Validation of a point of care lipid analyser using a hospital based reference laboratory

M Carey,[1]
C Markham,[1]
P Gaffney,[2]
G Boran,[2b]
V Maher[1]

Depts of Cardiology[1] *and Chemical Pathology*[2]*, The Adelaide and Meath Hospital incorporating The National Children's Hospital, Dublin*

ABSTRACT

Background Lipid measurements are essential in cardiovascular disease management in primary care. However, utilizing hospital laboratories may result in excess patient travel, sample loss and repeat clinic visits. Point of care (POC) lipid measurement would overcome these difficulties but has not been validated in an Irish setting.

Aim To compare POC lipid profile measurements using a cholestech LDX analyser with a hospital reference laboratory (Lab).

Method One-hundred subjects (30 men, 70 women) participated. Finger prick and venous samples were analysed directly by Cholestech LDX and the Lab.

Results A broad range of lipid values were measured. Absolute differences between POC and Lab measurements were insignificant except for a small over-estimation by the POC method of triglyceride 0.25mmol/l (95% CI 0.17 to 0.24), and an underestimation of HDLc – 0.11mmol/l (95% CI – 0.143 to –0.078). There were significant correlations between POC and Lab. levels; total cholesterol r=0.92, triglyceride r=0.93, HDLc r=0.92 and LDLc r=0.86 (all p< 0.0001).

Conclusion These results validate the use of the Cholestech LDX® analyser for point of care lipid measurements in clinical practice, provided well trained operators are supported by a hospital laboratory delivering quality assurance support.

INTRODUCTION

Ireland has a very high incidence of premature coronary heart disease (CHD) mortality [1]. Risk factor assessment and in particular lipid screening has become increasingly important. Since the primary care setting is where the main battle against CHD will be fought,[2] risk assessment and prevention needs to be optimized at this point. However, in order to measure lipid levels, patients often have to travel to hospitals or their blood samples are sent to laboratories. This can result in excess patient travel, sample loss and a repeat visit to their GP. The latter can result in a delay in initiation of treatment and loss of continuity of care. A point of care lipid measurement would avoid these potential problems and afford better patient care.

Point of care lipid analysers have been available for some time in Ireland [3] but the accuracy of this method has yet to be established. We therefore conducted a study to compare the results of full lipid profiles using the point of care lipid analyser (Cholestech LDX) to that of the reference hospital laboratory.

SUBJECTS AND METHODS

100 subjects were recruited. These subjects were either attending the Cardiac Risk Factor Clinic at the Adelaide Meath incorporating the National Children's Hospital because of coronary heart disease or hypertension, or were hospital staff members who volunteered to participate in the study. Seventy per cent of the subjects were female and 30% male. All subjects were Caucasian.

Fasting (80%) and non-fasting lipid profile samples (20%) were taken so that a broad range of lipid measurements could be compared. Following informed consent, subjects donated both a finger-prick sample and a venous whole blood sample. The finger prick sample and a portion of the venous sample were subjected to analysis in the point of care (POC) analyser. The rest of the venous sample was sent to the reference laboratory. All POC samples were analysed for a full lipid profile (total cholesterol, triglyceride, low-density lipoprotein cholesterol, high density lipoprotein cholesterol) and glucose level. Laboratory samples were analysed for lipid (n=100) and glucose levels (n =40).

Performance characteristics of the Cholestech LDX analyser – analytical limits, imprecision, interference factors are outlined in the Cholestech LDX System Procedure manual (www.cholestech.com).

Two research nurses who were trained in the use of the Cholestech machine performed all the blood sampling and data collection. Finger-prick sampling involved ensuring the subject was seated and their hands warmed to ensure good circulation. This was achieved by subjects washing their hands with warm water or gently massaging their fingers from the base to the tip to help the circulation in their fingertips. The finger was cleansed with an alcohol swab and dried thoroughly with a gauze strip before pricking the side of the finger (to reduce pain) with a lancet. To determine if alcohol swabbing interfered with the lipid results, a number of subjects (n=30) did not have the finger site swabbed with alcohol.

