Analytical Quality of Near-Patient Blood Cholesterol and Glucose Determinations

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Background: Screening for diabetes and hypercholesterolemia is widely advocated, and extra-laboratory testing could play a major role in cost-effective population screening. We wished to assess the analytical quality and interchangeability of capillary blood cholesterol and glucose assays as performed on near-patient devices in pharmacies in Pretoria, South Africa.

Methods: Accuracy of near-patient and laboratory analyzers was assessed by analyses of human-serum-based reference material. To assess interchangeability in routine use, six volunteers visited each of 12 randomly selected pharmacies consecutively during a 3-week period to have their fasting blood glucose and cholesterol concentrations determined. For comparison purposes, a similar procedure was followed to evaluate the eight clinical chemistry laboratories servicing Pretoria and surroundings.

Results: The analytical performances in our laboratory of a single point-of-care instrument and of a laboratory analyzer compared well. Nevertheless, between-pharmacy analytical variation was larger than between-laboratory variation (11% vs 6.1% for cholesterol; 10% vs 7.6% for glucose). For glucose measurements, near-patient testing in pharmacies demonstrated a bias of −48.1% to 16.2%, whereas bias for laboratory measurements was −1.0% to 7.4%. Cholesterol assays showed a bias of −5.6% to 16.6% in pharmacies compared with −10.6% to 3.7% in laboratories. The percentage of closeness to the homeostatic set point for a single glucose and cholesterol determination done in any pharmacy was 24.6% and 23.6%, respectively. The corresponding values for laboratories were 16.9% and 15.6%, respectively.

Conclusions: Although modern point-of-care instruments allow high-quality blood analyses under ideal conditions, performance goals often are not achieved in practice as indicated by a higher uncertainty of cholesterol and glucose blood results when determined in pharmacies. Nonuniformity of calibration procedures, deficiencies in training, a lack of internal quality control, and the absence of an external quality assessment program may all contribute to the current state of affairs.

Healthcare worldwide is moving in a direction of prevention through screening and educational programs as part of primary care, rather than treatment of an established condition. Although prevention is not always possible in all cases, complications and the cost of healthcare can nevertheless be reduced and quality of life can be improved by early diagnosis and treatment.

An awareness of the importance and possible advantages of population screening for diabetes and hypercholesterolemia has increased in recent years. According to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, all individuals 45 years and above should be screened for diabetes mellitus, and testing should be repeated every 3 years if initially normal. In certain high-risk patients, testing should be carried out at a younger age and with shorter intervals (1). Early detection, and consequently early treatment, might well reduce the burden of especially type 2 diabetes and its complications because epidemiological evidence suggests that retinopathy begins to develop at least 7 years before the clinical diagnosis of diabetes (2). Although extra-laboratory blood glucose measurement with a test strip system is not recommended for the primary diagnosis of diabetes mellitus, it may be used for screening purposes, followed by laboratory confirmation (3).

In the second report of the National Cholesterol Education Program (Adult Treatment Panel 2), it is stated that serum total cholesterol should be measured in all adults 20 years of age and older at least once every 5 years. Both blood cholesterol and other coronary heart disease risk factors are considered in classification of individuals and in deciding on follow-up intervals and appropriate intervention (4).
so-called “reagent test strip”, have produced a proliferation of inexpensive and convenient systems for the measurement of blood glucose and cholesterol outside the laboratory (5). The analytical performance of the reagent strips and meters can be evaluated easily in the laboratory, but this gives no information about the performance of the individual user who lacks professional training. The establishment of a quality-control program adapted to this new approach to biochemical testing remains an important challenge because conventional quality-control procedures with lyophilized materials or liquid ethylene glycol-based materials often are inappropriate (6).

In cost-effective population screening, extra-laboratory testing by, for example, primary care clinics, pharmacies, or general practitioners, could play a major part, provided that their results are reliable, accurate, and reproducible. Although the accuracy and precision of high-quality near-patient testing devices in the hands of trained laboratory technicians are excellent, little information exists on the accuracy and reproducibility of results obtained under day-to-day conditions at various point-of-care centers, e.g., pharmacies.

The objective of this study was to assess the analytical quality (accuracy and precision) and interchangeability of capillary blood total cholesterol and glucose assays as performed in 12 different pharmacies in Pretoria, South Africa.

**Materials and Methods**

**STUDY DESIGN**

Accuracy and within-subject total variance for capillary whole blood and venous serum/plasma total cholesterol and glucose measurements were determined under the following circumstances: (a) in our laboratory using a single point-of-care analyzer; (b) using 12 randomly selected pharmacies that offer near-patient testing facilities; (c) in our laboratory using a single clinical chemistry analyzer; and (d) using all 12 diagnostic clinical chemistry laboratories in the Pretoria area.

