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Comparison of the Reflectance Method (Reflotron® Reflectance Photometer) with the Absorbance Method (Automatic Analysers) for the Determination of Cholesterol

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Summary: The European Atherosclerosis Society (1) and the Expert Panel of the US National Cholesterol Education Program (2) have issued detailed guide values for recognition and management of hyperlipidaemia in adults. In these guidelines, the diagnosis of dyslipidaemia based on the measurements of total cholesterol, triacylglycerols, HDL and LDL cholesterol plays an important role.

A prerequisite for the desired success of interventive measures is the reliability of the analytical data. The aim of this study was to investigate the precision and accuracy of Reflotron® Cholesterol, a method based on the dry chemistry principle.

Accuracy was assessed by establishing the correlation with the standardized automated methods used in routine lipid diagnosis. In addition, it was also examined whether the Reflotron® Cholesterol results in plasma and blood are comparable. The Reflotron® cholesterol (sample: blood) showed a good correlation with the CHOD/PAP method on a Hitachi 737 instrument (sample: plasma). The median value of the differences of the test results was −0.4%. Similarly, the method comparison of Reflotron® Cholesterol (sample: blood) versus CHOD/PAP method on a SMAC instrument (sample: plasma) showed that Reflotron® produces slightly (1.8%) higher results. The Refloton® Cholesterol values obtained from blood samples were slightly lower than those from plasma samples (median value of the differences: −2.2%).

The results suggest that for routine purposes Reflotron® Cholesterol provides results which are in good agreement with those obtained by standardized wet chemistry methods.

Introduction

The Laboratory Standardization Panel of the National Cholesterol Education has recently published a report with the title "Current Status of Blood Cholesterol Measurement in Clinical Laboratories in the United States" (3). This report shows the considerable variance of enzymatic cholesterol determinations on the usual laboratory instruments when the same sample material is used. A total of 25 systems were examined. Seven systems (1884 samples) showed system-

[1]) On the occasion of the 25th anniversary of the German Society for Clinical Chemistry (cf. Editorial, this J. *27*, 529 (1989)).

atic deviations $< -5\%$, and 7 systems (425 samples) showed deviations $> +5\%$, the coefficients of variation (CVs) lying in the 2.2% to 8.8% range with a median value of 5.4%, i.e. the CVs of 14 systems (56%) were > 5% (3). According to the requirements of the Laboratory Standardization Panel, the coefficients of variation for cholesterol determinations should be < 5% and the deviations $< \pm 5\%$ of the "true" values. Coefficients of variation and deviations of $\leq 3\%$, however, are aimed at l.c. (3).

With respect to the new reflectance photometers, the Laboratory Standardization Panel takes the view that they should be further tested. Particular importance is attached to the comprehensive instructions for the user and to the flawless completion of quality assurance (3).

The objective of our examinations was to check the precision and accuracy of the Reflotron® system for cholesterol determination, in comparison with the Hitachi 737 and SMAC automatic analysers.

Materials

- Reflotron® Cholesterol, Lot 234 196 31/function curve 2638
- Reflotron® No. 29952
- Precinorm® U, Lot 158 096
  Assigned values: Reflotron® Cholesterol 5.31 mmol/l
  method of comparison 5.23 mmol/l
- Precipath® U, Lot 158 019
  Assigned values: Reflotron® Cholesterol 5.44 mmol/l
  method of comparison 5.57 mmol/l
- Hitachi® 737 using the CHOD/PAP method, Boehringer Mannheim (Calibrator: Beckman Level III)
- SMAC using the CHOD/PAP method, Boehringer Mannheim (Calibrator: Technicon Cal II)

Methods

Quality assurance

On five successive days, 10-fold determinations were carried out using Precinorm® U, Precipath® U and two pool sera on the three systems, using samples with cholesterol concentrations between 3.11 and 11.65 mmol/l. Only 10 samples had concentrations > 7.77 mmol/l, while the distribution below this value was rather dense.

Method comparison

Cholesterol concentrations were determined in 85 samples from hospitalized patients. The venous blood, treated with the anticoagulant lithium heparinate, was first analysed on the Reflotron®. The blood was then centrifuged and the supernatant plasma was used to determine the cholesterol value in the three systems.

Precinorm® U and Precipath® U were run daily for quality control.

Statistical methods

The significance of the differences of values was tested by the paired t-test. Regression analyses were performed according to the method of *Passing & Bablok* (6).

