Care by Using Fresh Whole Blood

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Desktop analyzers and single-test meters used in primary healthcare are mainly calibrated to measure whole blood. To minimize matrix effects of control materials in external quality assessment schemes, we stabilized fresh human EDTA-treated blood with sodium iodoacetate (1.8 mmol/L). The whole-blood based control material was useful for control of hemoglobin and glucose, the two most common analyses in primary care, even after storage at room temperature for at least 10 days and at 5°C for 3 weeks. The material was also useful for control of cholesterol and creatinine analyses for samples stored as long as 3 days at ambient temperature.

Indexing Terms: iodoacetate/flouride/quality assurance/sample treatment/control materials/glucose/hemoglobin/cholesterol/creatinine

Hospital laboratories perform regular calibration and quality control to assure reliable test results. Desktop biochemical analyzers or single-test meters are now frequently used in primary healthcare (1, 2). Although such instruments in the recent years have been constantly improved, the general practitioners who use them have often received minimal training in handling the instruments and are unfamiliar with the quality control of biomedical analyses that helps ensure accurate results (1–6).

The ideal quality-control material should be identical to the patient’s specimen to avoid matrix effects (2, 5, 7). Specimens analyzed in primary care are most often whole blood due to the ease of obtaining capillary blood and the desire to avoid centrifugation. Although some “dry-chemistry” analyzers can measure serum or plasma samples, desktop analyzers and single-test meters primarily are calibrated to measure whole-blood specimens. Thus, use of serum-based control specimens might produce different results on different analyzers (8). Given that the objective of standardization is to ensure that the same result is obtained regardless of how, where, or by whom the test is done (2), the assignment of different mean values of control samples to different analyzers in external quality schemes is not desirable.

The two most commonly performed tests in primary care in Denmark are blood hemoglobin and glucose measurements (3). A whole-blood-based control material for external quality assurance that could be used for both analyses would be valuable to avoid problems with matrix effects. In this study, we wanted to design such a stable whole-blood control medium for this purpose and to investigate its usefulness for other common analyses such as cholesterol (6, 9) and creatinine on the Reflotron analyzer.

Materials and Methods

Preparation of whole-blood control material. Blood from blood donors was obtained by venipuncture into sodium EDTA-containing tubes (final concentration 170 mmol/L). Then 9.4 mmol/L sodium fluoride or 1.8 mmol/L sodium monooiodoacetate was added. To obtain blood with low glucose in the experiments of recovery and linearity, or to establish a certain concentration of glucose, blood was left at room temperature for 2 days before addition of stabilizer. A sample of the blood taken before addition of stabilizer was tested for antibodies to human immunodeficiency virus and hepatitis C and for hepatitis B surface antigen. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Measurement of glucose. We used the following analyzers in our study, all kindly loaned by their manufacturers: Accutrend (Boehringer Mannheim, Mannheim, Germany), Glucometer Elite (Bayer Diagnostics, Copenhagen, Denmark), Hemocue B-glucose Photometer (Hemocue, Ängelholm, Sweden), Medisense Companion (MediSense, Copenhagen, Denmark), One Touch (LifeScan, Milpitas, CA), and Reflotron (Boehringer Mannheim). As the comparison method for glucose measurement in this study we used the Gluc-DH kit (Merck, Darmstadt, Germany; cat. no. 13886) in hemolysates (10) assayed on a Cobas Mira (Hoffmann-La Roche, Basel, Switzerland). The method was validated against a Reference Method value (obtained by gas chromatography–isotope dilution mass spectrometry at the Zen- trale Referenz Institution, Bonn, Germany) for a control serum used by Labquality, Helsinki, Finland in the Nordic surveys.

Measurement of hemoglobin. We used the Hemocue B-hemoglobin photometer and the Reflotron analyzer to measure hemoglobin. The comparison method was the sodium lauryl sulfate method for hemoglobin on a Sysmex K-1000 (Toa Medical Electronics, Kobe, Japan) (11), which was validated against the national external quality assessment scheme with a Reference Method target value (12).