Precision was determined for a single point-of-care instrument and single laboratory analyzer.

**POINT-OF-CARE CENTER EVALUATION**

During the first part of the study, six apparently healthy volunteers visited independently and unannounced each of 12 randomly selected pharmacies in Pretoria to have their fasting capillary blood glucose and cholesterol concentrations determined. Visits took place randomly during a 3-week period according to a predetermined schedule.

After each pharmacy visit, the participants had the same determinations done by one trained person at the Institute of Pathology. Either a right- or left-handed finger-prick blood sample was obtained according to appropriate guidelines for reliable finger-stick collection (7); the blood sample was analyzed immediately for glucose and cholesterol, using one near-patient device (Accutrend GCT; Roche Diagnostics, Mannheim, Germany). To minimize and standardize biological variation, all blood samplings were performed between 0900 and 1100 after an overnight fast of at least 10 h.

Two serum pools with concentrations at important decision cutoff values for glucose (2.5 and 7.0 mmol/L) and total cholesterol (5.2 and 6.2 mmol/L) were prepared from fresh human serum received at the Institute of Pathology for routine analyses. After being appropriately aliquoted, serum pools were frozen at −70 °C until being assayed. The respective in-house serum pools were assayed daily for 12 consecutive days on the single point-of-care instrument to assess between-day analytical variation.

After this 3-week period, each pharmacy was informed by telephone about the objective of the project. Permission was requested to assess the accuracy of each pharmacy’s testing device. This was performed by using three human-serum-based Standard Reference Materials (SRMs) with certified concentrations for glucose (SRM 965) and cholesterol (SRM 1952a) obtained from NIST (Gaithersburg, MD). The certified concentrations of glucose were determined using the NIST definitive method for glucose (isotope dilution/gas chromatography/mass spectrometry), whereas total cholesterol was determined by a modification of the isotope dilution/mass spectrometric definitive method.

The various point-of-care instruments usually measure glucose and cholesterol concentrations in capillary whole blood, whereas the reference material utilized in this study was human serum. For instruments using test strip systems incorporating a porous outer layer that allows only plasma to penetrate into the reagent layer, the same reference values are valid for whole blood, serum, and plasma. However, in instruments without this plasma separation procedure and which were calibrated to yield plasma results, when whole blood was used as the specimen for analysis, the value obtained from the instrument for the serum-based reference material was divided by 1.12 (8) before calculation of the percentage of bias.

The following point-of-care instruments were used by participating pharmacies. For cholesterol assays, nine pharmacies used an Accutrend analyzer (Roche Diagnostics), two pharmacies used the Lipotrend C (Roche Diagnostics), and one pharmacy used the Reflotron (Roche Diagnostics). For glucose assays, eight pharmacies used an Accutrend analyzer (Roche Diagnostics). The remaining pharmacies used either the Precision (Novo Nordisk), Glucometer Elite (Bayer), One Touch basic (Lifescan), or Reflotron analyzer (Roche Diagnostics).

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1 Nonstandard abbreviations: SRM, Standard Reference Material; ADA, American Diabetes Association; and SMBG, self-monitoring of blood glucose.
LABORATORY EVALUATION
Evaluation of the eight clinical chemistry laboratories took place 2 months after the evaluation of the point-of-care centers. The six volunteers visited each of the eight laboratories independently and unannounced over a 2-week period for venous blood sampling to determine their fasting serum total cholesterol and plasma glucose concentrations. After each visit, the participant went to the Institute of Pathology to have the same analyses done on a single laboratory instrument (Synchron CX7; Beckman Instruments). The above-mentioned in-house serum controls were also assayed for 12 consecutive days on the single laboratory analyzer to assess between-day analytical variance.

After completion of the laboratory visits, the accuracy of each laboratory’s clinical chemistry analyzer was assessed as described previously, using the NIST SRM. The following instruments were used by the participating laboratories: six used Beckman analyzers (Synchron CX7 or LX20) for both glucose and total cholesterol analyses, whereas the other two laboratories used the Hitachi 917 (Roche Diagnostics) and the Olympus AU 600 (Olympus Diagnostica), respectively.

ETHICS
All participants gave written consent for participation in the study. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, University of Pretoria.

CALCULATIONS AND STATISTICS
Results from repeat analyses of the serum pools on the in-house instruments were used to calculate the between-day analytical variance ($\sigma_{AC}^2$) for both the point-of-care ($\sigma_{AC}^2$) and laboratory analyzer ($\sigma_{AV}^2$).