Results

Quality assurance

The results for quality assurance are represented in table 1, which shows that all systems meet the demands of precision and congruence from system to system. The CVs for the Reflotron® were between 1 and 2.5%, those for the Hitachi 373 between 0.7 and 1.6% and those for the SMAC between 0.2 and 1.7%.

The values obtained on the Reflotron® with pool sera 1 and 2 lay approx. 3% and 2% respectively above those obtained with the two automated instruments. When the Hitachi and SMAC cholesterol values were compared with each other, the mean deviation was found to be only 0.026 mmol/l.

Quality assurance during the method comparison

Table 2 represents the day-to-day precisions obtained with control sera in the three systems examined. With the Reflotron® the variance hardly increases compared with the within-series precision, whereas the day-to-day CVs of the methods of comparison are in the same range as those of the Reflotron®.

Method comparison

The mean values, standard deviations and ranges for the cholesterol measurements with the four methods are given in table 3.

The regression analyses for method comparisons with Reflotron® (cholesterol determination in plasma or blood respectively) and comparison methods (CHOD/PAP method on a Hitachi 737 or a SMAC instrument respectively) were performed according to the method of *Passing & Bablok* (6).

Table 4 gives a summary of the six method comparison studies, while figure 1 to figure 6 represent the results in detail: CHOD/PAP method on a Hitachi 737 vs. Reflotron® (sample plasma) (fig. 1), CHOD/PAP method on a SMAC vs. Reflotron® (sample: plasma) (fig. 2), Reflotron® (sample: blood) vs. Reflotron® (sample: plasma) (fig. 3), CHOD/PAP method on a Hitachi 737 vs. Reflotron® (sample: blood) (fig. 4), CHOD/PAP method on a SMAC vs. Reflotron® (sample: blood) (fig. 5), CHOD/PAP method on a Hitachi 737 vs. CHOD/PAP method on a SMAC (fig. 6).

None of the computed slopes of the regression curves was significantly different from 1.

Tab. 1. Quality assurance by means of control and pool sera

	Day 1		Day 2		Day 3		Day 4		Day 5	
	$\bar{x}$ mmol/l	CV %	$\bar{x}$ mmol/l	CV %	$\bar{x}$ mmol/l	CV %	$\bar{x}$ mmol/l	CV %	$\bar{x}$ mmol/l	CV %
Reflotron®										
Precinorm® U	5.00	1.5	5.00	1.4	5.23	1.3	4.92	1.3	5.00	1.3
Precipath® U	4.74	2.1	4.88	2.2	4.92	1.6	4.79	1.4	4.89	1.5
Pool 1	3.73	1.1	3.73	1.4	3.73	2.0	3.73	1.6	3.68	2.5
Pool 2	8.78	2.2	9.04	1.3	8.83	1.6	8.54	1.8	8.86	1.3
Hitachi® 737										
Precinorm® U	5.23	0.9	5.18	0.8	5.31	0.9	5.31	1.5	5.31	0.9
Precipath® U	5.39	0.8	5.39	0.7	5.36	0.7	5.49	0.7	5.41	1.1
Pool 1	3.57	1.0	3.57	0.7	3.57	1.6	3.65	1.2	3.65	1.0
Pool 2	8.57	1.1	8.52	0.7	8.57	1.1	8.67	1.0	8.86	0.9
SMAC®										
Precinorm® U	5.26	0.6	5.13	0.2	5.31	0.3	5.26	0.2	5.13	0.3
Precipath® U	5.41	0.9	5.31	0.4	5.34	0.4	5.41	0.2	5.31	0.2
Pool 1	3.63	1.7	3.57	0.4	3.65	0.3	3.65	0.3	3.63	0.3
Pool 2	8.60	0.8	8.52	0.31	8.62	0.3	8.70	0.5	8.65	0.4

Tab. 2. Day-to-day precision

	Reflotron®		Hitachi® 737		SMAC®	
	$\bar{x}$ (mmol/l)	CV (%)	$\bar{x}$ (mmol/l)	CV (%)	$\bar{x}$ (mmol/l)	CV (%)
Precinorm® U	5.02	2.0	5.20	1.5	5.13	2.0
Precipath® U	4.82	2.4	5.36	2.0	5.31	1.0

Tab. 3. Cholesterol determination (mmol/l) on Reflotron® and automatic analysers

Method	Arithmetric mean	Standard deviation	Median	Range
CHOD/PAP* on Hitachi 737	5.85	1.81	5.49	2.99–13.16
CHOD/PAP on SMAC	5.98	1.80	5.62	3.09–12.77
Reflotron® (sample: plasma)	5.95	1.78	5.72	2.76–12.06
Reflotron® (sample: blood)	5.84	1.81	5.51	2.63–12.66