Measurement of cholesterol and creatinine. We also used the Reflotron analyzer to measure cholesterol and creatinine.
Results

Stabilization of glucose in whole blood. The following concentrations were optimal to obtain acceptable recovery (>97%) of glucose measured by the comparison method after 2 days of storage at ambient temperature: ≥9.4 mmol/L sodium fluoride or ≥1.8 mmol/L sodium iodoacetate. Thus, the percentage recoveries of glucose in a blood sample initially containing 10 mmol/L glucose and various concentrations of sodium fluoride were:
- at 0 mmol/L sodium fluoride, 6%;
- at 1 mmol/L, 53%;
- at 2.5 mmol/L, 82%;
- at 5 mmol/L, 93%;
- at 7.5 mmol/L, 96%;
- at 10 mmol/L, 97%;
- and at 15 mmol/L, 98%. With sodium iodoacetate, the results were:
- at 0 mmol/L, 6%;
- at 1 mmol/L, 93%;
- at 1.5 mmol/L, 98%;
- at 2.0 mmol/L, 98%;
- at 2.5 mmol/L, 97%;
- and at 7.5 mmol/L, 95%.

To verify the stabilizing effect on different samples for a longer period, blood glucose was measured in five patients' samples (stripped of glucose and then supplemented with 8 mmol/L glucose) stored at room temperature for 10 days. Without stabilizer the glucose dropped rapidly after 1 day (recovery = 30% (95% confidence interval 23–37%), whereas 9.4 mmol/L sodium fluoride and 1.8 mmol/L sodium iodoacetate both showed a stabilizing effect, most pronounced for iodoacetate (Table 1).

At 5°C the same samples were stable for at least 3 weeks: with fluoride, mean recovery was 99% (C.I. 97–102%); with iodoacetate, the mean was 100% (C.I. 97–102%).

Evaluation of fluoride and iodoacetate as stabilizers on seven selected glucose analyzers. We investigated the most commonly used analyzers in Denmark, all of which were designed for fresh blood without anticoagulants. The manufacturers of Reflotron and Hemocue report no interferences from EDTA; therefore, we studied the effect of EDTA on those four instruments for which no information was available from the manufacturer. Setting the mean values of 10 different fresh blood samples without EDTA to 100% (mean glucose, 4.8 mmol/L) we obtained the following recoveries for the same samples with EDTA added: MediSense 94% (C.I. 84–105%), One Touch 101% (C.I. 97–105%), Elite 99% (C.I. 95–104%), and Accutrend 100% (C.I. 94–107%).

To evaluate a possible influence of the stabilizer and the variation of this influence on various blood samples, we assayed 10 different patients' samples in the absence and in the presence of stabilizer (Fig. 1). The addition of fluoride significantly reduced the glucose concentration measured with the One Touch (P < 0.05). The Accutrend gave a lower value for glucose than the other instruments did, whether stabilizers were present or not. Sample-to-sample variation seemed to be higher for the MediSense than for the other instruments.

Influence of the stabilizers on hemoglobin measurements with selected analyzers. No interference from iodoacetate (1.8 mmol/L) was observed on the Sysmex 1000, the Hemocue, or the Reflotron analyzer, but fluoride (9.4 mmol/L) caused an overestimation of hemoglobin on the Reflotron (see below). The following mean hemoglobin values were obtained for 10 different whole-blood samples with the Sysmex 1000: with no additives, hemoglobin was 7.4 mmol/L (C.I. 6.9–7.9); with added fluoride, 7.3 mmol/L (C.I. 6.5–7.5); and with added iodoacetate, 7.3 mmol/L (C.I. 6.9–7.7). On Reflotron the results were:
- 7.0 (6.3–7.6), 7.8 (C.I. 7.4–8.7), and 7.0 (C.I. 6.4–7.5) mmol/L, respectively.
- The respective Hemocue results were:
- 7.0 (C.I. 6.5–7.4), 6.9 (C.I. 6.5–7.4), and 7.1 (C.I. 6.5–7.5) mmol/L.

