Accuracy of Three Dry-Chemistry Methods for Lipid Profiling and Risk Factor Classification

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The purpose of this project was to determine the accuracy in lipids measurement and risk factor classification using Reflotron, Cholestech, and Ektachem DT-60 dry-chemistry analyzers. Plasma and capillary venous blood from fasting subjects (n = 47) were analyzed for total cholesterol (TC), high density lipoprotein (HDL-C), and triglycerides (TG) using these analyzers and a CDC certified laboratory. Accuracy was evaluated by comparing the results of each portable analyzer against the CDC reference method. One-way ANOVAs were performed for TC, HDL-C, and TG between all portable analyzers and the reference method. Chi-square was used for risk classification (2001 NIH Guidelines). Compared to the reference method, the Ektachem and Reflotron provided significantly lower values for TC (p < .05). In addition, the Cholestech and Ektachem values for HDL-C were higher than the CDC (p < .05). The Reflotron and Cholestech provided higher values of TG than the CDC (p < .05). Chi-squares analyses for risk classification were not significant (p > .45) between analyzers. According to these results, the Ektachem and Cholestech analyzers met the current NCEP III guidelines for accuracy in measurement of TC, while only Ektachem met guidelines for TG. All 3 analyzers provided a good overall risk classification; however, values of HDL-C should be only used for screening purposes.

Key Words: cholesterol, HDL-cholesterol, triglycerides, CHD-risk factors

Introduction

Studies have consistently found an association between coronary heart disease (CHD) and elevated total cholesterol (TC), elevated low-density lipoprotein (LDL-C), or low high-density lipoprotein (HDL-C; 8). In addition, elevated serum total triglycerides (TG) are nowadays classified as an independent risk factor for CHD (5). Because blood lipids can be improved by initiating therapeutic lifestyle changes or medications that result in a reduction in heart disease (5), lipid profiling has become even more important in the treatment and prevention of CHD. Guidelines established in 2001 by the National Institutes of Health (NIH) suggested that adults...
(20 years and older) should have a fasting lipoprotein profile (TC, HDL-C, LDL-C, and TG) measurement once every 5 years (5).

Lipid profiling can be done in a variety of settings, including hospitals, health care offices, as well as non-clinical lab sites such as shopping centers, churches, schools, job-sites, and community centers (14). Because lipid profiling could be performed outside of a laboratory setting, it is imperative to determine if the devices being used to measure these lipids are accurate. TC, HDL-C, and TG are directly measured, while LDL-C can be directly measured or can be calculated utilizing the Friedewald equation (6), which requires measurements of TC and TG. Accuracy in measurement of all these lipids is essential to avoid misclassification of individuals that could affect early detections and interventions in case of risk.

The Reflotron®, the Ektachem® DT-60, and the Cholestech® are portable dry-chemistry analyzers commonly used to measure cholesterol for screening population in non-medical situations (fitness centers, health promotion programs, community centers, and physicians’ offices). The Reflotron and the Cholestech can utilize whole blood, while the Ektachem DT-60 requires plasma or serum. All three units measure cholesterol and TG concentrations by dry-chemistry reflectance spectrophotometry. The three analyzers have been investigated in numerous studies (1, 2, 4, 7, 10, 12, 13, 17, 18); however, direct simultaneous comparisons between the three systems and a CDC approved laboratory have not been simultaneously made for TC, HDL-C, and TG. Therefore, the present study has three purposes: (a) to compare the accuracy of the Ektachem DT-60 and the Cholestech L.D.X. for estimating TC, HDL-C, and TG and the Reflotron for estimating TC and TG with a standardized laboratory procedure, (b) to determine if these analyzers met the NCEP recommendations for accuracy of TC (≤3% bias), of HDL-C (≤5% bias), and of TG (≤5% bias), and (c) to determine if these portable analyzers allowed for proper classification of individuals into desirable, borderline high, or high-levels according to new guidelines by NIH (5; see Table 1).

**Methods**

**Subjects**

Study participants included 47 fasting healthy volunteers: 24 women and 23 men. The women were younger (35 ± 10 y) than the men (44 ± 10 y). They weighed 65.3 ± 6.8 kg, while the men weighed 83.0 ± 7.7. All subjects read and signed an informed consent previously approved by the Internal Review Board of the University of North Carolina at Chapel Hill.