* CHOD/PAP = Cholesterol oxidase/*p*-aminophenazone

Tab. 4. Method comparison with regression analysis according to *Passing & Bablok*

Method x	CHOD/PAP** on Hitachi 737	CHOD/PAP on SMAC	Reflotron® (blood)	CHOD/PAP on Hitachi 737	CHOD/PAP on SMAC	CHOD/PAP on Hitachi 737
Method y	Reflotron® (plasma)	Reflotron® (plasma)	Reflotron® (plasma)	Reflotron® (blood)	Reflotron® (blood)	CHOD/PAP on SMAC
Slope	1.00	1.00	0.98	1.03	1.04	1.00
y intercept (mmol/l)	0.104	0.0	0.202*	−0.206	−0.339*	0.129*
S. D. of residuals (mmol/l)	0.363	0.349	0.286	0.341	0.338	0.106
Difference of means (mmol/l)	0.091*	−1.104	0.104*	−0.012	−0.137*	0.124
Median of differences (%)	1.9	0.0	2.2	−0.4	−1.8	2.3
Median of differences (mmol/l)	0.100	0.0	0.112	−0.010	−0.104	0.137

* $p < 0.05$ ** CHOD/PAP = Cholesterol oxidase/*p*-aminophenazone

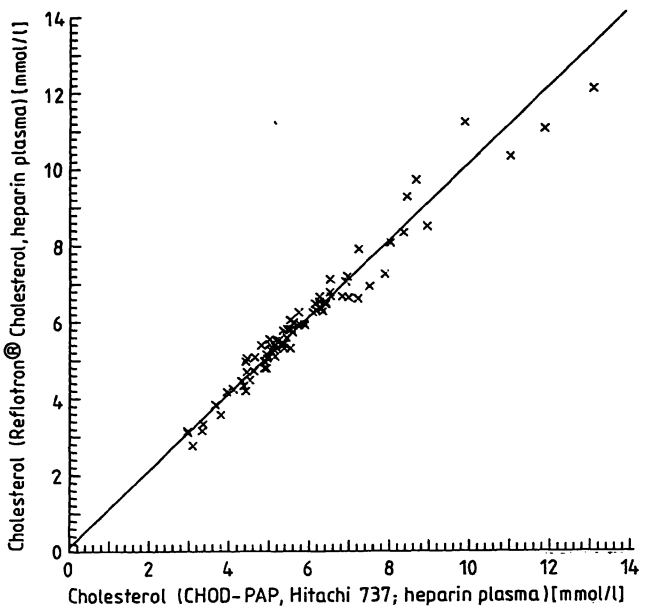

Fig. 1. Method comparison cholesterol
x: CHOD-PAP Hitachi 737, heparin plasma;
y: Reflotron® Cholesterol, heparin plasma
N = 85
y = 0.103 + 1.00 x

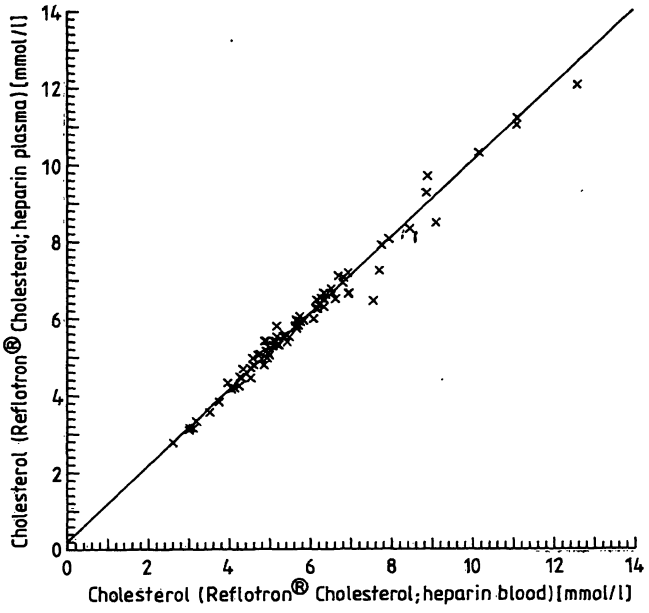

Fig. 3. Method comparison cholesterol
x: Reflotron® Cholesterol, heparin blood;
y: Reflotron® Cholesterol, heparin plasma
N = 85
y = 0.201 + 0.984 x

