COMPARATIVE PERFORMANCE OF TWO POINT-OF-CARE ANALYSERS FOR LIPID TESTING

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SUMMARY

The aim of this study was to compare the analytical performance of the Cholestech LDX and CardioChek PA lipid point-of-care devices to a CDC-certified laboratory. Inter-assay imprecision (n=10) for blood samples from 2 patients with different lipid profiles was 3.0% for total cholesterol, 2.6% for triglyceride, 5.2% for HDL cholesterol and 6.2% for calculated LDL cholesterol on the Cholestech, and 4.4% for total cholesterol, 4.8% for triglyceride, 7.0% for HDL cholesterol and 7.4% for calculated LDL cholesterol on the CardioChek. In a patient comparison study (n=100), correlation coefficients (r) between the POCT and laboratory methods were greater than 0.90 for all tests for the Cholestech and greater than 0.84 for all tests for the CardioChek. The mean difference (bias) between the results obtained on the Cholestech LDX and the laboratory method was not statistically significant; however the mean difference between the CardioChek and the laboratory method was statistically significant for total, HDL and LDL cholesterol (one way analysis of variance with Scheffe post-hoc test). The Cholestech LDX met the NCEP goal for total error for all analytes except LDL cholesterol. The CardioChek PA system met the NCEP total error goal for triglyceride but not the other lipid analytes. We conclude that the Cholestech LDX device is a suitable POCT device for cardiovascular risk assessment in the primary care setting, while the CardioChek device requires more study and refinement. (Clin. Lab. 2007;53:561-566)

KEY WORDS

point-of-care testing, lipids, cardiovascular disease, accuracy and precision, total analytical error

INTRODUCTION

Blood lipid measurements are widely accepted biochemical markers for cardiovascular risk assessment and management. There is a positive relationship between total and LDL cholesterol concentrations and the risk of both future coronary events and mortality from coronary heart disease (CHD). Elevated triglyceride concentrations above 2.0 mmol/L are predictive of CHD, particularly when associated with either an increased concentration of LDL cholesterol or a decreased concentration of HDL cholesterol [1].

In Australia, the recent national Australian Diabetes, Obesity and Lifestyle (AUSDIAB) study found the prevalence of elevated total cholesterol concentrations (greater than or equal to 5.5 mmol/L) and elevated triglycerides (greater than or equal to 2.0 mmol/L) were 51% and 21% in more than 11,200 adults studied.

In Australia’s indigenous population, age-adjusted CHD death rates are three times higher than non-Aboriginal Australians overall and ten-times higher for Indigenous people aged 25-44 [3,4].

In recent years our work has focussed on the application of point-of-care testing models for chronic disease prevention and management in the rural and remote Indigenous community setting. In 2001 we conducted the first evaluation of the Cholestech LDX lipid device (Cholestech, Hayward, CA, USA) in this country [5]. The Cholestech LDX performed a full lipid profile (total, HDL, and calculated LDL cholesterol and triglyceride) on 35 μL of capillary or venous whole blood in 5 minutes. Recently a second POCT lipid device, the CardioChek PA analyser (Polymer Technology Systems Inc, IN, USA)
Table 1: Assessment of the accuracy of two lipid POCT analysers relative to a CDC-certified laboratory method

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Lab Method versus</th>
<th>No. of Patients</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>CardioChek PA</td>
<td>103</td>
<td>1.07</td>
<td>0.32</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Cholesteck LDX</td>
<td>102</td>
<td>0.88</td>
<td>0.47</td>
<td>0.97</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>CardioChek PA</td>
<td>101</td>
<td>1.05</td>
<td>-0.03</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Cholesteck LDX</td>
<td>101</td>
<td>1.02</td>
<td>0.09</td>
<td>0.99</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>CardioChek PA</td>
<td>103</td>
<td>0.83</td>
<td>0.09</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Cholesteck LDX</td>
<td>99</td>
<td>0.92</td>
<td>0.12</td>
<td>0.91</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>CardioChek PA</td>
<td>95</td>
<td>1.04</td>
<td>0.62</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Cholesteck LDX</td>
<td>92</td>
<td>0.85</td>
<td>0.18</td>
<td>0.94</td>
</tr>
</tbody>
</table>

became available in Australia. The hand-held CardioChek PA analyser performed a full lipid profile on 40 μL of blood in 2 minutes.

