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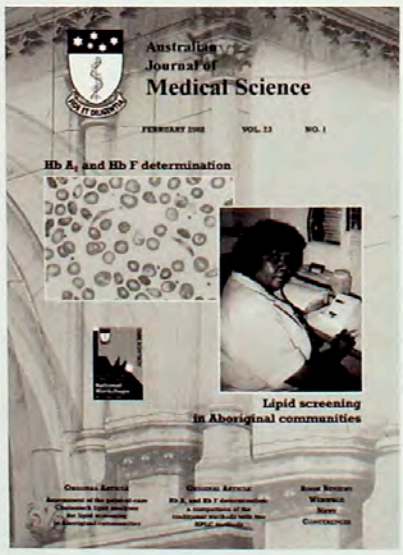

Hb A_2 and Hb F determination
Red cells from a patient with sickle haemoglobin (blood smear, May-Grunwald-Geimsa stain).

Lipid screening in Aboriginal communities
The Cholestech point-of-care analyser being used by Sandy Wilson, Aboriginal Health Worker, Meningie and Districts Memorial Hospital and Health Services Inc, South Australia. Ms Wilson is working in partnership with Mark Shephard to screen Aboriginal community members from Meningie for their risk of cardiovascular disease using the Cholestech.
Photograph by permission of Sandy Wilson

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Assessment of the point-of-care Cholestech lipid analyser for lipid screening in Aboriginal communities

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Abstract

Cardiovascular disease is the leading cause of mortality in Aboriginal Australians. Screening for cardiovascular disease risk factors, notably elevated blood lipids, is urgently needed. The small, portable Cholestech machine (Point-of-Care Diagnostics) can enzymatically measure total cholesterol, triglyceride and HDL cholesterol (without the prior need for precipitation of other lipoproteins) on 35µL of capillary or venous whole blood in under 5 minutes. It also calculates LDL cholesterol. Its suitability for use in Aboriginal communities was assessed. Fifty-one volunteers had their lipids measured on capillary and venous whole blood samples on the Cholestech. These results were compared with those obtained by Center for Disease Control (CDC)-certified methods on the Hitachi 917. The correlation (r^2) between the Cholestech and Hitachi machines for both capillary and venous whole blood samples was ≥ 0.96 for total cholesterol, ≥0.99 for triglyceride, and ≥0.92 for HDL- and LDL cholesterol. The mean percentage difference between results on the Cholestech and Hitachi for both sample types was less than 2% for total cholesterol and triglyceride analyses and less than 5% for HDL- and LDL cholesterol. A positive bias of 6% was observed on the Cholestech at HDL cholesterol concentrations greater than 1.2 mmol/L. Within-day precision (CV%) on whole blood samples ranged from 0.9 to 3.5% for total cholesterol, 1.6 to 2.5% for triglyceride and 6.3 to 7.9% for HDL cholesterol. There was no significant difference between capillary and venous whole blood lipid measurements performed on the Cholestech. With its simple operation, fully automated nature, sound analytical performance and ability to produce a full lipid profile in under 5 minutes, the Cholestech would be suitable for the Aboriginal health care setting.

Keywords - Cholestech, point-of-care technology, screening, lipids

Introduction

Cardiovascular disease is the leading cause of mortality in Aboriginal Australians, with mortality rates due to coronary heart disease and stroke being twice those of non-Aboriginal Australians (1-3). Of particular concern are the high death rates from coronary heart disease among young and middle-aged Aboriginal people, with death rates for people aged 25-44 years being more than 10-times those of other Australians (2). Risk factors for cardiovascular disease in Aboriginal people include hypertension,

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diabetes, obesity, tobacco and alcohol consumption, lack of physical activity and high blood lipids (2-7).

Screening for cardiovascular disease risk factors, notably elevated blood lipids, has been carried out in selected Aboriginal communities and parts of Australia. For example, the prevalence of dyslipidaemia was examined in a study of over 1000 Aboriginal people from Central Australia, the Kimberley in Western Australia and Cape York in Queensland was 36% (4).

In another study of over 350 Aboriginal people from a community west of Alice Springs, 68% of men and 46% of women over 35 years of age had raised cholesterol levels, while 51% of men and 27% of women had elevated triglyceride concentrations (5). In a country Victorian screening program, 30% of over 120 Aboriginal males tested had cholesterol levels greater than 6.5 mmol/L while 56% had triglycerides greater than 2.0 mmol/L (6). In the Tiwi Islands, 50% of over 850 adults screened had dyslipidaemia, notably high triglycerides (7).

