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Determination of Cholesterol in Blood. Part 2

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Abstract—The chronological development of procedures for determining the cholesterol concentration of in plasma, serum, and whole blood is presented in the review. It is stated that, since the correlation between the risk of development of cardiovascular diseases and the concentrations of total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in human blood was established by numerous medical studies, procedures for the measurement of these parameters have been developed most actively. A brief overview of these procedures and the results of their comparative testing in patient medical examinations are given. Classifications are proposed for procedures for determining total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in the blood. The mechanism of action of the chemical reactions taking place in these procedures, advantages and disadvantages, and priorities for their application are considered. Promising directions for the development and improvement of procedures to ensure more accurate measurements of the blood cholesterol concentration, are mentioned, and alternative means of determining the risk of cardiovascular disease are discussed.

Keywords: cholesterol, plasma, serum, blood, measurement, concentration, procedure, analysis, clinical diagnostic laboratory.

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CONTENTS

1. RESEARCH IN THE 1980s

In 1980, Warnick et al. compared the results from the determination of HDL cholesterol in blood plasma and serum samples obtained in 14 clinical diagnostic laboratories. To separate HDL from VLDL and LDL, solutions of heparin and manganese(II), phosphotungstic acid and magnesium(II), and phosphotungstic acid and manganese(II) were used; electrophoresis was also applied. The HDL cholesterol concentration was measured with Liebermann–Burchard reagent and a solution containing iron(III) chloride or enzymes. Assessment of the determination of the HDL cholesterol concentration in plasma and serum showed the need to improve both the procedures and reference samples for quality control [1].

In 1980, Hohenwallner et al. explored the use of three samples for quality control (after their reduction as solutions) in determining the HDL cholesterol concentration in serum by two enzymatic spectrophotometric procedures. According to the first procedure, 500 μL of serum was mixed with 50 μL of a solution containing phosphotungstic acid (40 g/L) and magnesium chloride (0.5 M). The resulting mixture was maintained at room temperature for 10 min and then centrifuged for 20 min to remove sedimented VLDL and LDL. One hundred microliters of a solution containing 4-aminophenazone, phenol, cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7) was added to 25 μL of the centrifuged liquid, and the absorbance of the resulting mixture was measured after 30 min at a wavelength of 546 nm. In accordance with the second procedure, 500 μL of serum was mixed with 25 μL of a solution of dextran sulfate (20 g/L) and 50 μL of a solution of magnesium sulfate (1.1 M). The resulting mixture was maintained at room temperature for 5 min and then centrifuged for 10 min to remove sedimented VLDL and LDL. One hundred microliters of a solution containing 4-aminophenazone, phenol, cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7) was added to 25 μL of the centrifuged liquid, and the absorbance of the resulting mixture was measured after 30 min at 546 nm. The first test sample for quality control was unsuitable for

determining the HDL cholesterol concentration in serum by the former procedure, while the second test sample was unsuitable by the latter procedure. Quality control of HDL cholesterol measurements in serum by both methods could only be made with the third sample [2].

Scottolini et al. studied the concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol in the blood serum of patients in 1980. The total cholesterol concentration was determined by a spectrophotometric procedure with solutions of cholesterol esterase (EC 3.1.1.13) and cholesterol oxidase (EC 1.1.3.6). The HDL cholesterol concentration was found by a procedure including VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II), and the LDL cholesterol concentration was determined by the procedure with the formula of Friedewald et al. [3].

In 1980, Wood et al. compared the measurements of the total cholesterol concentration in blood plasma and serum by the following spectrophotometric procedures:

• A procedure developed by Abell et al. in 1952 (Procedure 1);

• A procedure using iron(III) chloride implemented in an automatic flow device with a capacity of 40 tests per hour (Procedure 2);

• A procedure using Liebermann-Burchard reagent implemented in an automatic flow device with a capacity of 50 tests per hour (Procedure 3).

The discrepancy between measurements of the total cholesterol concentration by the above procedures in freshly drawn serum and plasma samples or in samples stored frozen (–15°C) for no more than one month was less than 5%. The determinations of total cholesterol concentrations by Procedures 1 and 3 in plasma and serum samples stored frozen for 1 to 2 years did not differ. However, the results for the total cholesterol concentrations in plasma and serum samples kept frozen for 1–2 years obtained by Procedure 2 were markedly superior to the results obtained by Procedure 1 [4].