This study compared the analytical performance of the two POCT lipid devices relative to a CDC-certified laboratory method and analytical goals recommended by the National Cholesterol Education Program (NCEP) [6]. The study represented the first evaluation of the CardioChek PA system in Australia.

**MATERIALS AND METHODS**

The CardioChek PA and Cholesteck POCT measurement systems

The CardioChek PA system uses a single dry reagent strip to measure the lipid profile. The Cholesteck LDX uses a plastic reagent cassette. Both analysers initially separate plasma from red blood cells, then a portion of plasma is directed to analyte-specific reaction pads and lipid concentrations are determined by reflectance photometry. The two POCT devices share similar method chemistries. HDL is initially separated from LDL and VLDL fractions following precipitation by either phosphotungstic acid (CardioChek PA) or dextran sulphate and magnesium acetate (Cholesteck LDX). Total cholesterol and HDL cholesterol are both converted enzymatically to cholesterol-4-en-3-one and hydrogen peroxide. The peroxide then reacts with different chromogens (disubstituted aniline in the CardioChek PA system and N-ethyl-N-sulphonypropyl-m-toluidine with the Cholesteck LDX) to form quinoneimine dyes. Triglyceride also undergoes enzymatic conversion to dihydroxyacetone phosphate and hydrogen peroxide, and its concentration is determined using the same colour reaction as cholesterol and HDL cholesterol. LDL cholesterol is calculated using the Friedewald formula for samples with triglyceride concentrations less than 4.5 mmol/L.

Both analysers are pre-calibrated by the manufacturers. Calibration information is encoded on a brown magnetic strip on each Cholesteck LDX cassette. The CardioChek PA has a code chip supplied with each box of test strips.

**Comparative Laboratory Method**

Plasma lipids were measured at the Clinical Trials Laboratory, SouthPath, Flinders Medical Centre, Adelaide using methods certified for their analytical performance through participation in the Centres for Disease Control-National Heart, Lung and Blood Institute (CDC-NHLBI) Lipid Standardisation Program. Total cholesterol and triglyceride were measured enzymatically on the Hitachi 917 analyser (Roche Diagnostics, Germany). HDL cholesterol was also measured enzymatically on the Hitachi following precipitation of VLDL and LDL from plasma by phosphotungstic acid. LDL cholesterol was calculated using the Friedewald formula.

**Assessment of Precision**

Inter-assay imprecision was assessed on both POCT analysers by repeated analysis (n=10) of venous whole blood samples from 2 patients with different lipid profiles (patient 1: total, HDL and LDL cholesterol 5.0, 1.1 and 2.5 mmol/L respectively and triglyceride 3.2 mmol/L, patient 2: total, HDL and LDL cholesterol 6.9, 1.8 and 4.1 mmol/L respectively and triglyceride 2.1 mmol/L).

**Assessment of Accuracy**

Heparinised venous whole blood samples were collected from 103 patients with a wide range of lipid concentrations over a four week period. POCT analyses were performed on the day of sample collection. Prior to laboratory analysis, the samples were centrifuged at 2300 g for 5 minutes and venous plasma separated for subsequent testing within 48 hours.
Agreement between methods was assessed by Passing Bablok linear regression and Bland Altman plots [7] using the Analyse-It statistical package (Analyse-It Software Ltd., Leeds, UK). The slope, intercept and correlation coefficient (r) were calculated from Passing Bablok analyses. The difference in the mean values obtained by the laboratory and POCT methods was assessed using one way analysis of variance (ANOVA) with Scheffe post-hoc test. Linear regression analysis on the Bland Altman difference plots was used to determine whether the observed bias was constant or concentration dependent (p<0.05, difference from zero of slope).

Bi-level commercial quality control materials supplied by each POCT manufacturer (Cholestech LDX Lipid Controls, lot number 3274 and PTS Multi-Chemistry and HDL Cholesterol controls, lot numbers 24603 and 17603 respectively) were run daily with each batch of patient samples analysed across the test period.

RESULTS

Assessment of Precision

The average inter-assay imprecision for patient blood samples recorded by the Cholestech LDX device was 3.0% for total cholesterol, 2.6% for triglyceride, 5.2% for HDL cholesterol and 6.2% for calculated LDL cholesterol. Corresponding imprecision recorded for the CardioCheck PA was 4.4% for total cholesterol, 4.8% for triglyceride, 7.0% for HDL cholesterol and 7.4% for calculated LDL cholesterol.