The mean within-subject total variance ($\sigma_S^2$) was determined using data obtained from the in-house instruments for capillary blood ($\sigma_{SC}^2$) and venous serum/plasma ($\sigma_{SV}^2$) cholesterol and glucose assays, respectively, as described by Fraser and Harris (9).

The average within-subject biological variance ($\sigma_B^2$) was then calculated by adjusting within-subject total variance for analytical variance. Because the determinations for this study were carried out in singletons, the general formula suggested by Holzel (10) was used for calculation of within-subject biological variance.

The within-subject biological variance for capillary blood ($\sigma_{IC}^2$) was determined as follows:

$$\sigma_{IC}^2 = \sigma_{SC}^2 - \sigma_{AC}^2$$

The within-subject biological variance for venous serum or plasma ($\sigma_{IV}^2$) was calculated similarly:

$$\sigma_{IV}^2 = \sigma_{SV}^2 - \sigma_{AV}^2$$

Using the data from all of the participating pharmacies for each individual, we again calculated the within-subject total variance. This variable was designated $\sigma_{SV}^2$. By correcting $\sigma_{SV}^2$ for the capillary blood within-subject biological variance ($\sigma_{IC}^2$), we could calculate the “between-pharmacy analytical variance” ($\sigma_{AP}^2$) according to the following formula:

$$\sigma_{AP}^2 = \sigma_{SV}^2 - \sigma_{IC}^2$$

The between-pharmacy analytical variance included true within-instrument analytical variance, between-instrument variance, sampling variance, and operator-specific variance (i.e., variance introduced by having multiple operators of a given analyzer, or variance attributable to different analyzers being used by different single operators).

The appropriate CVs were subsequently calculated for each of the above variables.

For a capillary blood glucose or cholesterol determination done at any of the participating pharmacies, the percentage of closeness to the homeostatic set point (95% dispersion of the mean value) was calculated as follows (9, 11):

$$D^2 = \frac{Z^2(\text{CV}_{AP}^2 + \text{CV}_{IC}^2)}{n}$$

Where $D$ is the percentage of closeness to the homeostatic set point; $Z$ is the number of standard deviations required for a stated probability (for $p = 0.05$, $Z = 1.96$); $n$ is the number of specimens (in this case, $n = 1$); $\text{CV}_{AP}$ is the between-pharmacy analytical CV; and $\text{CV}_{IC}$ is the within-subject biological CV, determined on capillary whole blood.

The magnitude of a significant ($P = 0.05$) difference between two subsequent cholesterol determinations (critical difference) was calculated (12) for the following scenarios:

(a) using the same point-of-care center:

$$CD = K \times (\text{CV}_{AC}^2 + \text{CV}_{IC}^2)^{1/2}$$

Where $CD$ is the critical difference; $K = 1.96 \times (2)^{1/2} = 2.77$ for $p = 0.05$; $\text{CV}_{AC}$ is analytical CV, as determined on a single instrument; and $\text{CV}_{IC}$ is within-subject biological CV for capillary whole blood.

(b) using different point-of-care centers:

$$CD = K \times (\text{CV}_{AP}^2 + \text{CV}_{IC}^2)^{1/2}$$

Where $\text{CV}_{AP}$ is between-pharmacy analytical CV.

Similar calculations were performed using data from the participating laboratories for each individual. In this case, $\sigma_{SL}^2$ designates within-subject total variance calculated from the data of all participating laboratories for each individual, and $\sigma_{AL}^2$ indicates between-laboratory analytical variance, i.e., a combination of true within-instrument analytical variance, between-instrument variance, sampling variance, and operator-specific variance.

Accuracy was determined by means of duplicate analyses of the SRMs for glucose and cholesterol, respectively. The results were used to calculate the percentage of bias according to the following formula:
Bias (%) = \frac{\text{Value obtained} - \text{Certified value}}{\text{Certified value}} \times 100

**Results**

Using the CVs as summarized in Table 1, we calculated that the percentage of closeness to the homeostatic set point was 24.6% for a single glucose determination done on capillary whole blood in any one of the participating pharmacies, whereas it was 16.9% if performed on plasma in any one of the participating laboratories. For a total cholesterol assay, the percentage of closeness to the homeostatic set point was 23.6% when performed on capillary whole blood in any one of the participating pharmacies and 15.6% when performed on serum in any one of the participating laboratories.