In 1980, Kohl et al. studied the characteristics of the following procedures for determining the HDL cholesterol concentration in blood plasma and serum:

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium(II) (Procedure 1);

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid (Procedure 2);

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with solutions of heparin and manganese(II) (Procedure 3);

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with a solutions of polyethylene glycol (Procedure 4);

• Nonenzymatic (Liebermann–Burchard reagent) spectrophotometric procedure using VLDL and LDL sedimentation with solutions of heparin and manganese(II) (Procedure 5);

• Electrophoretic procedure (Procedure 6).

The mean-square deviation of the relative error of measurement of the HDL cholesterol concentrations in plasma corresponded to 6.5% (Procedure 1), 3.3% (Procedure 2), 14.2% (Procedure 3), 3.7% (Procedure 4), and 2.3% (Procedure 5). The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in serum was at the level of 8 (Procedure 1), 5.2 (Procedure 2), 4.6 (Procedure 3), 5.5 (Procedure 4), and 2.8% (Procedure 5). The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in serum by Procedure 6 was 14.3%. The determinations of the HDL cholesterol concentration in serum obtained by Procedures 1–3 exceeded the Procedure 5 by 111–136 mg/L (0.29–0.35 mM). The correlation coefficient between the results obtained for HDL cholesterol concentrations in 25 serum samples by Procedures 1 and 4 corresponded to 0.95, but those for Procedure 1 were on average 19 mg/L (0.05 mM) higher than those of Procedure 4 [5].

In 1980, Clark and Grooms proposed an amperometric procedure to determine the total cholesterol concentration in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3)).

At a temperature of 50°C, serum was diluted by approximately 13 times with a buffer solution including cholesterol esterase and cholesterol oxidase, and the hydrogen peroxide concentration that formed was measured using a platinum electrode. Some steroids (5α-androstan-3β-ol-17-one, 5-androstene-3β-ol-17-one, 5-androsten-17α-methyl-3β, 17β-diol, and others) presented in serum interfered with the determination of total cholesterol concentrations [6].

In 1980, Baillie et al. studied the characteristics of the following procedures for determining the HDL cholesterol concentration in blood plasma and serum:

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium(II) (Procedure 1);

• Electrophoretic procedure using plates with an agarose gel (Procedure 2);

• Electrophoretic procedure using plates with cellulose acetate (Procedure 3).

The mean-square deviation of the relative error of determinations of HDL cholesterol concentrations of 592 mg/L (1.53 mM) in serum by Procedure 1 was 7.9%; it was 5.4%; for the concentration of 601 mg/L (1.55 mM) by Procedure 2 and 10.1% for the concentration of 586 mg/L (1.52 mM) by Procedure 3 [3]. The mean-square deviation of the relative error of an HDL cholesterol quantifications in blood serum of 379 mg/L (0.96 mM) by Procedure 1 was 9.1%; it was 8.4%, for concentration of 348 mg/L (0.9 mM) by Procedure 2 and 14.9% for 350 mg/L (0.91 mM) by Procedure 3. The measurements of the HDL cholesterol concentration in 97 serum samples according to Procedure 1 were in agreement with the results obtained by Procedure 2 with a correlation coefficient of 0.94 and with the Procedure 3 with a correlation coefficient of 0.87 [7].

Cohen et al. developed a chromatography–mass spectrometric procedure for determining the total cholesterol concentration in the blood serum in 1980. The serum was mixed with 1 mL of a cholesterol-d_7 (cholest-5-ene-25,26,26,26,27,27,27-d_7-3-ol) solution in ethanol. A 0.6-mL portion of an aqueous potassium hydroxide solution (8.9 M) and 4 mL of ethyl alcohol were added to the mixture. The resulting mixture was kept at 37°C for 3 h for the hydrolysis of β cholesterol, yielding α cholesterol. Next, 5 mL of distilled water and 10 mL of hexane were added, the mixture was evaporated, and the precipitate formed was dissolved in 2 mL of methanol. Then, 50 μL of the resulting solution were evaporated again, and the formed precipitate was treated with 50 μL of *N*,*O*-bis(trimethylsilyl)acetamide at room temperature for 0.5 h. The solution (3-μL portion) was injected into a gas chromatograph equipped with a mass spectrometric detector that recorded ions with mass-to-charge ratios (m/z) of 458 and 465 of chemical compounds separated in the analytical column; the total cholesterol concentration in serum was then calculated. The mean-square deviation of the relative error of determinations of total cholesterol concentrations of 3.4298, 4.719, 6.1494, 7.4556, and 8.7881 mM in serum corresponded to 0.37, 0.35, 0.38, 0.34, and 0.36% [8].