Assessment of Accuracy

The mean (range) of lipid concentrations in the 103 patient samples tested by the laboratory was: total cholesterol 4.83 mmol/L (1.9 to 7.8), triglyceride 2.06 mmol/L (0.61 to 6.63), HDL cholesterol 1.28 mmol/L (0.4 to 3.1) and LDL cholesterol 2.59 mmol/L (0.85 to 5.9).

For cholesterol, one Cholestech result was below the lower measuring range of the device (2.6 mmol/L) and could not be included in comparative studies. For triglyceride, three results were not included in the comparison for each POCT device because they exceeded either the lower or upper limits of the measuring ranges of the devices. For HDL cholesterol, four Cholestech results exceeded the upper limit of the device’s measuring range (2.6 mmol/L). For LDL, results were not obtained on 8 samples with the CardioChek and 11 samples with the Cholestech because either the triglyceride exceeded 4.5 mmol/L or a quantitative HDL cholesterol result was not available for use in the Friedewald formula calculation.

The slope, intercept, and correlation coefficient (r) of the two POCT methods versus the laboratory method are shown in Table 1. Correlation coefficients (r) between the POCT and laboratory methods were greater than 0.90 for all tests for the Cholestech and greater than 0.84 for all tests for the Cardiochek.

For total cholesterol there was a statistically significant difference between methods [F(2, 305)=9.08, p=0.0001]. Post-hoc comparison using Scheffe test indicated that the mean score for the Cardiochek (mean 5.38, standard deviation [SD] 1.29) was significantly different from that of the Cholestech (mean 4.75, SD 1.06) and significantly different from the laboratory method (mean 4.80, SD 1.20). There were no significant differences between the Cholestech and the laboratory method for cholesterol. For HDL cholesterol there was a statistically significant difference between methods [F(2, 302)=5.47, p=0.005]. Post-hoc comparison using Scheffe test showed that the mean score for the Cardiochek (mean 1.18, SD 0.34) was significantly different from the Cholestech (mean 1.32, SD 0.32) and from the laboratory method (mean 1.32, SD 0.42). There were no significant differences between the Cholestech and the laboratory method. For LDL cholesterol there was a statistically significant difference between methods [F(2, 287)=22.54, p=<0.0001]. Post-hoc comparison using Scheffe test indicated that the mean score for the Cardiochek (mean 3.24, SD 0.96) was significantly different from the Cholestech (mean 2.39, SD 0.82) and significantly different from the laboratory method (mean 2.53, SD 1.00). However again there were no significant differences between the laboratory method and Cholestech. For triglycerides, there were no significant differences between the methods [F(2, 302)=0.47, p=0.62].

Bland Altman plots of the differences between the two POCT devices and the laboratory method are provided in Figure 1. There was considerably more scatter in the difference between POCT and laboratory results with the Cardiochek device, particularly for cholesterol and triglyceride. For the Cholestech, the observed bias was concentration dependent for total and LDL cholesterol and triglyceride, while for the Cardiochek bias was concentration dependent for HDL cholesterol (p<0.05, linear regression, difference of slope from zero).

Performance of Laboratory Methods During the Evaluation Period

During the evaluation period, the imprecision and bias of the laboratory methods, as assessed by the repeated analysis of samples from the CDC-NHLBI Standardisation Program was as follows: total cholesterol, imprecision <2%, bias <2%; triglyceride imprecision <2.5%, bias <4%; HDL cholesterol imprecision <2.5%, bias <2%. This observed performance met the CDC analytical performance criteria for all three tests.
Figure 1: Bland-Altman difference plots comparing differences in results between the Cholestech and Cardiochek POCT devices and a CDC-certified laboratory method. Difference, mmol/L = POCT minus laboratory result; dotted line = mean bias; dashed line = limits of agreement; solid grey line = zero bias.
Table 2: Imprecision, bias and total error of two POCT analysers relative to performance goals recommended by the NCEP

