

Accuracy of analyses for lipid profile parameters as measured with the CR3000 system

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Abstract. – Total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) levels are positively related to coronary heart disease (CHD), and high-density lipoprotein cholesterol (HDLC) levels are negatively related to CHD. Efforts to identify and treat people at increased risk based on cholesterol and lipoprotein levels have led to more lipid testing and the need for very reliable test results. Point-of-care testing (POCT) has developed from the demand for analytical information more fastly than is available from central laboratories. By carrying the analysis closer to the patient some process steps have been eliminated, facilitating a shorter time to result and faster management response with improved outcomes. Thus benefits include better therapeutic turnaround times (TATs), decreased blood loss as a result of reduced phlebotomy secondary to clinical improvement and diminished resource utilization. These effects depend on acceptable analytical performance in comparison with central laboratory techniques and in relation to clinical criteria.

Key Words:

Total cholesterol, Coronary heart disease, Low-density lipoprotein, High-density lipoprotein, Point-of-care testing, Turnaround times.

Introduction

Point-of-care diagnostic testing, or testing (POCT) performed at the patient's bedside, allows physicians to diagnose patients more rapidly than traditional laboratory-based testing. Rapid results can enable better patient management decisions, improved patient outcomes, and a reduction in the overall cost of care. These tests are utilized in hospitals, clinics, doctor offices, pharmacies and research institutions for the purpose of diagnosis and monitoring of disease.

Although clinicians may associate point-of-care testing with critical care, the reality is that POCT (bedside, decentralized, or near-patient testing) is already being performed in virtually every clinical setting.

POCT began more than 30 years ago, although the acronym came into use within the past 15 years. The driving force behind this type of testing has always been to improve patient care through rapid availability of reliable results. The ability to obtain clinical laboratory test results at the site of care in 2 minutes has immediate medical management benefits as well as resource and time benefits.

The potential benefits of POCT include earlier and more appropriate diagnosis, fewer tests, earlier treatment, and reduction or elimination of unnecessary treatment. An unquantifiable benefit of POCT is the convenience and decreases in the time spent in a department or clinic, which are advantages for providers and patients alike.

Lipid metabolism disorders are an extremely relevant chapter of screening diagnosis with point of care devices: in fact, the association of high concentrations of blood lipids with premature coronary heart disease (CHD) and the effectiveness of lowering the blood cholesterol concentration in decreasing coronary-disease risk are now well established¹⁻⁴.

Being cholesterol a relevant risk factor for atherosclerosis and CHD, the determination of the lipid panel is essential for primary and secondary prevention of cardiovascular diseases. CHD is the world's leading cause of death, and as such, represents a serious global health problem. It is caused by the natural ageing process and the excesses of a western lifestyle. The major risk factors for CHD are high blood pressure, hypercholesterolemia, unhealthy diet (too much salt, sugar and saturated fatty acids, and not enough fresh fruit and vegetables), tobacco use, male sex, dia-

betes, obesity, advancing age, genetic disposition, excess alcohol consumption and sedentary lifestyle. The priority in tackling the CHD epidemic is therefore prevention. In this way, several countries have put forth treatment guidelines that emphasize risk factor modification for the prevention of CHD. Beyond lifestyle factors such as smoking, obesity, and physical inactivity, dyslipidemia and hypertension are considered key targets for intervention.

Consequently, the general recommendations to operators in the management and prevention of CHD⁵⁻⁶ should include complete lipid profile testing, that is total cholesterol, HDL, LDL and triglycerides.

In an attempt to facilitate the application of these recommendations, the introduction of Point of Care systems, such as the CR3000, is desirable. Designed to quickly measure lipid panel parameters in microlitre quantities of blood, this instrument can be used in non-laboratory settings such as physician's office, pharmacies or field-testing sites. We have assessed the accuracy of the CR3000 lipid panel parameter measurements compared against measurements performed in a licensed hospital clinical laboratory.

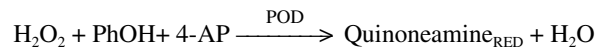
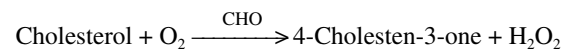
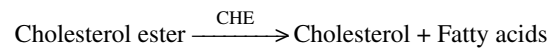
Principle of Methods

To execute the lipid profile assays, the Callegari S.p.A. (Parma, Italy) system employs wet chemistry and photometric technology to perform absorbance readings which measure the colour of the sample. The absorbance is converted automatically into concentrations based on the standard calibration curves stored by the instrument's microprocessor.