The first drop of blood from the puncture site was wiped away to reduce the potential influence of interstitial fluid [4]. The second drop of blood was then collected in a capillary tube. The capillary tube was filled with 35µl of blood for analysis within 10 seconds of blood sampling. This was to avoid blood coagulation interfering with the capillary action. Care was taken to avoid the collection of air bubbles. It was necessary to insert the blood from the capillary tube into the sample well of the cassette within 5 minutes to avoid blood clotting. The cassettes were placed in a cassette holder and analysed by a fully automated procedure in less than 10 minutes. The concentration of each lipid fraction was proportional to the intensity of the coloured product obtained and measured by reflectance photometry [5]. Results were automatically printed (Figure 1). The storage and handling of the cassettes is important and clear guidelines accompany each analyser. Cassettes were stored in a fridge at 2 to 8°c and only removed 10 minutes before use. Unused cassettes were never returned to the fridge for future use.

The hospital laboratory staff were blinded as to which samples were part of this validation study. Total cholesterol, HDL cholesterol, triglycerides and plasma glucose were analysed in the laboratory using a Hitachi Modular P® analyser and Roche® reagents. All methods have a between run imprecision of <3%. Results outside the analytical limits of the methods are automatically diluted by the analyser and the corrected result reported. Both analyser and laboratory LDL cholesterol was calculated by the Friedewald equation[6] ([LDL-chol] = [Total chol] - [HDL-chol] - ([TG]*.458). Laboratory results were downloaded from the hospital computers.

This study had ethical approval from Trinity College ethical committee.

STATISTICAL ANALYSIS

All statistical analysis was performed using JMP statistical software 5.1.2, www.jmp.com. Univariate correlations between like variables (measured by POC or Laboratory) were performed using the Spearman rank correlation method. Absolute differences between samples were compared by paired t-tests. P values less than 0.05 were deemed significant. Bland Altman difference plots were used to evaluate differences of values between methods. Passing Bablock Regression was used to compare the methodologies used for lipid measurements.

RESULTS

Seventy women and 30 men mean age 46 ± 13 years participated in the study. Their range of lipid values were as follows: total cholesterol (3.3 to 9.2 mmol/l), triglyceride (0.38 to 6.32 mmol/l), high density lipoprotein cholesterol (HDLc) (0.84 to 2.97 mmol/l) and low density lipoprotein cholesterol (LDLc) (0.9 to 7.3 mmol/l).

Figure 2 demonstrates the strong correlations between lipid levels measured by POC and the hospital laboratory; total cholesterol: $r = 0.92$ ($p < 0.0001$), triglyceride: $r = 0.93$ ($p < 0.0001$) HDLc $r = 0.92$ ($p < 0.0001$) and LDLc $r = 0.86$ ($p < 0.0001$) . The LDLc values were determined indirectly using the Friedewald equation except for two samples where triglycerides exceeded 4 mmol/l. From the graphs, it is evident that the correlations were true throughout the range of measured values. However, there were two outliers evident in triglyceride measurements.

The outliers from the finger prick sample analysed were no longer evident when the venous samples from the same two patients were analysed in the Cholestech LDX (Figure 3). Both patients were female and on recollection admitted to using hand cream before the lipid testing had been carried out.

In absolute terms, the mean difference between the Cholestech LDX values of finger prick samples and the laboratory values were small.

The methodologies for lipid measurements were also compared using Passing Bablock Regression with the following results for finger prick vs laboratory : cholesterol: slope 1.02 (95% CI 0.95 to 1.11), intercept -0.145 (95% CI - 0.53 to 0.23), triglyceride: slope 0.93 (95% CI 0.88 to 0.98), intercept -0.086 (95% CI -0.15 to -0.02), HDLc: slope 1.07 (95% CI 1.0 to 1.16), intercept -0.001 (95% CI -0.123 to 0.105), LDLc slope 0.96 (95%