Perhaps even more disturbing is the prevalence of lipid disorders found in a recent study in the Kimberley region of 74 Aboriginal children and adolescents (mean age 18.5 years). Nearly a quarter of these young Aboriginal people had hypercholesterolaemia, while 12% had elevated LDL cholesterol concentrations (8). Clearly, further well co-ordinated, community-controlled and culturally-appropriate lipid screening programs are urgently needed in Aboriginal communities nationally to identify people with disorders of lipid metabolism and to provide these people with the opportunity to participate in intervention programs aimed at lowering their risk for cardiovascular disease.

A major barrier to effective lipid screening in many rural and remote Aboriginal communities is limited access to pathology laboratories. Aboriginal health services may be several hundred, even thousands, of kilometres from the nearest pathology service and blood samples may take up to several days to reach that service, particularly if air transport is limited or unavailable. The return of results to the community and then to the individual patient incurs further delays, while patient follow-up may not be possible.

The ability to perform on-site testing for blood lipids using point-of-care technology would address many of the current problems associated with effective delivery of a lipid screening service. The small, portable Cholestech machine (Point-of-Care Diagnostics) can measure total cholesterol, triglyceride and HDL cholesterol, without the prior need for precipitation of other lipoproteins, on 35µL of capillary or venous whole blood in under 5 minutes. It also calculates LDL cholesterol. The analytical performance of the Cholestech machine was assessed in the laboratory, with the view to testing the machine in the Aboriginal health care setting for lipid screening and on-going management.

Materials and Methods

Samples

Fifty-one volunteers had their lipid levels measured on capillary (fingerprick) and venous whole blood samples on the Cholestech (Point-of-Care Diagnostics, Artarmon, NSW) and on venous plasma by the SouthPath laboratory, Flinders Medical Centre, South Australia.

After sitting for five minutes, each subject had a capillary and venous whole blood sample collected less than five minutes apart. The capillary sample for the Cholestech was taken from the upper side corner of the chosen finger, with the first drop of blood being wiped away. Venous whole blood was collected by venepuncture into a heparinised blood tube. After testing the venous whole blood sample on the Cholestech, the remaining blood was sent to SouthPath, where it was centrifuged for five minutes at 5,000 rpm and venous plasma separated for subsequent analysis.

The Cholestech system

Thirty-five microlitres of sample (capillary or venous whole blood) is placed in the sample well of the Cholestech reagent cassette, and loaded into the instrument. Once in the machine, plasma is separated from red blood cells using a glass fibre

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screen. Plasma is directed to individual analyte-specific, solid phase reagent pads containing reactants. Resultant colour is measured by reflectance photometry.

Total cholesterol and triglyceride are measured enzymatically using a Trinder's indicator system with N-ethyl-N-sulfohydroxypropyl-*m*-toluidine sodium salt (9).

HDL cholesterol is isolated from other lipoproteins following their precipitation in the cassette using dextran sulphate/magnesium acetate (10). The filtrate containing HDL cholesterol is then directed to the HDL cholesterol reaction pad, where cholesterol in this fraction is measured enzymatically as above.

LDL cholesterol is calculated using the Friedewald formula (LDL cholesterol = Total Cholesterol - HDL cholesterol - Triglyceride/2.2) (11). This formula provides an adequate indirect estimate of LDL cholesterol, provided the sample has a triglyceride concentration less than 4.5 mmol/L and is free of chylomicrons (11). For these reasons, fasting samples are the specimen of choice for LDL cholesterol calculation by the Friedewald formula.

Calibration information is encoded on a magnetic strip on each cassette and is read by the analyser during each analysis. Calibration is set by the manufacturer using pooled human whole blood or sera that have assigned values traceable to the National Committee for Clinical Laboratory Standards (for total cholesterol) and Centres for Disease Control Reference Methods (for triglyceride and HDL cholesterol).

The Cholestech is capable of measuring analyte concentrations within the following ranges: total cholesterol 2.6 to 12.9 mmol/L, triglyceride 0.5 to 7.3 mmol/L, and HDL cholesterol 0.4 to 2.6 mmol/L. Patients with results exceeding the instrument's wide assay limits for any of these analytes should have a fresh, fasting venous sample collected and sent to the laboratory for accurate quantitation.