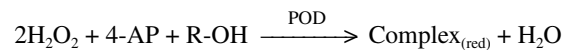
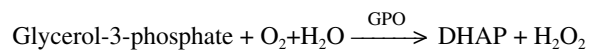
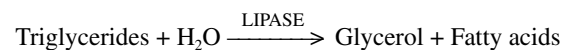
All the assays are based on enzymatic methodologies⁷⁻¹¹.

Cholesterol esters in the sample are enzymatically cleaved by the cholesterol esterase (CHE) into free cholesterol and fatty acids. Further oxidation by cholesterol oxidase (CHO) origins cholest-4-ene-3-one and hydrogen peroxide (H₂O₂), which (in presence of a peroxidase, POD) converts phenol and 4-aminophenazone (4-AP) into a red substance (quinoneamine). The intensity of the colour of this substance is directly proportional to the cholesterol concentration. In the HDL test, VLDL and LDL fractions are precipitated with a polianionic buffer. Thereafter, enzymes react only on the HDL

fraction present in the supernatant fraction obtained by centrifugation.



Triglycerides are converted by lipoprotein lipase to fatty acids and glycerol. Glycerol then in the presence of glycerol kinase (GK), ATP and glycerol-oxidase (GPO) produces H₂O₂ and dihydroxy-acetonphosphate (DHAP). H₂O₂ in the presence of 4-AP and the sensitive chromogen subsequently produces a red phenolic derivative using POD. The intensity of the colour of the red substance is directly proportional to the triglycerides concentration in the sample.



Study Design

The accuracy was assessed for the total and HDL cholesterol and triglycerides measurements made in venous whole-blood samples using the CR3000 system and these were compared against readings obtained using venous serum in a licensed hospital clinical laboratory.

We screened approximately 375 adults, aged 18 years and above – unselected individuals for whom any of the test panels were previously requested by the physician. All subjects fasted for 12-14 h, and venous blood was collected under standardized conditions between 08:00 and 10:00.

Blood samples were obtained by vein puncture, collected into evacuated tubes (Vacutainer Tubes, Becton Dickinson, Franklin Lakes, NJ, USA) containing sufficient potassium EDTA to give a final concentration of 1.8 mg/mL. A second venous blood sample was obtained, drawing blood into evacuated tubes containing no anticoagulant to separate serum samples.

The EDTA-blood samples were tested using the CR3000 system. For all the experiments a unique reagent batch was used for each CR3000 test to

avoid possible sources of variability due to the reagent's production cycle. The serum samples were analyzed on VITROS 350 instrument (Ortho-Clinical Diagnostics, a Johnson & Johnson Company, Raritan, NJ, USA) which uses multilayer slide dry chemistry technology. All measurements were performed within 4 hours.

Statistics

The significance of the differences between the two methods (reference laboratory and CR3000) was calculated by using analysis of variance (F-test). A value of $p < 0.05$ was considered significant. In addition, a linear regression analysis was performed.

Results

One of the most important parameters in the validation of an analytical method is the assessment of accuracy. The accuracy (or "trueness" in the recent nomenclature) of the method was estimated by BIAS analyses using laboratory measurements as reference that is VITROS 350. The term BIAS means the difference between the expectation of the test results and a true value.

All samples were tested on the CR3000 and on the Reference Laboratory (RL) instrument. Concentrations of specimen are real concentrations of the patients involved in the experiment without any further treatment.

After a preliminary analysis, the average BIAS was evaluated in different ranges of concentration dividing the entire range of concentrations into three groups (K) of approximately the same number of measures (Table I).

Table I. Analyte Concentration Groups employed for the accuracy statistical analysis.