The critical differences for cholesterol determinations were 33.3% and 22.1% when two subsequent determinations were done in any of the pharmacies or any of the laboratories, respectively. For two subsequent determinations performed at the same pharmacy or the same laboratory, the critical differences were 16.2% and 16.9%, respectively. The latter two values are based on the in-house evaluation of a point-of-care instrument and a clinical chemistry analyzer and will vary from pharmacy to pharmacy or laboratory to laboratory, depending on the analytical CV for the instruments in use.

Figs. 1 and 2 summarize the observed bias for glucose measurements in pharmacy point-of-care centers and in laboratories. Bias in pharmacies was −48.1% to 16.2%, whereas bias for laboratory measurements was −10.0% to 7.4%.

Because of the limited analytical range of the Accutrend analyzer (3.9–7.8 mmol/L), only the medium concentration of the SRM for cholesterol (certified value, 6.04 ± 0.04 mmol/L) was used to assess bias. Bias was −5.6% to 16.6% in pharmacies and −10.6% to 3.7% in laboratories for the cholesterol assay (Figs. 1 and 2).

**Discussion**

This study is an attempt to quantify the variation between point-of-care centers for two important blood components, total cholesterol and glucose. The data on variation allowed us to assess the utility of a screening test at a point-of-care center (pharmacy) in terms of the expected closeness of the result to the individual’s homeostatic set point. Similar data were also obtained for all eight of the clinical chemistry laboratories in Pretoria. Data obtained from the laboratories served as a reference with which the pharmacies could be compared.

For cholesterol analyses, the instrument most popular in pharmacies (Accutrend) yielded a CV_A of 2.5%, whereas a CV_A of 3.3% was achieved with the in-house clinical chemistry analyzer (Synchron CX7).

The within-subject biological CVs for capillary and venous specimens for total cholesterol (5.3% and 5.1%, respectively) confirmed the previous observations by Kafka et al. (13) that there is no significant difference in the coefficient of biological variation between capillary and

**Table 1. Components of variation for point-of-care and laboratory glucose and cholesterol analyses.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Capillary blood</th>
<th>Venous plasma/serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>(CV_{AD}), %</td>
<td>5.7</td>
<td>2.5</td>
</tr>
<tr>
<td>(CV_{SD}), %</td>
<td>8.6</td>
<td>6.0</td>
</tr>
<tr>
<td>(CV_{DC}), %</td>
<td>6.9</td>
<td>5.3</td>
</tr>
<tr>
<td>(CV_{SP}), %</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>(CV_{AP}), %</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

\(CV_{SD}\), within-subject total CV for capillary blood; \(CV_{SP}\), within-subject total CV for capillary blood analyses between pharmacies; \(CV_{SP}\), analytical CV for venous serum/plasma as obtained from a single laboratory instrument; \(CV_{AP}\), within-subject total CV for venous serum/plasma; \(CV_{AL}\), within-subject biological CV for venous serum/plasma analyses between laboratories; \(CV_{AL}\), between-laboratory analytical CV for venous serum/plasma.

**Fig. 1. Bias for pharmacy point-of-care centers.**

Observed bias (%) for glucose (at three concentrations: low, medium, and high) and total cholesterol (Chol) measurements at different pharmacies. Point-of-care instruments: D, Accutrend; B, Glucometer Elite; O, One Touch basic; +, Precision; *, Reflotron; square, Lipotrend.

**Fig. 2. Bias for clinical chemistry laboratories.**

Observed bias (%) for glucose (at three concentrations: low, medium, and high) and total cholesterol (Chol) measurements at different laboratories. Laboratory instruments: D, Synchron CX7; +, Synchron LX20; O, Hitachi 917; B, Olympus AU 600.
venous specimens with respect to total cholesterol concentration. The results also compare well with the average value from previous studies by Fraser (CV\textsubscript{i} = 5.8\%) (14).

The CV\textsubscript{AP} for total cholesterol was 11\% compared with a CV\textsubscript{AL} of 6.1\%, indicating a higher between-pharmacy variation than experienced between laboratories. This implies an increased uncertainty of the significance of a pharmacy cholesterol measurement, as reflected by a percentage of closeness to the homeostatic set point of 23.6\% if a single capillary blood specimen was analyzed for cholesterol. The corresponding value obtained in laboratories was 15.6\% (relative reduction of 50\% from pharmacy value).

Given that the within-instrument analytical performance for point-of-care and laboratory analyzers is similar, it seems that the higher between-pharmacy variation may be attributed to differences in sampling (sampling variance) or nonuniformly calibrated instruments.

Further evidence supporting that calibration bias may have contributed to a higher between-pharmacy variation compared with between-laboratory variation is derived from analyses of certified serum material obtained from the NIST. For the cholesterol assay, the bias range spanned 22.2\% for pharmacy analyzers compared with 14.3\% for laboratory analyzers. Between-analyzer matrix effects cannot be excluded for the laboratory analyses (designed to assay whole blood samples), and these effects could have contributed to the wider bias range observed for the point-of-care instruments.