CI 0.87 to 1.06), intercept –0.07 (95% CI -0.21 to 0.29). These results are all highly significant. Table 1A demonstrates that total cholesterol measurements using these methods differed by 0.037 (CI -0.03 – 0.11 mmol/l) which were small and not statistically significant. Triglyceride values were higher using POC than laboratory by 0.25 mmol/l (CI 0.17 to 0.24 mmol/l) ($p<0.001$) and HDL cholesterol values were lower using POC than the laboratory by -0.11 mmol/l (CI -0.143 to -0.078 mmol/l) ($p<0.001$). Therefore, there was a small but significant overestimation of triglyceride values and a small but significant underestimation of HDL cholesterol levels. As evident in Table 1B, the difference in HDL measurement was insignificant if a venous sample was directly analysed in the Cholestech LDX. Differences in triglyceride levels were also less marked but still significant. This would indicate that the small differences were due to differences in the laboratory methods. The differences between the finger prick and venous samples analysed in the Cholestech LDX most likely reflected differences due to tissue fluid lipoproteins present in the finger prick sample.

Use or non-use of an alcohol swab before sampling did not influence the degree of correlation between the methods or absolute differences. The correlations between methods and the absolute differences between methods were unchanged whether subjects were fasting or not. The differences in lipid values using POC and laboratory did not correlate with subjects lipid levels. This would indicate accuracy at all lipid levels. This is evident from the difference plots for the lipid measurements showing that the differences were equally spread across the concentration range (Figure 4).

The learning curves of the two different operators did not result in significantly greater differences between the methodologies in the early part of the study compared to the lipid measurements of subjects recruited later in the study.

In 40 subjects, corresponding hospital laboratory glucose measurements were made. There was a significant correlation between finger prick and hospital laboratory glucose levels $r = 0.54$ $p < 0.0005$. Absolute differences between analyser whole blood glucose levels and laboratory plasma glucose levels were small 0.2 (0.05 – 0.34) mmol/l and not statistically significant.

DISCUSSION

This study confirms that near patient lipid testing using the Cholestech LDX machine, in a hospital clinic setting, using dedicated and motivated operators with central laboratory support, yields results that correlate highly significantly to those measured independently in a standardized hospital laboratory. This was evident for total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol levels. There was a statistically significant overestimation of triglyceride levels and an underestimation of HDL cholesterol levels by the POC method but these differences were quite small. In practice, these differences are unlikely to be clinically significant.

The correlation coefficient for LDLc r value was lower than that for total cholesterol, triglyceride and HDLc. This is possibly due to the fact that LDLc was calculated using the Friedewald equation and the variations in triglyceride and HDL values between methods would have exaggerated LDL differences and hence reduced the LDLc correlation between methods.

A number of possible confounders were considered such as sampling technique, Haemoglobin levels, use or non–use of alcohol swabs, operator learning curve and differences between operators. None of these factors made any significant impact on the results obtained. The only recognized consideration was the influence of hand cream on sample triglyceride levels.

The implications of these results are that a relatively simple point of care assay provides accurate lipid profile results for clinical practice. Utilization of this technique could provide general practitioners and practice nurses with useful measurements with which they can make clinical judgments and instigate treatment at the time of first patient contact. This will naturally free up doctor and patient time and ensure better continuity of care. It should be noted, however, that any POC service should only be implemented in accordance with national and international guidelines [7-8].

Point of care testing can also be used in the out-patient setting in hospitals where decisions need to be made on pharmacological changes at the time of the patient visit thus reducing visit frequency.

While this study primarily examined lipid measurements, a sub-population of 40 patients revealed that there was a significant correlation between laboratory measured and point of care

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Table 1 (a)

LIPIDS	MEAN DIFFERENCE BETWEEN METHODS (POC - LAB.)	95% CI	P VALUE
Cholesterol mmol/l	0.037	(-0.030 to 0.110)	n.s.
Triglycerides mmol/l	0.200	(0.170 to 0.240)	p < 0.001
HDLc mmol/l	-0.110	(0.143 to -0.078)	p < 0.001

POC = point of care finger prick sample; Lab. = Laboratory measured; n.s. = non significant