**Instrumentation**

Reflotron® (Boehringer, Mannhein, Indianapolis, IN, USA). This analyzer requires approximately 30 µL of the whole blood, serum, or plasma, which is collected in lithium heparin coated capillary tube and then dispensed onto a reagent pad. The reagent strip is then inserted into the analyzer. The sample is absorbed through a glass fiber filter layer, which separates plasma from the erythrocytes in the whole blood. For the CHO reaction, the plasma then migrates into a zone containing cholesterol esterase, cholesterol oxidase, and a dye indicator. Chemical reactions are completed in 3 min, and the analyzer measures the reflectance and converts it into cholesterol concentrations by a calibration algorithm encoded in the tab. A
similar reaction is used to measure TG, but the reagent pad contains cholesterol esterase, glycerol kinase, adenosin triphosphate, L-α-glycerol phosphate oxidase, peroxidase, and a dye indicator. The triglycerides are cleaved into glycerol and free fatty acids. Then, the glycerol is phosphotized. The phosphotized glycerol is then oxidized and reacts with the dye indicator.

**Ektachem DT-60® (Ortho-Clinical Diagnostics, Rochester, NY, USA).** This analyzer requires previous centrifugation of the blood sample to obtain either serum or plasma. For TC and TG, 10 µL of serum or plasma are dispensed onto a multilayered film slide. Once the slide has been “spotted” with the specimen, the slide enters the analyzer where it is heated. For TC, cholesterol esters are hydrolyzed to cholesterol, which is then oxidized to generate hydrogen peroxide. The hydrogen peroxide oxidizes a leuco dye to produce a colored compound that is measured by reflectance spectrometry providing the cholesterol concentration. For TG measurement, the reaction is similar except that the TG’s slide uses glycerolphosphate. For HDL-C, the plasma or serum is previously treated to separate HDL-C from low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Initially, the proteins undergo a reaction during which VLDL and LDL proteins are precipitated and then removed by centrifugation. Afterwards, 10 µL of the supernatant containing the HDL-C are deposited on a slide containing cholesterol ester hydrolase and a dye indicator. The HDL-C reading is also obtained by colorimetric reflectance. Each analysis requires just over 5 min, and up to six specimens can be loaded into the analyzer at any one time.

**Cholestech L.D.X.® (Cholestech Corp., Hayward, CA, USA).** Blood from a finger stick is collected into a lithium heparin coated capillary tube and then dispensed onto a lipid profile Cholestech L.D.X. cassette containing the factory calibration information to convert reflectance readings to TC, HDL-C, and TG concentrations. The cassette is then placed into the L.D.X. analyzer, where a system on the cassette separates the plasma from the blood cells. Plasma flows to the right side of the cassette, where it is transferred to TG and TC reaction pads, and to the left side, where it precipitates LDL and VLDL. HDL-C remains in the filtrate, where it is transferred to the reaction pad that contains the reagents for its quantification. The Cholestech uses cholesterol esterase for TC and HDL-C. For the TG reaction, it also utilizes cholesterol esterase to hydrolyze TG to glycerol and free fatty acids. The glycerol goes under further reactions containing glycerol kinase plus magnesium and glycerol phosphate. Whole blood, plasma, or serum could be used as specimens.

**Reference Technique.** A Hitachi model 911 semi-automated spectrophotometer (Roche Diagnostics Corp., Indianapolis, IN, USA) was used for the reference method. The Hitachi 911 uses a spectrophotometric technique to determine total cholesterol that is based on the method developed by Roeschlau et al. (11). The Hitachi reports a sensitivity of 3 to 800 mg/dl, with an imprecision of ≤ 1.4% at the upper limits of detection. For HDL-cholesterol, the Hitachi uses the method developed by Sugiuchi et al. (15). The sensitivity is 3 to 120 mg/dl with an imprecision of ≤ 4% error at the detectable upper limit. Triglyceride determination by the Hitachi uses methods developed by Wahlefeld et al. (19), that has a sensitivity of 4 to 1000 mg/dl and an imprecision of < 2% at the upper detectable limit. These methods used in conjunction with the Hitachi model 911 meet the CLIA (Clinical Laboratories Improvement Amendments) and the National Institute of Health, National Cholesterol Education Program goals for acceptable performance.
Procedures

Subjects reported to the testing site after an overnight fast. Each subject was seated for at least 5 min, and then two capillary samples and an 8.5 ml venous sample were collected from an antecubital vein without stasis using an EDTA vacutainer. All three samples were obtained from the same limb and capillary samples were obtained following manufacture’s recommended procedures for utilization of the portable analyzer in a clinical setting. Milking was not utilized. The capillary samples were immediately analyzed using the Cholestech and the Reflotron. The 8.5 ml of venous blood was separated in two tubes and centrifuged to obtain plasma. One of the tubes contained the plasma to be used immediately with the Ektachem to measure TC, HDL-C, and TG, and the other tube was sent to the reference laboratory (Centers for Disease Control–[CDC] certified University of North Carolina Hospitals laboratory) for measurement of TC, HDL-C, and TG.