K	Analyte	Concentration Range (mg/dL)
1	Cholesterol	123-204
	HDL	25-44
	Triglycerides	76-160
2	Cholesterol	205-245
	HDL	45-60
	Triglycerides	165-270
3	Cholesterol	245-352
	HDL	61-112
	Triglycerides	270-590

For each group, the average BIAS was obtained by the formula 1 with a 95% confidence interval by the formula 2 and 3 (see below).

$$\bar{B}k = \frac{\sum_{m=1}^{Nk} (ym-xm)}{Nk} \tag{1}$$

$$I.C. = \bar{B}k \pm 2 \frac{SDk}{\sqrt{Nk}} \tag{2}$$

$$SDk = \sqrt{\frac{\sum [(ym-xm) - \bar{B}k]^2}{Nk - 1}} \tag{3}$$

Formulas. K = group number, xm = CR3000 result, ym = RL result

To provide an immediate idea of BIAS entity, three quantities are calculated taking the average BIAS: $\bar{B}k$ of each K-th group and the relative I.C. extremes calculating what percentage they represents of the minimum concentration in that group (Ck_{min}):

$$B_{max} (\%) = \frac{\bar{B}k}{Nk} \times 100$$

$$B - (\%) = \frac{\bar{B}k - 2SDk / \sqrt{Nk}}{Ck_{min}} \times 100$$

$$B + (\%) = \frac{\bar{B}k + 2SDk / \sqrt{Nk}}{Ck_{min}} \times 100$$

In Table II a summary of the results obtained are reported.

The scatter plots of lipid panel assays for samples processed on the CR3000 analyzer and VITROS 350 are displayed in Figures 1-3.

Table II. BIAS percentage (%) on the response. SD = Standard Deviation.

Analyte	B _{max}	Overall mean BIAS	SD BIAS
Cholesterol	6.4	0.5	7.6
HDL	18.8	-1.6	10.5
Triglycerides	2.3	1.0	11.1

Good correlations were found between the CR3000 and the Reference Laboratory instrument.

For ease of comparison the correlation coefficients, slope and intercepts are summarized in Table III. As suggested by NCCLS¹² regressions with $R^2 > 0,95$ was retained satisfactory to compensate errors, but for some parameters we also accepted regressions with a worst R^2 value until the accuracy value (in particular the max BIAS) obtained from the data, matched the desired value.

The angular coefficient of the linear regression (Slope in the Table III) is close to 1 for all the three parameters, showing an excellent correlation between the two methods. This is also confirmed by the statistical analysis of the data (Pearson coefficient of correlation, r in the Table III, $p < 0.01$).

The CR3000 results for total cholesterol, HDL and triglycerides were, on average, 0.4% higher, 0.02% lower and 3.1% higher than those ob-

Table III. Comparison of CR3000 and VITROS 350 – Linear regression factors.

Analyte	Coefficient correlation r	Slope	Intercept
Cholesterol	0.978	1.0189	-3.07
HDL	0.92	1	-0.013
Triglycerides	0.98	0.96	+7.9

tained with the VITROS 350, respectively. Table IV shows the average values obtained from patients tested with the two type of instruments. Also the dispersion within the series of data collected was reported (SE, standard error, Table IV).

For Total Cholesterol, 95% of the readings were between $\pm 15\%$ from the expected value. No data showed differences above 20% with respects to the reference value.

Table IV. Statistical summary of parameter concentrations obtained with the CR3000 and VITROS 350.

Analyte	CR3000 (mg/dl)		VITROS 350 (mg/dl)	
	Average	SE	Average	SE
Cholesterol	202.7	4.3	203.5	3.9
HDL	55.28	1.33	55.35	1.44
Triglycerides	168.6	10.37	166.2	11.08