Comparative method

Total cholesterol, triglyceride and HDL cholesterol were also measured on separated venous plasma by routine methods at the SouthPath laboratory, Flinders Medical Centre. Total cholesterol and triglyceride were measured enzymatically on the Hitachi 917 analyser (Roche Diagnostics, Germany). HDL cholesterol was measured enzymatically on the Hitachi following precipitation of very low and low density lipoproteins from plasma with phosphotungstic acid/magnesium chloride (12).

All methods are certified through participation in the Centers for Disease Control- National Heart, Lung and Blood Institute (CDC-NHLBI) Lipid Standardisation Program (Centers for Disease Control and Prevention, Atlanta, Georgia).

Precision studies

Within-day precision studies (n=10) were performed on fresh venous whole blood samples from three volunteers. Between-run precision studies (n = 10) were conducted using liquid quality control material (Cholestech LDX Controls, Level 1 and Level 2, Catalogue Number 10-983). During the evaluation, Level 1 and Level 2 controls were run in an alternate manner every time a new reagent kit was opened. A single lot number of reagent was used throughout the evaluation.

Statistical analyses

Passing/Bablock linear regression analysis was used to assess the correlation between analytical measurements on the Cholestech and Hitachi analysers (13,14). Slope, intercept, correlation coefficient (r^2) and standard error (Sy.x) are calculated using the Passing/Bablock analysis. If the test method (Cholestech) showed good agreement with the comparative method (Hitachi), the slope would be close to 1.00, the intercept near to 0, the correlation coefficient close to 1.00, and the standard error small.

Results

Range of analyte concentrations tested

The range of analyte concentrations found in the 51 subjects tested in this study was: total cholesterol 3.1 to 8.5 mmol/L, triglyceride 0.5 to 7.8 mmol/L, HDL cholesterol 0.7 to 2.6 mmol/L and LDL cholesterol 1.6 to 5.7 mmol/L (Hitachi, venous plasma values).

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Method comparison

The correlation between the Cholestech and Hitachi machines is shown for each analyte and each sample type using Passing/Bablock linear regression plots (Figure 1).

The correlation (r^2) between the Cholestech and Hitachi for both capillary and venous whole blood samples was ≥0.96 for total cholesterol, ≥0.99 for triglyceride, and ≥0.92 for HDL cholesterol and LDL cholesterol.

The mean % difference between results on the Cholestech and Hitachi for both sample types was less than 2% for total cholesterol and trigylceride analyses and less than 5.5% for HDL cholesterol and LDL cholesterol. A positive bias of 6% was observed with capillary HDL cholesterol on the Cholestech at concentrations greater than 1.2 mmol/L.

Comparison between capillary and venous whole blood

The mean percentage difference between capillary and venous whole blood lipid measurements performed on the Cholestech was 0.8% for total cholesterol, 0.4% for triglyceride, 2.3% for HDL cholesterol and 2.7% for LDL cholesterol ($p>0.05$, not significant by the paired *t*-test, except for HDL cholesterol where $p=0.029$). This indicates that capillary sampling provides lipid results equivalent to those obtained using venous whole blood.

Precision

Within-day precision (coefficients of variation) on three fresh whole blood samples ranged from 0.9 to 3.5% for total cholesterol, 1.6 to 2.5% for triglyceride and 6.3 to 7.9% for HDL cholesterol. Between-run precision on the two liquid controls (Levels 1 and 2) were 3.2 and 3.4% for total cholesterol, 3.1 and 3.0% for triglyceride, 2.2 and 2.4% for HDL cholesterol.

The median imprecision achieved by all Australasian laboratories using all methods in the most recent cycle of the Royal College of Pathologists of Australasia-Australasian Association of Clinical Biochemists General Serum Chemistry Quality Assurance Program was 2.5% for total cholesterol, 3.8% for triglyceride and 5.2% for HDL cholesterol (J Gill, personal communication).

Practicability of the Cholestech

The Cholestech machine proved very robust during the laboratory evaluation. Across more than 200 analyses, no instrument breakdowns or cartridge error messages were recorded. An administrative staff member with no previous laboratory experience performed much of the evaluation. This person was able to conduct analyses after less than half an hour's training, and commented on its ease and simplicity of use.

Discussion

With heart disease being today's greatest single cause of Aboriginal mortality, the need to characterise cardiovascular risk profiles among Aboriginal people is clearly a pressing concern. Screening for elevated blood lipids is an important component of identifying risk.