Accuracy Evaluation

In this study, a CDC-certified hospital laboratory (University of North Carolina Hospitals, Chapel Hill, NC, USA) was used as the “criterion” method. According to Kaplan and Pesce, accuracy is defined as the extent to which a measurement is close to the true value (9). The mean difference between the methods being investigated is referred to as bias and can be positive or negative. Bias represents the degree of systematic error between the methods; the smaller the bias, the more accurate the method (16). For this study, accuracy was defined as agreement between cholesterol values obtained with the Cholestech L.D.X., the Reflotron, and the Ektachem DT-60, with those from the CDC standardized laboratory. The results are presented using Bland-Altman plots, which plot the mean differences between the reference method and each portable analyzer (3). One must remember that in method-comparison studies, there is some inherent measurement error in both methods.

Statistical Analysis

One-way analyses of variances (ANOVAs) were performed comparing the TC, HDL-C, and TG values obtained from the portable analyzers and the reference method. In case of significant finding (p < .05), Tukey post hoc tests were conducted between the portable analyzers and the reference method. Paired t tests using the Bonferroni correction factor were conducted to examine percent bias in measurement for the three variables (TC, HDL-C, and TG) between each analyzer and the reference method. Percent bias was determined using the following equation \[
\frac{\text{(portable analyzer-reference method)/reference method}}{100}
\]. Percent bias was also compared against the NCEP III guidelines. Chi-square was used to determine misclassification of subjects into risk categories comparing each portable analyzer with the reference method. Classification categories can be found in Table 1. Spearman correlations were also obtained for each pair-wise comparison. For all statistical comparisons, alpha was set at p < .05.

Results

The mean values and standard deviations for the reference method and each portable analyzer for TC, HDL-C, and TG are reported in Table 2. These are absolute values
Rubin et al.

and do not reflect any plasma volume shift that may have occurred. However, since all samples from the same individual were taken simultaneously from the arm and finger, the plasma volume status for each sample should have been similar. The reference method reported the highest mean value for TC and the lowest mean value for HDL-C. For TC, significant differences were observed between the results from the reference method and the results from the Ektachem and the Reflotron ($p < .05$).

### Table 1 Risk Classifications for Total Cholesterol, HDL-Cholesterol, and Triglycerides

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Desirable</th>
<th>Borderline risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>&lt; 200</td>
<td>200–239</td>
<td>≥ 240</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>&lt; 5.17</td>
<td>5.17–6.18</td>
<td>≥ 6.20</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>&gt; 60</td>
<td></td>
<td>&lt; 40</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>&gt; 1.55</td>
<td></td>
<td>&lt; 1.03</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>&lt; 150</td>
<td>150–199</td>
<td>200–499</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>&lt; 1.69</td>
<td>1.69–2.25</td>
<td>2.26–5.63</td>
</tr>
</tbody>
</table>

### Table 2 Lipid Concentrations of Total Cholesterol (TC), HDL Cholesterol (HDL-C), and Triglycerides (TG) in mg/dl Comparing the Reference Method With the Ektachem, Cholestech, and Reflotron Portable Analyzers ($n = 47$)

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>TC</th>
<th>HDL-C</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference method</td>
<td>193.2 ± 45.1</td>
<td>49.0 ± 14.1</td>
<td>103.0 ± 86.4</td>
</tr>
<tr>
<td></td>
<td>(5.00 ± 1.17)</td>
<td>(1.27 ± 0.36)</td>
<td>(1.16 ± 0.97)</td>
</tr>
<tr>
<td>Ektachem</td>
<td>189.2 ± 44.7*</td>
<td>54.6 ± 17.0*</td>
<td>99.8 ± 57.6</td>
</tr>
<tr>
<td></td>
<td>(4.89 ± 1.16)</td>
<td>(1.41 ± 0.44)</td>
<td>(1.13 ± 0.65)</td>
</tr>
<tr>
<td>Cholestech</td>
<td>190.0 ± 43.0</td>
<td>52.4 ± 14.8*</td>
<td>118.0 ± 87.7*</td>
</tr>
<tr>
<td></td>
<td>(4.91 ± 1.11)</td>
<td>(1.36 ± 0.38)</td>
<td>(1.33 ± 0.99)</td>
</tr>
<tr>
<td>Reflotron</td>
<td>186.8 ± 50.1*</td>
<td>—</td>
<td>113.9 ± 85.1*</td>
</tr>
<tr>
<td></td>
<td>(4.83 ± 1.30)</td>
<td></td>
<td>(1.29 ± 0.96)</td>
</tr>
</tbody>
</table>