We therefore chose iodoacetate as stabilizer because it led to better recovery and fewer interferences than fluoride. Stability studies of hemoglobin measurements on Sysmex 1000 with 1.8 mmol/L iodoacetate showed no significant changes on storage for ≤10 days at ambient temperature. For five different patients' samples the mean recovery was 99% (C.I. 98–101%) on the 10th day (initial mean hemoglobin, 8.2 mmol/L).

Linearity of glucose and hemoglobin measurements after addition of iodoacetate. Iodoacetate did not affect the linearity of measurements of hemoglobin in the

Table 1. Effect of fluoride and iodoacetate on stability of glucose in whole-blood samples stored at ambient temperature.

<table>
<thead>
<tr>
<th>Days stored</th>
<th>Fluoride</th>
<th>Iodoacetate</th>
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<tbody>
<tr>
<td>0</td>
<td>100 (97–103)</td>
<td>100 (97–103)</td>
</tr>
<tr>
<td>1</td>
<td>98 (97–101)</td>
<td>100 (98–101)</td>
</tr>
<tr>
<td>2</td>
<td>97 (94–98)</td>
<td>99 (97–101)</td>
</tr>
<tr>
<td>3</td>
<td>93 (90–98)</td>
<td>100 (98–103)</td>
</tr>
<tr>
<td>4</td>
<td>89 (87–92)</td>
<td>97 (95–100)</td>
</tr>
<tr>
<td>5</td>
<td>88 (87–90)</td>
<td>97 (95–99)</td>
</tr>
<tr>
<td>6</td>
<td>90 (88–92)</td>
<td>97 (95–99)</td>
</tr>
<tr>
<td>10</td>
<td>87 (84–90)</td>
<td>97 (94–100)</td>
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To five whole-blood samples stripped of glucose we added 8 mmol/L glucose, divided into aliquots, and added either 9.4 mmol/L fluoride or 1.8 mmol/L iodoacetate. Mean recoveries (four measurements on each sample) were calculated as the percentages of the glucose concentration on day 0 after storage.

Fig. 1. Influence of fluoride and iodoacetate on glucose measured with seven instruments (left to right): Cobas Mira, Reflotron, Hemocue, MediSense, OneTouch, Glucometer Elite, and Accutrend. Means and 95% confidence intervals of 10 different whole-blood samples supplemented with glucose to 10 mmol/L are shown for: samples without addition (white bars), samples with 9.4 mmol/L fluoride (light-shaded bars), and samples with 1.8 mmol/L iodoacetate (dark-shaded bars).
concentration range 0–11 mmol/L. Glucose was also unaffected in the concentration range 0–15 mmol/L in whole blood with the tested analyzers (not shown), except for Elite; the regression line, Elite = 0.81 (±0.02) Cobas Mira + 1.34 (±0.14) (S_{y|x} = 0.28 mmol/L, n = 14), indicates a constant positive bias and low recovery.

Imprecision after addition of iodoacetate. The imprecisions of the measurements of glucose and hemoglobin with the investigated analyzers were not increased by the addition of iodoacetate (Table 2).

Interference of iodoacetate on cholesterol and creatinine analyses on the Reflotron analyzer. Iodoacetate did not affect the measurements of cholesterol and creatinine in blood on the Reflotron analyzer. Comparison studies on cholesterol in 25 blood samples (range 2.7–10 mmol/L) in the presence (y) and absence (x) of iodoacetate gave the regression line: y = 0.99 (±0.01) x + 0.08 (±0.09), with S_{y|x} = 0.15 mmol/L, compared with a within-run analytical imprecision (SD) of 0.18 mmol/L (mean cholesterol concentration, 6.50 mmol/L; n = 10). The regression line for creatinine determined in 47 blood samples (range 44–730 μmol/L) with (y) and without (x) iodoacetate was: y = 1.04 (±0.01) x − 1.5 (±1.7); the S_{y|x} was 8.6 μmol/L, compared with a within-run SD of 8.2 μmol/L (mean creatinine, 150 μmol/L; n = 10).