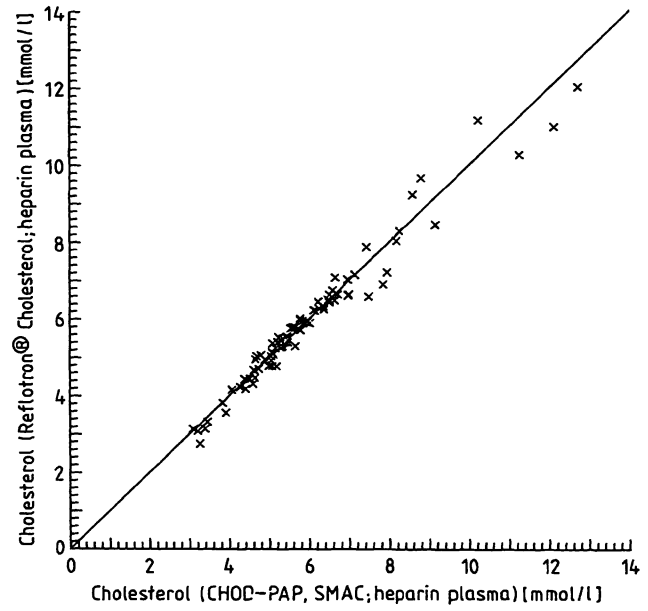

Fig. 2. Method comparison cholesterol
x: CHOD-PAP SMAC, heparin plasma;
y: Reflotron® Cholesterol, heparin plasma
N = 85
y = $-3.81 \cdot 10^{-6}$ + 1.00 x

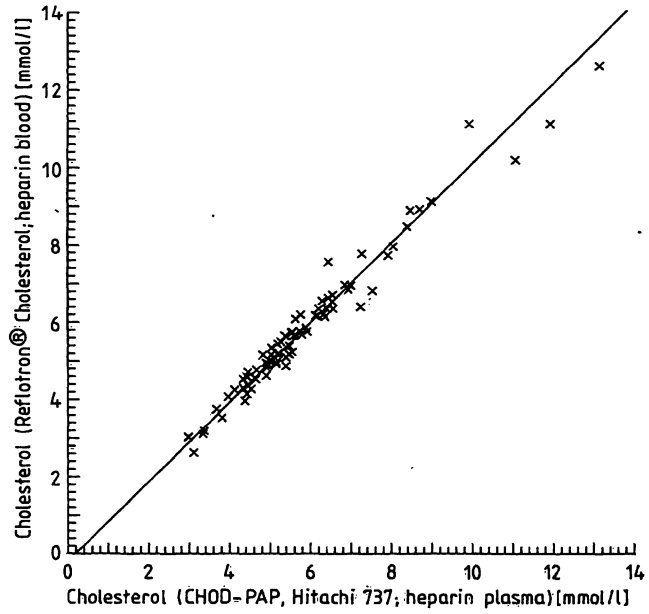

Fig. 4. Method comparison cholesterol
x: CHOD-PAP Hitachi, heparin blood;
y: Reflotron® Cholesterol, heparin plasma
N = 85
y = −0.205 + 1.03 x

The cholesterol values obtained with Reflotron® (sample: plasma) were slightly higher than obtained with CHOD/PAP on a Hitachi 737 (0.091 mmol/l or 1.9%, $p < 0.05$). All the results lay within the range of ± 15%. The y intercept did not differ significantly from 0.

The corresponding results of the method comparison with the SMAC analyser are represented in figure 2.

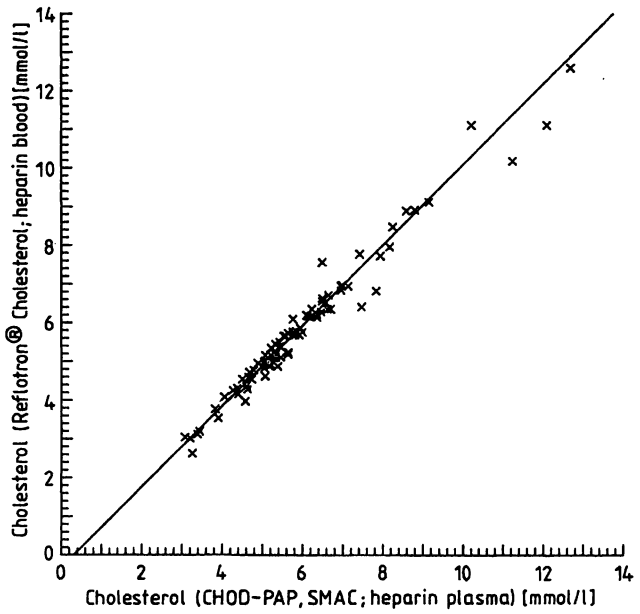

Fig. 5. Method comparison cholesterol
x: CHOD-PAP SMAC, heparin plasma;
y: Reflotron® Cholesterol, heparin blood
N = 85
y = −0.338 + 1.04 x