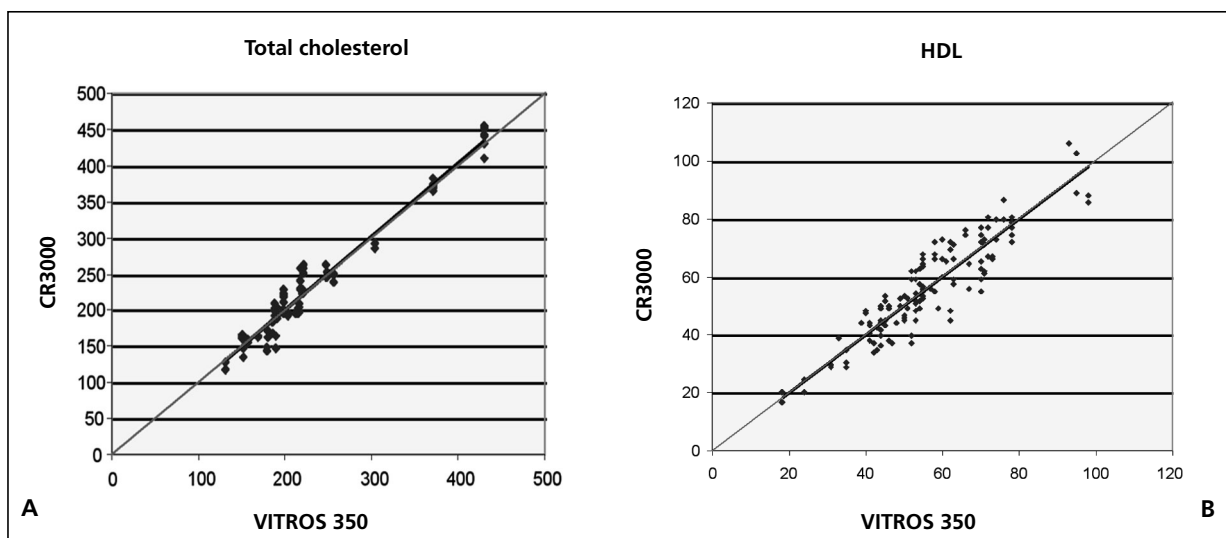
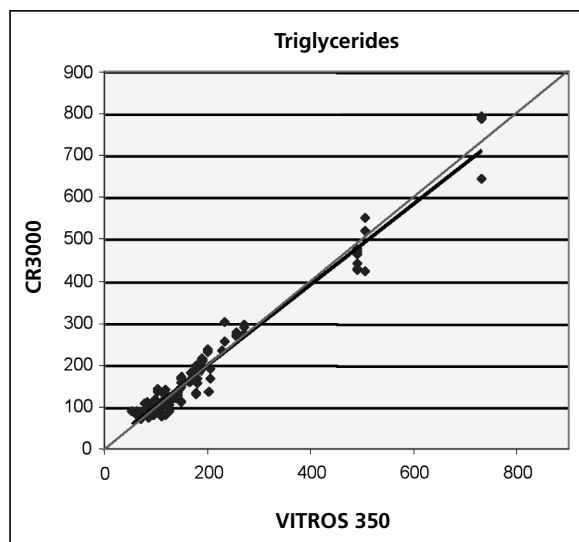


Figure 1. A, B, Correlation obtained between CR3000 and VITROS 350.

Figure 2. Correlation obtained between CR3000 and VITROS 350.



All HDL results obtained had a less than 25% deviation from the expected result and in 95% of cases the measured difference remained below 20%. In summary 90% of the triglycerides values showed less than 18% difference respect to the reference value.

In brief, the deviation percentages confirm that the instrument performs lipid panel readings in an accurate manner and positions the instrument as both a product for the use of screening and monitoring of lipid panels through time at near patient testing areas.

Discussion

POC systems should be validated in terms of accuracy in order to provide a suitable feed back concerning clinical evaluation and therapeutic perspectives.

The accuracy is essentially an agreement between the tested diagnostic device and standardized laboratory systems.

According to the National Cholesterol Education program laboratory standardization panel, every clinical method to detect blood cholesterol, must show an accuracy with an average bias (recommended deviation of standardized reference method value) not greater than 3%.

Another recommendation of the National Cholesterol Education program relates to the full lipid profile analysis of total errors, which accepts a global variation in the accuracy in the range of 8,9% of the reference value; this final

tolerance range is due to 95% tolerance interval considering maximum bias 3% and variation coefficient $\pm 3\%$.

The values obtained with the CR3000 falls between the recommended guidelines.

In fact, the CR3000 lipid panel data correlate well with those from the reference laboratory.

Indeed, the analysis of the linear regression of the VITROS 350 in dry chemistry, for all the analytes examined, shows excellent correlation coefficients (from a minimum of $r=0.92$ to a maximum of $r=0.98$). It can be concluded that the CR3000 has proved to be a reliable system which guarantees a high quality performance in its field both for screening and monitoring of lipid panels with rapid TAT times.

Conclusions

Point of care systems offer several practical advantages if compared with standard laboratory instruments: they are easy to use, widen patient access to improved patient care in the surgery, pharmacy or at the bedside, can lower the cost of testing and increase TATs of results.

The employment of point of care systems in widespread disease management programs such as screenings, to select individuals which are then to be addressed to physicians and provided treatment is very effective especially in prevention and early treatment, provided that adequate education plans and patient referral to specialists are accomplished.

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