Stability studies of cholesterol measurements by the Reflotron with 1.8 mmol/L iodoacetate showed no significant changes during storage for ≤4 days at room temperature. For five different patients’ samples the mean recovery (four measurements on each sample each day) at room temperature was 100% (C.I. 97–104%) on the fourth day (initial mean cholesterol, 4.2 mmol/L).

In the presence of iodoacetate the Reflotron creatinine measurements were stable ≤3 days at room temperature. The mean recovery for five patients’ samples (four measurements on each sample each day) at room temperature was 101% (C.I. 98–105%) on the third day (initial mean creatinine, 99 μmol/L).

Discussion

The most common biochemical analyses in general practice in Europe are measurements of glucose, hemoglobin, and cholesterol (3, 6, 9). For glucose measurements many instruments, based on various principles, are available. Although many of these were intended for home monitoring by diabetics, the apparatuses are also used in primary care, in hospital nursing departments, and for outpatients. Hemoglobin and cholesterol are mainly measured on larger desktop analyzers. Most of these instruments are precalibrated by the manufacturers and cannot be recalibrated by the users. Obviously, some instruments in this study were not calibrated to give the same reference values as the “wet-chemistry” methods. The control materials supplied are generally unusable on instruments from other companies. A common whole-blood-based control material would therefore be valuable for detecting operator dependent mistakes or instrument errors.

To be used for external quality assessment, the whole-blood-based control material must fulfill two criteria: sufficient stability during mailing at ambient temperature, and minimal or at least correctable interferences.

For glucose and hemoglobin determinations, the whole-blood-based control material used in this study was stable at room temperature for 10 days or more. This is sufficient for testing if the samples sent to the practitioners arrive by mail within 1–4 days. High temperature and freezing should be avoided. At 37°C the glucose and hemoglobin concentration were stable for 6 days of storage (data not shown).

Fluoride and iodoacetate are well-known inhibitors of glycolysis. Boehringer Mannheim does not recommend use of fluoride for glucose and hemoglobin measurements on Reflotron because of interferences. In our study, the hemoglobin measurements by Reflotron, although deviating from those by the comparison method, were linear in the presence of fluoride (data not shown). Consequently, interferences might be eliminated by correction. This is also true for measurements of glucose in the presence of fluoride by the One Touch and the Elite. Iodoacetate interfered with glucose measurement only on the MediSense. Because this latter deviation also could be corrected, we consider it preferable to manage the problem by corrections instead of assigning different values to this apparatus.

Although the ideal requirement for a stabilizer, i.e., showing no interferences in any instruments, was not obtained, iodoacetate still had a good stabilizing effect on glucose with minimal instrument interferences.

Both fluoride and iodoacetate are inhibitors of enolase and pyruvate kinase, enzymes that participate late in the glycolysis pathway. Therefore, a small initial de-

<table>
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<th>Table 2. Instrument imprecision for 20 hemoglobin and glucose measurements* in whole blood with and without iodoacetate.</th>
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<tr>
<td><strong>Glucose, mmol/L</strong></td>
</tr>
<tr>
<td>Cobas Mira</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>No addition</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>CV, %</td>
</tr>
<tr>
<td>Iodoacetate, 1.8 mmol/L</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>CV, %</td>
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*Five samples, measured four times each on the same day.
crease in the glucose concentration cannot be avoided when these stabilizers are used (13). Consequently, the glucose target value determined by the internal comparison method in the control laboratory should first be performed after 4–6 h and preferably after 2 days, to best match the testing time by the practitioners. Iodoacetate did not interfere with the cholesterol and creatinine analyses on the Reflotron, which are commonly used for these analyses in primary care (2, 6). Other instruments were not investigated. For unknown reasons the cholesterol and creatinine were unstable at 5°C. The whole-blood control material stabilized with iodoacetate at room temperature therefore appears to be useful for quality control of these two analytes as well. Thus, some of the most frequently performed analyses in primary care (3, 6, 9) can be checked with the same control sample.

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References