Table 1 (b) — Absolute differences between Lipid measurements

A Difference between finger prick lipid measurements using LDX cholestech and Laboratory measurements

	mmol/l	95% CI	p values
Cholesterol	0.037	(-0.37 to 0.11)	ns
Triglyceride	0.25	(0.18 to 0.33)	p<0.001
HDLc	-0.11	(-0.143 to -0.078)	p<0.001

B Difference between venous lipid measurements using LDX Cholestech and Laboratory measurements

	mmol/l	95% CI	p values
Cholesterol	0.024	(-0.05 to 0.95)	ns
Triglyceride	0.155	(0.08 to 0.23)	p<0.001
HDLc	-0.015	(-0.065 to -0.03)	ns

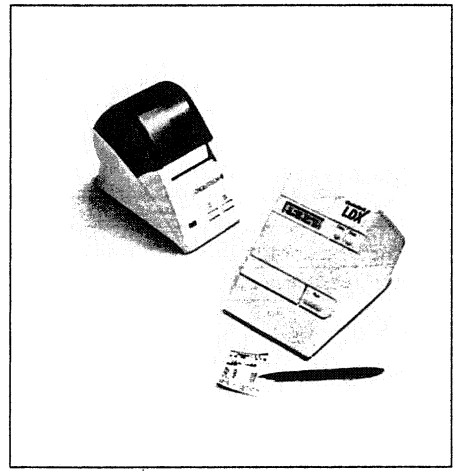

Figure 1 — CHOLESTECH LDX ANALYZER

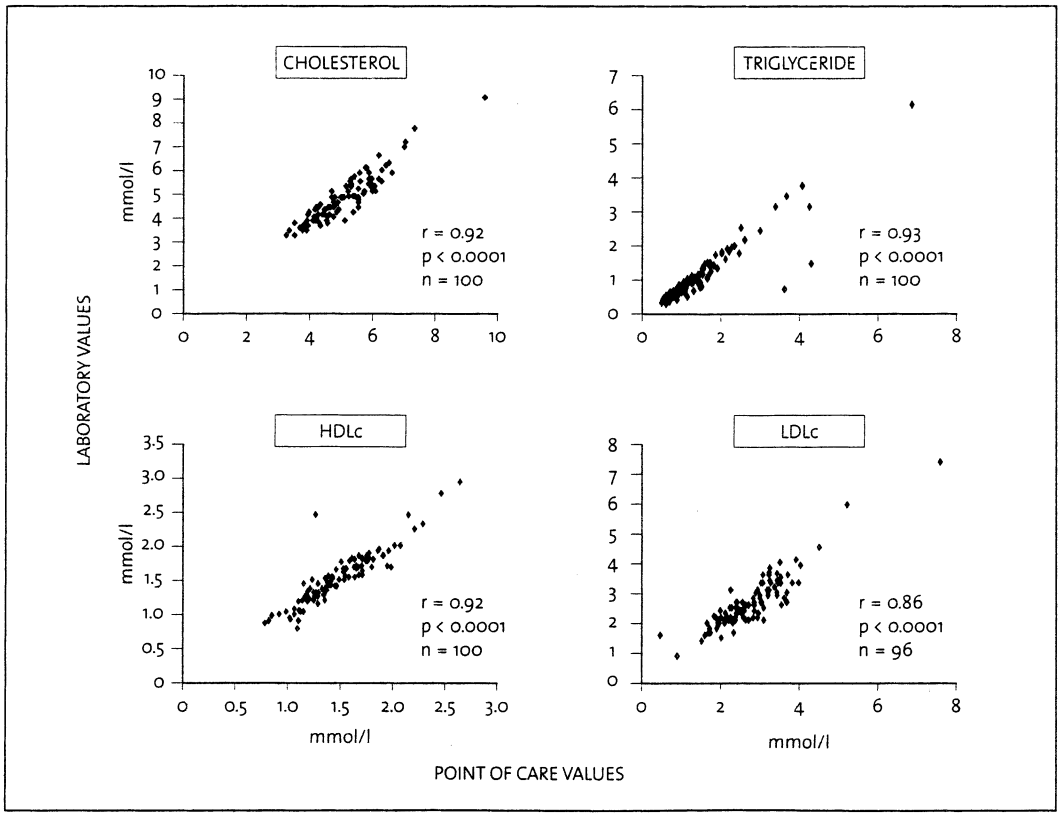

Figure 2 — COMPARISON OF LABORATORY AND POINT OF CARE TESTING

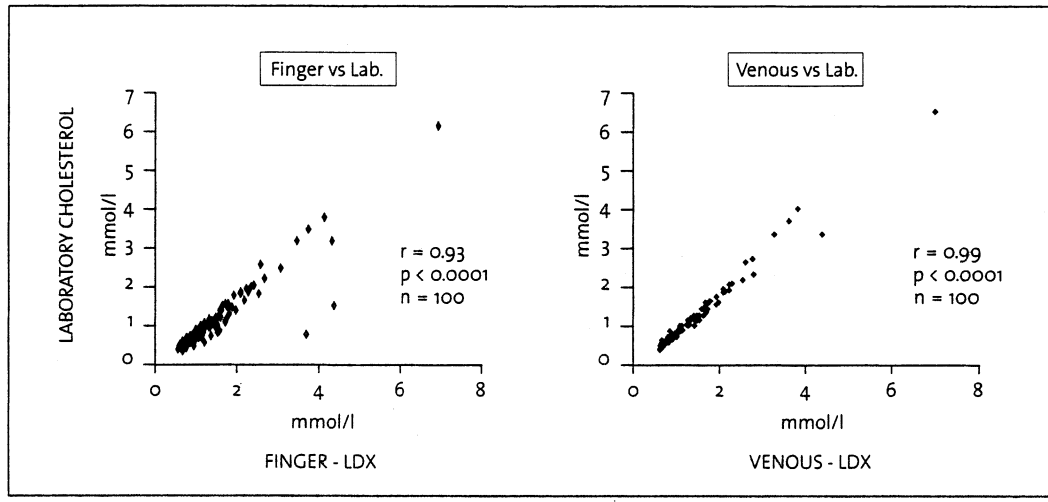

Figure 3 — TRIGLYCERIDE MEASUREMENT

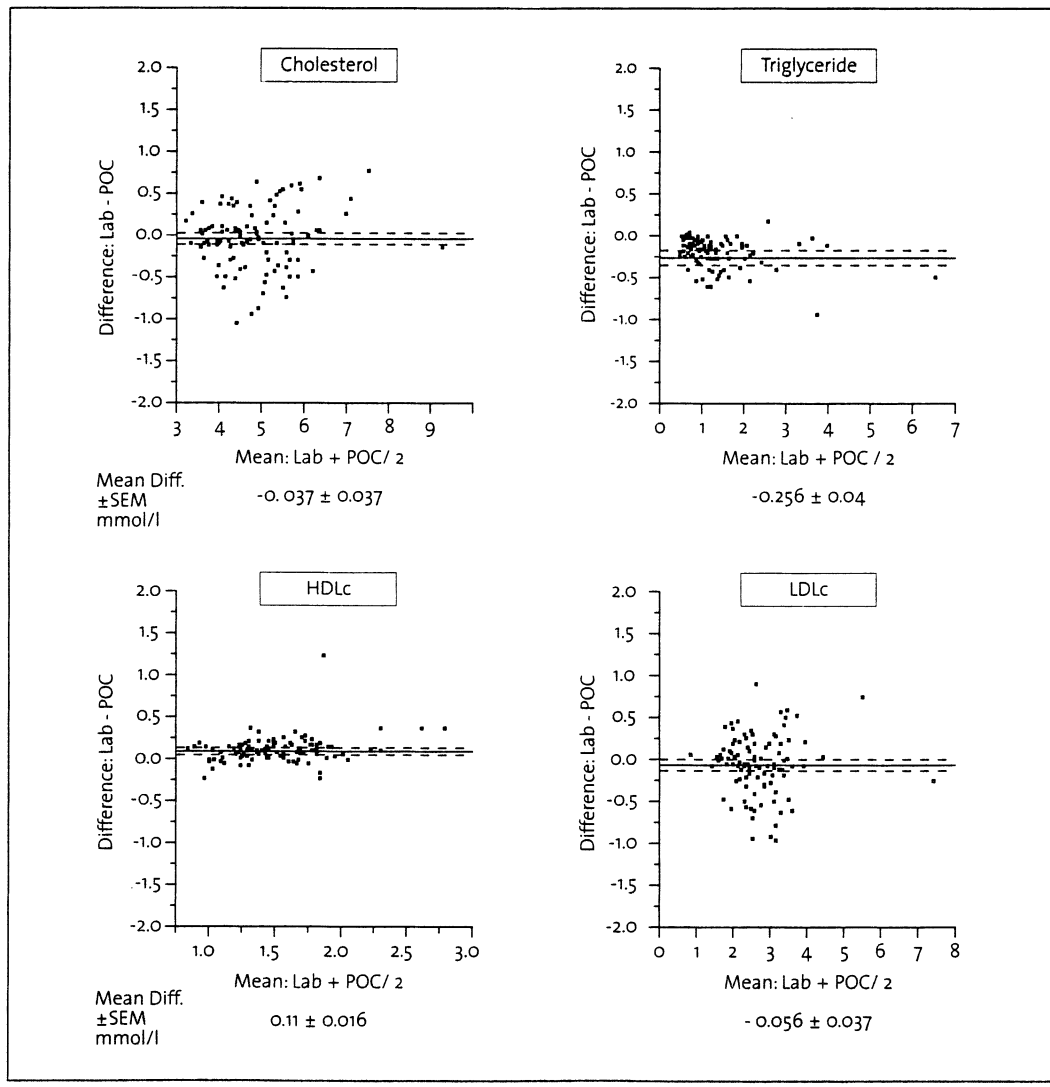

Figure 4 — DIFFERENCE PLOTS OF LABORATORY AND FINGER PRICK LIPID MEASUREMENTS

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measured glucose levels. Absolute differences in glucose measurements were not statistically significant. However, this was not the primary aim of this study and a more detailed study of a larger sample of glucose measurements would be needed to confirm its accuracy.