The ability to perform on-site lipid testing in the Aboriginal community setting offers considerable advantages to both Aboriginal patient and doctor. In particular, the ability to conduct testing and have the result immediately available to the patient means that the results are more relevant and timely for the patient, while the doctor can act more expediently on the results and tailor subsequent management accordingly.

In our laboratory evaluation, the Cholestech point-of-care lipid analyser demonstrated excellent analytical performance characteristics for total cholesterol and triglyceride. HDL cholesterol measurements on the Cholestech showed a small positive bias at concentrations greater than 1.2 mmol/L and greater imprecision than total cholesterol and triglyceride analyses on whole blood samples. However, overall performance for this analyte was considered satisfactory for screening purposes where the combination of low HDL cholesterol (less than 1.0 mmol/L) and high triglyceride (greater than 2.0 mmol/L) is of particular clinical concern.

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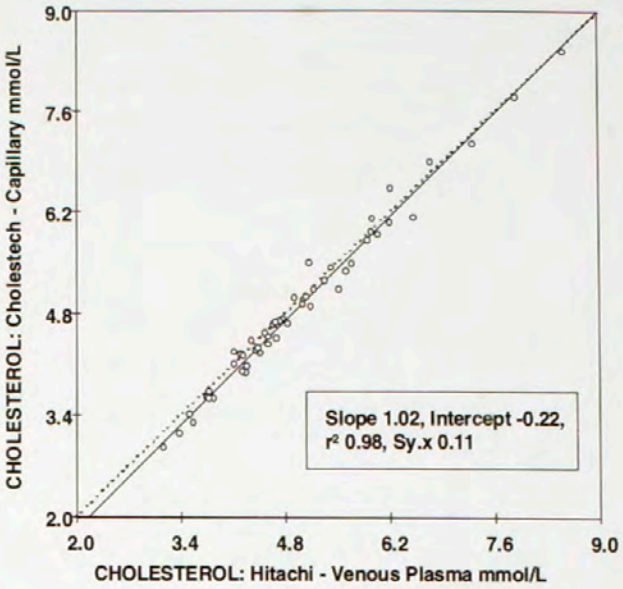
The machine's ability to measure HDL cholesterol in less than five minutes, without the need for prior precipitation of other lipoproteins, is a major practical advantage for field use.

Previous studies have suggested that lipid analyses performed on capillary fingerprick samples produced results that were lower than those found on equivalent venous whole blood samples (15). This was considered to be due in part to contamination (dilution) of capillary whole blood with tissue fluid, and to the dependence of fingerprick sampling on good technique.

However, with the development of better guidelines for fingerprick sampling (including adherence to wiping away the first drop of blood), recent data, including the present study, shows that the more convenient capillary (fingerprick) whole blood sampling is a suitable analytical surrogate for the more invasive, more technically difficult venepuncture sampling (16).

With its simple operation, fully automated nature, sound analytical performance and ability to produce a full lipid profile in under 5 minutes, the Cholestech offers a robust, practical option for first-line lipid screening in the Aboriginal health care setting.

Together with the concurrent development of community education programs targeting nutrition, exercise and smoking and alcohol reduction strategies, the Cholestech machine has the potential to play an integral role in reducing the burden of cardiovascular risk that currently afflicts Aboriginal people.



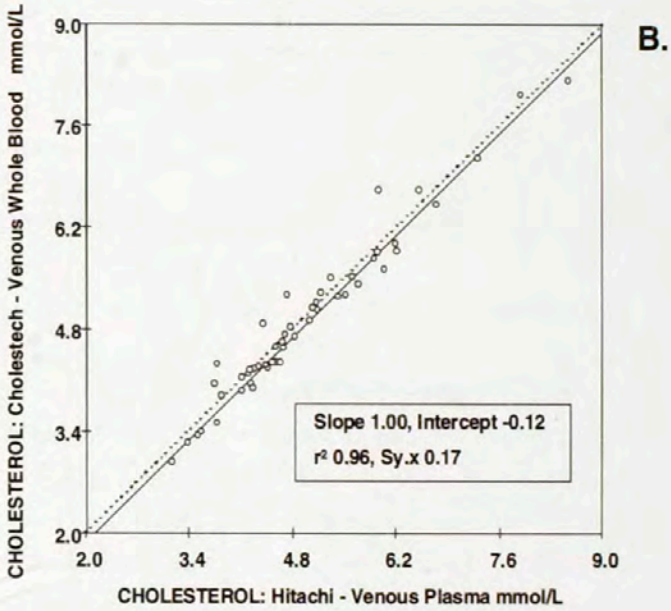


Figure 1. (A-H) Passing/Bablock linear regression plots showing the correlation observed between the Cholestech (y-axis) and the Hitachi (x-axis) for each lipid analyte (cholesterol, triglyceride, HDL cholesterol and LDL cholesterol) and for each sample type tested on the Cholestech (capillary and venous whole blood). The solid line represents the line of best fit between the two instruments as determined by the linear regression equation, while the hatched line represents the line of equivalence.