*Note.* Results in parentheses are presented in S.I. units of mmol/L. *$p < .05$ versus the reference method.
the reference method ($p < .05$). For TG measurement, the Ektachem produced the lowest mean value; however, only the values provided by the Cholestech and Reflotron were significantly different from the reference values ($p < .05$).

The results for accuracy/bias testing, the mean differences between the reference method and the portable instruments, are presented in Table 3 and Figures 1–3. All the $t$ tests for bias between the three portable analyzers and the reference method were statistically significant for measurements of TC and HDL-C. For TG, only the Cholestech and the Reflotron were significantly biased ($p < .05$). The current NCEP III guidelines for accuracy allows a bias of ±3% for TC, ±5% for HDL-C, and ±5% for TG. Our results showed that for TC, the Ektachem and the Cholestech both met current NCEP guidelines. Conversely, the Reflotron did not meet the NCEP III guidelines, underpredicting TC by more than 3%. With respect to HDL-C measurement, neither the Ektachem nor the Cholestech met the NCEP III guidelines for accuracy. Finally, only the Ektachem DT-60 met the NCEP III guidelines for accuracy in TG measurement.

Another way to detect accuracy is to determine the ability of the portable analyzers to correctly classify individuals by risk category (Table 1). The percentages of misclassification for TC categories were: 10% for Cholestech, 15% for Ektachem, and 6% for Reflotron. Although misclassification occurred, Chi-square results for TC were not significant, thus none of the portable analyzers differed in classification of subjects compared to the reference method ($p > .60$). Concomitantly, Spearman correlation coefficients between the reference method and each of the portable analyzers were high: Cholestech $r = 0.930$, Ektachem $r = 0.921$, Reflotron $r = 0.942$.

Misclassification percentages were greater for HDL-C: 23% for Cholestech and 21% for Ektachem. Chi-square results for HDL-C classification by the portables analyzer also did not differ from the reference method of classification ($p > .45$). The Spearman correlation coefficients were: Cholestech $r = 0.782$, Ektachem $r = 0.822$.

Finally, proportions of TG misclassified were lower than for the other two lipids: 6% for Cholestech, 2% for Ektachem and Reflotron. The chi-squares for TG classification were also not statistically significant ($p > .80$), with Spearman correlation coefficients of $r = 0.889$ for the Cholestech, $r = 0.904$ for the Ektachem, and $r = 0.937$ for the Reflotron.

Table 3  Bias of the Three Portable Analyzers for Total Cholesterol (TC), HDL Cholesterol (HDL-C), and Triglycerides (TG) Using the Reference Method As the Standard for Comparison

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ektachem</th>
<th>Cholestech</th>
<th>Reflotron</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>−2.09*</td>
<td>−1.67*</td>
<td>−3.35*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>+11.42*</td>
<td>+7.03*</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>−3.1</td>
<td>+14.6*</td>
<td>+10.6*</td>
</tr>
</tbody>
</table>

Note. Bias is expressed as a percentage of deviation from the standard. *$p < .05$. 
Discussion

This study compared the same blood sample by three portable analyzers against a CDC-approved laboratory as the reference method. Although the TC percent bias was statistically different for all three analyzers, our results showed that the Ektachem and the Cholestech met the current NCEP guidelines for accuracy and the Reflotron did not. This has been observed in other studies for the Cholestech (4, 7, 12, 17) and
for the Ektachem (7, 10). Rogers et al. (12) found that when Cholestech was used to measure TC in whole blood, it met the accuracy standards for whole blood, but it did not meet standards for plasma or serum. In contrast to our Reflotron results, a study by Asmman et al. (1) found that the Reflotron did meet the Laboratory Standardization Panel guidelines, and a study by Miller et al (10) also found that Reflotron met the earlier NCEP guidelines for accuracy when venous samples were used but not with capillary samples.