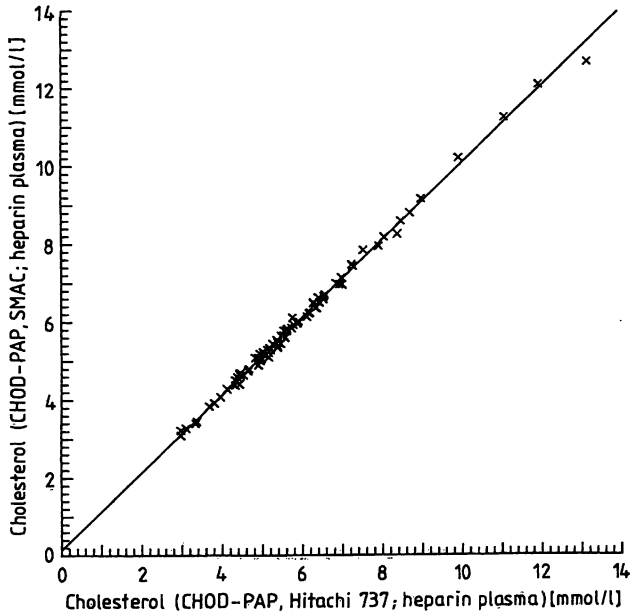

Fig. 6. Method comparison cholesterol
x: CHOD-PAP Hitachi, heparin plasma;
y: CHOD-PAP SMAC, heparin plasma
N = 85
y = 0.129 + 1.00 x

Except for one value (3.276 mmol/l vs. 2.756 mmol/l), all results also came to lie within the range of ± 15%. No systematic differences between the two methods were found. The median value of 0 was found for both the methodical and the relative methodical differences.

The concentration differences between blood and plasma for the cholesterol determinations on the Reflotron® are represented in figure 3. Values obtained with blood are slightly higher than those obtained with plasma (0.104 mmol/l on average, $p < 0.05$). The median value of the differences was 0.112 mmol/l or 2.2%. All the results lay within the range of ± 15%.

For the analysis of blood on the Reflotron® and the analysis of plasma with the methods being compared, pairs of test values are obtained which are symmetrically distributed around $y = x$. While the values obtained with the Hitachi 737 did not differ significantly from those obtained on the Reflotron®, the SMAC results were slightly higher. The differences of means was 0.137 mmol/l, $p < 0.05$. The median value of the differences was 0.104 mmol/l or 1.8%.

In each case, one result differed by more than + or −15% (Hitachi 737: 6.448 mmol/l, SMAC: 6.526 mmol/l, Reflotron® (sample: blood): 7.592 mmol/l and Hitachi 737: 3.120 mmol/l, SMAC: 3.276 mmol/l, Reflotron® (sample: blood): 2.626 mmol/l).

A comparison of cholesterol concentrations in plasma obtained with the two automatic instruments is shown in figure 6. The SMAC values were systematically higher than the Hitachi 737 values, and the difference of means was 0.124 mmol/l, $p < 0.05$. The median value of the differences was 0.137 mmol/l or 2.3%.

Discussion

Since the introduction of Reflotron® Cholesterol, greatly differing results have been reported on its precision and accuracy (4, 5, 7, 8). Frequently, the authors came to the conclusion that the test results obtained with this system were too low, thus falsely classifying a patient requiring treatment as healthy (7, 8).

Our examinations revealed a close agreement between the Reflotron® Cholesterol results and those of automatic systems, when all systems were working under strictly observed conditions. Prerequisites are an experienced team in control of the analytical problems of the cholesterol determination (pre-analytics) on the automatic systems, and a good instruction in the use of the Reflotron® system, as already shown by *M. Rohac* (9).

These results confirm that the Reflotron® Cholesterol test meets the rigid demands of the Laboratory Standardization Panel for cholesterol determination in the clinical laboratory.

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