Although finger-stick sampling is considered easier to perform, requires less training, and is less invasive than conventional venous collection, there is nevertheless considerable potential for technique-related variability, which often is responsible for the discrepant results between finger-stick and venous results. Finger-stick specimens are subject to dilution by interstitial fluid, giving a lower cholesterol concentration. Hemolysis is more likely to occur with finger-stick collection. Sensitivity to hemolysis varies by method but is usually the cause of falsely increased values (7). In recent years, improved lancets and collection devices have become available, and with appropriate techniques, finger-stick results can be equivalent to venous results (7).

The National Cholesterol Education Program recommends a performance of 3% CV with a 3% bias for determination of total cholesterol concentration (15, 16). This becomes a total allowable error of 8.9\% [total error = % absolute bias + (1.96 × CV\textsubscript{A})] vs the true value in situations where cholesterol is determined as a single measurement affected by both systematic and random error (17). This implies that only two pharmacies did not meet the 8.9\% total error goal for determination of total cholesterol.

Despite the larger variation in pharmacy determinations, they should nevertheless be adequate to screen for hypercholesterolemia, provided that an abnormal value is confirmed by repeat testing. As stated previously by Fraser (14), our results also stress that a patient cannot be classified into a certain risk category based on a single cholesterol determination, not even when determined in a clinical chemistry laboratory (15.6\% closeness to the homeostatic set point).

Changes in sequential results from a patient may be caused by analytical imprecision, within-subject biological variation, or by a change in the patient’s clinical condition. The “critical difference” between results gives an indication of the magnitude of the change that should occur before it is considered (with 95\% confidence) to be attributable to an altered clinical condition or to treatment of the patient.

Our results indicate that cholesterol testing at different pharmacies currently may have a limited utility in monitoring the efficacy of treatment for hyperlipidemia. The difference between two subsequent cholesterol measurements at two different pharmacies should be at least 33.3\% before it may be considered (with 95\% confidence) to be the result of intervention. For two different laboratories, the critical difference is substantially less at 22.1\%.

When the same pharmacy or laboratory is used to follow the result of intervention, the critical difference declines to ~17\% in both cases. This again serves to illustrate how between-pharmacy variation contributes to the uncertainty of results.

To assess pharmacy glucose screening, we compared our results to the American Diabetes Association (ADA) guidelines applicable to self-monitoring of blood glucose (SMBG). According to the ADA guidelines of 1986, the goal of all future SMBG systems should be to achieve a variability (system + user) of <10\% at glucose concentrations of 1.7–22.2 mmol/L. In 1986, the ADA stated that with current systems, SMBG measurements should be within 15\% of the results of the reference measurement (18). Later, at a second conference in 1993, they recommended tightening the limits on “analytic error” to within 5\% (19). Applying the 1986 ADA guidelines, only three pharmacies had results for all three SRMs within 10\% of the certified concentrations. In contrast, all of the laboratories reported values for the SRM materials within 10\% of the certified values.

For glucose determinations, the larger biological variance (CV\textsubscript{bi}) as well as analytical imprecision for capillary specimens (CV\textsubscript{A}) contributed to the larger total variance. However, a significant portion of the larger between-pharmacy variance could probably also be attributed to differences in sampling (sampling variance) and to the variety of instruments from different manufacturers being used.

The previously published within-subject biological CV (CV\textsubscript{i}) for fasting plasma glucose of 4.75\% (14, 20) was confirmed by our value of 4.0\% for plasma glucose on venous specimens. However, the value of 6.9\% for fasting capillary whole-blood glucose was considerably larger. No published information could be found for this specific type of specimen. The reason for the larger variance
observed for capillary whole-blood glucose vs venous plasma glucose is unknown but could in part be attributable to shifts over the red cell membrane and varying degrees of lymphatic dilution.

It has repeatedly been shown that a scheme involving training, internal quality control, and external quality assessment substantially improves the quality of near-patient testing (21–23). If this is combined with a universal system for calibration of near-patient devices and uniformity in reporting of results, pharmacy determinations should yield results comparable to the clinical chemistry laboratory. Unfortunately, in contrast to diagnostic clinical chemistry laboratories, near-patient testing in South Africa (as in many other countries) is not subject to compulsory external quality assessment programs or accreditation with a relevant authority. Appropriate quality-control schemes and accreditation requirements for point-of-care laboratory services will enhance quality and accuracy of blood test results.

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