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The author would like to thank Lily Mickalov and Karan Lavender for their technical assistance in performing lipid analyses on the Cholestech. Dr Malcolm Whiting, (SouthPath, Flinders Medical Centre) performed comparative Hitachi analyses.

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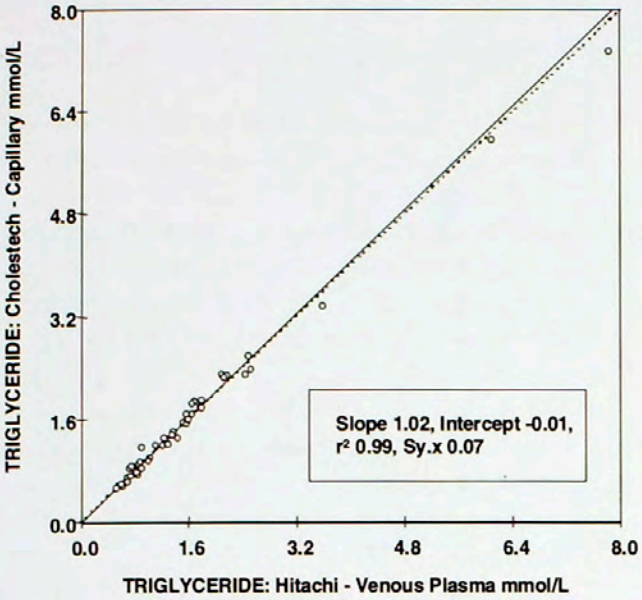