This study did not directly test how accurate the point of care results would be in the primary care setting as comparative samples would have had to be transported to hospital laboratories. Prolonged transport time, altered sample temperature and may have added variables which could have confounded the results. For this reason it was thought that it would be most appropriate to perform the study directly in the hospital setting to avoid sample transportation difficulties but the results would be applicable in all settings. One important consideration in the primary care setting is operator training which may differ from practice to practice. However, with careful instruction, as was the case for the two nurses who performed this study, there was no evidence of any measurement inaccuracies or of a learning curve effect. All operators need to be aware of possible confounders as already outlined. There is a need for a validation of the Cholestech LDX or other point of care lipid analysers in individual clinical practices before this service could be offered.

There should be sufficient training of staff in the safe operation and maintenance of the equipment with standard operating procedures fully documented. Quality assurance measures[9] both internal and external need to be established to ensure quality control in the clinical setting. Support structures with a local clinical laboratory are advocated for the long-term success of the service, particularly for the ongoing training and quality assurance. Use of point of care analysis in settings where medical input for result interpretation is not present or where appropriate medical guidelines[10] are unavailable, should be cautioned.

The cost-effectiveness of the service needs to be examined further taking into account the current cost of each cassette (€12 for each full lipid and glucose cassette) versus the cost to the patient for travel (€1 to €40 private car versus taxi) parking (€5) and hospital laboratory costs (€5) per sample for phlebotomy, reagents and staffing. This would nearly break even for a person with their own transport (€11 hospital lab attendance versus €12 per cassette at GP surgery). However, the cost of repeat GP visits (€20 – €50, GMS or Private) has not been considered. In addition, the inconvenience for patients, the potential for sample and result loss and the time delay in starting treatment need to be factored into the equation.

When used appropriately, point of care testing will improve patient management and fortify the services that are provided for patients at the primary care level.

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Correspondence: Vincent Maher, Dept of Cardiology, Adelaide Meath Hospital, Tallaght, Dublin 24 Ireland; Tel. +353 1 414 2112; Fax +353 1 414 3052; Email: vincent.maher@amnch.ie