<table>
<thead>
<tr>
<th>Analyte</th>
<th>POCT Instrument</th>
<th>Observed Imprecision (%)</th>
<th>NCEP Goal for Imprecision (%)</th>
<th>Observed Bias (%)</th>
<th>NCEP Goal for Bias (%)</th>
<th>Observed Total Error (%)</th>
<th>NCEP Goal for Total Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>CardioChek PA</td>
<td>4.35%</td>
<td>3%</td>
<td>12.1%</td>
<td>3%</td>
<td>19.3%</td>
<td>9%</td>
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<tr>
<td></td>
<td>Cholestech LDX</td>
<td>3.03%</td>
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</tr>
<tr>
<td>Triglyceride</td>
<td>CardioChek PA</td>
<td>4.83%</td>
<td>5%</td>
<td>1.1%</td>
<td>5%</td>
<td>9.1%</td>
<td>15%</td>
</tr>
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<td></td>
<td>Cholestech LDX</td>
<td>2.61%</td>
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<tr>
<td>HDL Cholesterol</td>
<td>CardioChek PA</td>
<td>7.02%</td>
<td>4%</td>
<td>10.9%</td>
<td>5%</td>
<td>22.5%</td>
<td>13%</td>
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<td></td>
<td>Cholestech LDX</td>
<td>5.22%</td>
<td></td>
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</tr>
<tr>
<td>LDL Cholesterol</td>
<td>CardioChek PA</td>
<td>7.37%</td>
<td>4%</td>
<td>27.4%</td>
<td>4%</td>
<td>39.5%</td>
<td>12%</td>
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<td></td>
<td>Cholestech LDX</td>
<td>6.24%</td>
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</tr>
</tbody>
</table>

Total Analytical Error

The National Cholesterol Education Program (NCEP) has recently recommended laboratory-based analytical goals for imprecision, inaccuracy and total error [6]. Based on the formula %Total Error = %Bias + 1.65xCV% [8], the total error for lipid measurements was calculated for both POCT analysers and compared to the NCEP goals (Table 2). The %CV was derived from the results of the precision study, while % bias was calculated from paired Bland Altman data.

The Cholestech LDX met the NCEP goals for total error for all analytes except LDL cholesterol; however goals for imprecision and bias were not met for HDL cholesterol and triglyceride respectively. The CardioChek PA system met the NCEP goals for imprecision, bias and total error for triglyceride but not for the other lipid analytes where observed performance showed significant bias and total error well outside the recommended goals.

DISCUSSION

There is a strong clinical need for regular lipid testing as part of cardiovascular risk assessment programs, particularly in Australia’s rural and remote Aboriginal communities where there is a significant excess mortality from CHD among young Aboriginal adults. It is currently recommended that all Aboriginal adults with hyperlipidaemia (total cholesterol greater than 6.5 mmol/L or total cholesterol greater than 5.5 mmol/L and HDL cholesterol less than 1.0 mmol/L) be offered lipid-lowering medication [3].

Point-of-care testing has a particular niche in the Australian Aboriginal community setting because Aboriginal health workers can be successfully trained as POCT operators and POCT facilitates a strong sense of community ownership, cultural factors crucial to the acceptance of health models in the Indigenous setting [9,10].

In this study, the analytical performance of two POCT lipid analysers was compared to a CDC-certified laboratory method and laboratory-based analytical goals promulgated by the NCEP. The Cholestech LDX readily met NCEP goals for total error except for LDL cholesterol. The performance of the Cholestech in this study is similar to the findings of a recent Australian Government-commissioned report on this analyser [11]. The CardioChek PA system only met the NCEP total error goal for triglyceride. The significant biases observed with the CardioChek device in this study are difficult to explain, especially for HDL cholesterol where the CardioChek and laboratory methods use the same precipitant. Following discussions with the manufacturer, possible explanations for the poor performance observed could be attributed to differences in calibration between the POCT device and the laboratory methods or relate to the glass in the strip holder area of the device being unclean or having a hairline crack over the reading window. Also the samples were applied with a plastic capillary tube, which was cumbersome to dispense. A better sample delivery system may improve device performance.

The observation that both POCT analysers did not meet the goal for total error for LDL cholesterol is of some concern, given the importance of this marker for cardiovascular risk management and the fact that laboratory methods are now available for direct measurement of LDL cholesterol [12]. A challenge exists for the manufacturers of POCT lipid devices to develop improved methods for direct LDL cholesterol determination.

In summary, the generally sound analytical performance of Cholestech LDX indicates it is suitable within the discussed limitations for cardiovascular risk assessment of Aboriginal communities in the primary care setting, but the CardioChek PA device requires further study and refinement.
ACKNOWLEDGEMENT

Dr Malcolm Whiting from the Clinical Trials Laboratory, SouthPath, Flinders Medical Centre, is thanked for performing laboratory lipid analyses in this study.

CONFLICT OF INTEREST

None declared.

References


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