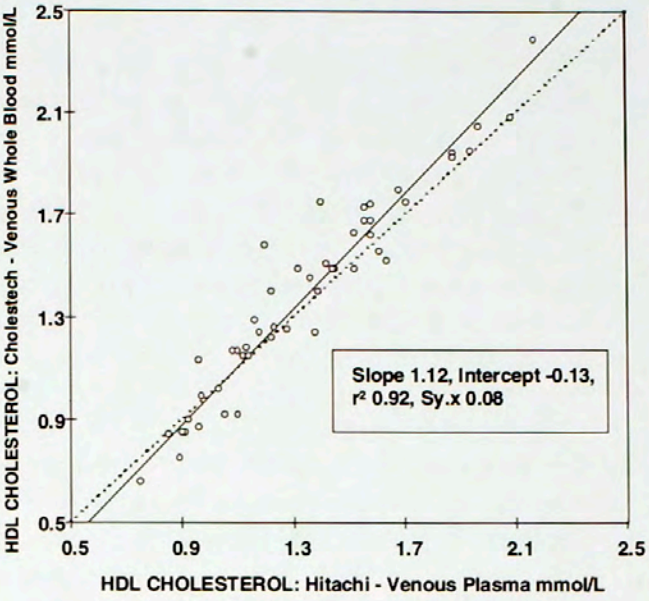

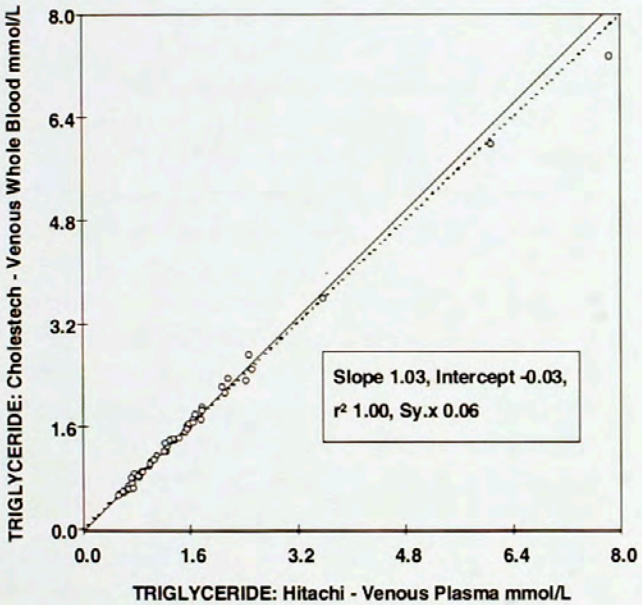

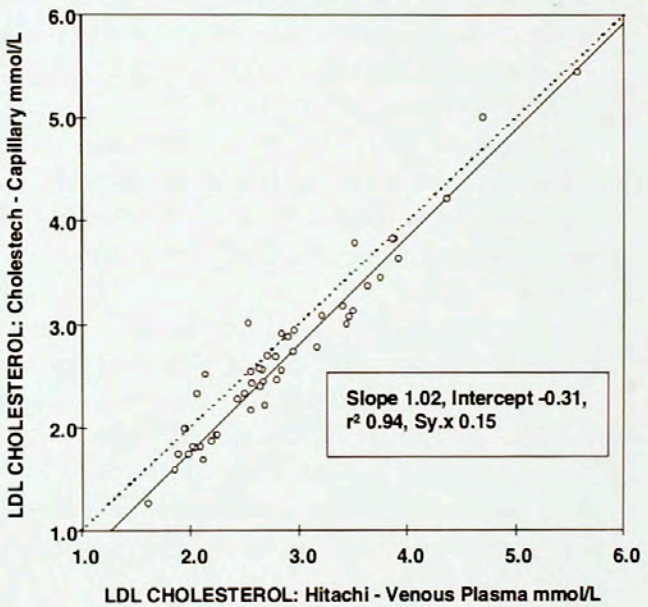

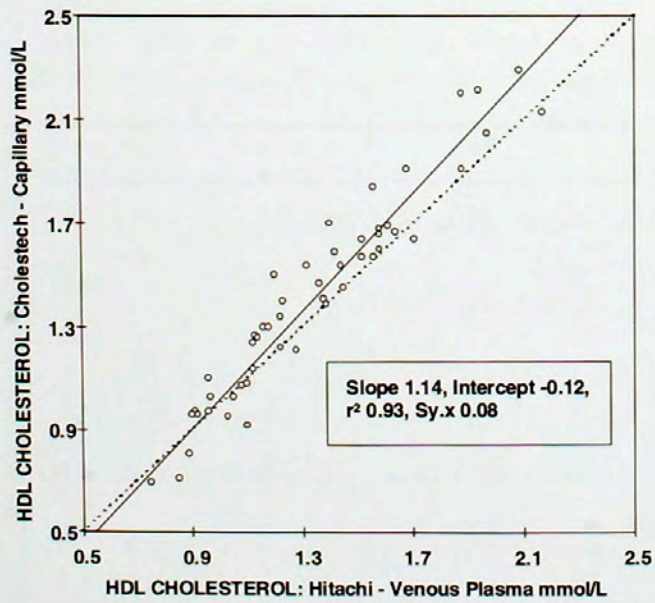

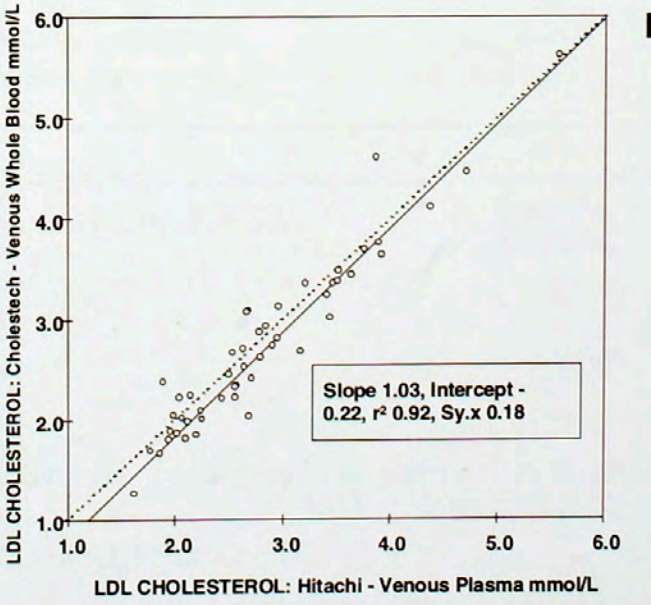

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