A trend was observed in negative bias for TC measurement in all three analyzers (Figure 1). Under-prediction was observed in 31.9%, 36.2%, and 42.5% of the samples (Ektachem, Cholestech, and Reflotron, respectively). As shown in Figure 1, the majority of the under-prediction occurred in the lower values in the Reflotron (<200 mg/dl) but was scattered across all values for the other two analyzers. This reflects the possibility that these analyzers would tend to put someone that is at risk in a lower risk category because of the underestimation of the values. Assman et al. (1) in their study of the Reflotron, observed that when whole blood was used instead of plasma, TC results were lower than expected. Since the Cholestech, as well as the Reflotron, uses whole blood, a similar result might have occurred in our study. However, the reason for the under-prediction in the Ektachem is not understood.
The Bland-Altman plots show the distribution of values obtained from the portable analyzers compared to the reference method, and the acceptable variability. As evident in Figure 1, total cholesterol was overestimated throughout the whole range of values in 6.3% and in 10% of the samples for the Cholestech and the Ektachem, respectively. Overestimation was observed in 8% of the samples for the Reflotron and was mainly for values 180 to 320 mg/dl. Accuracy was observed in 58% of the samples for Ektachem, in 58% for the Cholestech, and in 48% for the

Figure 3 — Bland-Altman plot for the distribution of the triglycerides values (mg/dl) for each of the three units compared to the reference method. Top graph is the Cholestech, middle graph is the Ektachem, and the lower graph is the Reflotron.
Reflotron for TC measurement. Therefore, although the Cholestech and the Ektachem meet the NCEP III guidelines for accuracy, users of these portable analyzers need to realize that they can under-, and over-predict values.

Neither the Ektachem nor the Choletech met the current guidelines for HDL-C measurement (Figure 2). These results are in agreement with findings of Cobbaert et al. (4) and Bard et al. (2) for the Cholestech analyzer. The portable units accurately predicted the values of HDL-C (within 5%) in only 23% and 28% of the samples for the Ektachem and Cholestech, respectively. Both analyzers tended to overestimate HDL-C values: in 68% of the cases the Ektachem and in 60% of the cases the Cholestech. In addition, the Cholestech also under-predicted HDL-C values in 6.4% of the cases (3 out of 48).

Only the Ektachem met the guidelines for accuracy in measurement of triglycerides. This analyzer accurately measured 69% and overestimated 29% of the samples. Overestimation was observed across all values. The Reflotron also tended to overestimate samples (61%) but mainly at the lower end of the scale. The Reflotron was the only analyzer that under-predicted some results but only in 4% of the samples at the higher end of the spectrum. Thus, the Reflotron analyses were accurate in 35% of the cases. The Cholestech correctly measured only 13% of the samples while overestimating in 87% of the cases. The bias, although noticeable throughout the whole range of values, was mostly evident in the highest values. Concurrently, Bard et al. (2) found that the Cholestech analyzer did not meet the previous NCEP guidelines for accuracy in TG measurements. Overall, for TG measurement, all three analyzers tended to overestimate values. Overestimation of TG values could be a potential problem in borderline–high triglycerides cases, since the values obtained from these portable analyzers might place somebody at risk when, in reality, the person has a desirable value. Fortunately, from a prevention point of view, this situation is preferable to underestimation of values, resulting in no detection of risk.

Conclusions

Our results suggest that both Cholestech and Ektachem met the current NECP III guidelines for accuracy in measurement of TC, and the latter also met the guidelines for accuracy in TG measurement. For practical purposes, correct risk classification becomes the important outcome in lipid profile screening. Thus, highlighting the ability of the portable analyzers to correctly classify individuals in the stipulated ranges or risk categories is most relevant. All three analyzers had misclassifications ≤ 15% for TC and ≤ 6% for TG. Although the percentages of misclassification seem acceptable, caution should be taken when classifying subjects that have borderline high TC values. The results obtained by the Reflotron appear to be negatively biased.

If results are negatively biased, the risk classification could place somebody who might be borderline into the low risk group. Thus, the follow-up measurement, as suggested by NCEP III for borderline values, would not be indicated. Therefore, healthcare providers should become familiar with the weakness of each particular analyzer and adjust their routine procedures concurrently. According to our results, neither the Ektachem nor the Cholestech met the accuracy guidelines for HDL-C, and the values provided by them were significantly different from values provided by a reference method. In addition, greater than 20% of the samples were misclassified; thus, measurements of HDL-C with these portable analyzers should not be considered for diagnostic purposes but could possibly be used for population screening.
References


Acknowledgments

This research was supported by a grant of the National Institutes of Nursing Research of the National Institutes of Health # NR01837. We would also like to acknowledge the Biobehavioral Laboratory of the UNC School of Nursing for their assistance with the blood samples.