In 1980, MacAulay et al. proposed an enzymatic spectrophotometric procedure for determining the total cholesterol concentration in blood serum that was designed for implementation in an automatic flow device with a capacity of 60 tests per hour. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

A flow (0.16 mL/min) of serum was mixed with a flow (1.6 mL/min) of an aqueous solution of surfactant Brij-35 (0.05%). Further, a flow (0.1 mL/min) was separated from the resulting flow; it was mixed with flows of solutions containing 4-aminophenazone, phenol, and said enzymes. The resulting mixture was run into the flow reactor with a temperature of 37°C and was then supplied into the cuvette of a spectrophotometer (the optical path length was 15 mm) that functioned at a wavelength of 505 nm. The absorbance of the mixture was linearly dependent on the total cholesterol concentration in serum up to 4000 mg/L (10.35 mM). The mean-square deviations of the relative error of the total cholesterol determination at concentrations of 1270, 1620, 2020, 2440, and 3460 mg/L (3.28, 4.19, 5.22, 6.31, and 8.95 mM) in serum were 3.68, 2.84, 2.76, 3.96, and 2.57%. The presence of bilirubin and hemoglobin in serum affected the determination of the total cholesterol concentration. In particular, the presence of bilirubin in serum at a concentration of 10 mg/L resulted in an increase in the total cholesterol determination on average by 35 mg/L (0.09 mM) [9].

In 1980, Bullock et al. studied the use of 25 kinds of samples based on horse, bovine, and human serum (supplied by 15 different manufacturers) as quality control samples in determining the HDL cholesterol concentration in blood serum by two enzymatic spectrophotometric procedures. In one procedure, VLDL and LDL sedimentation with a solution of phosphotungstic acid and magnesium chloride was used; in another procedure, solutions of heparin and manganese chloride were applied. The data indicated that, among the studied serum samples, only eight could be used as quality control samples in determining the HDL cholesterol concentration by these procedures [10].

In 1980, Mao and Kottke analyzed the completeness of VLDL and LDL sedimentation by solutions of heparin and manganese chloride in the determination of the HDL cholesterol concentration in blood plasma [11].

In 1980, Smith et al. developed an enzymatic spectrophotometric procedure that uses VLDL and LDL sedimentation with solutions of heparin and manganese(II) for determining the HDL cholesterol concentration in serum. The mean-square deviation of the relative error of determination in serum was 4.3% for the HDL cholesterol concentration of 114 mg/L (0.29 mM) and 1.8% and for the concentration of 600 mg/L (1.55 mM). The HDL cholesterol determinations in 25 serum samples by the developed procedure were in agreement with results obtained by the enzymatic spectrophotometric procedure proposed by Ash and Hentschel in 1978; the correlation coefficient was 0.99 [12].

In 1980, Srinivasan et al. investigated the cholesterol concentrations (including HDL and LDL) in serum lipoprotein fractions of patients aged 5 to 17 years. In this research, the procedures based on VLDL and LDL sedimentation with solutions of heparin and calcium chloride and solutions of heparin and manganese chloride were used [13].

In 1980, Liedtke et al. proposed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood plasma and serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

Initially, serum or plasma was treated with solutions of heparin (500 mg/L) and manganese(II) (1 g/L), and the resulting mixture was centrifuged to remove sedimented VLDL and LDL. Two hundred and fifty microliters of a phosphate buffer solution (pH 7.24) containing 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase was added to 10 μL of the centrifuged liquid at 37°C; after 12 min, the absorbance of the resulting mixture was measured at 500 and 600 nm. The mean-square deviation of the relative error of the HDL cholesterol measurements of 450 mg/L (1.16 mM) in serum did not exceed 3% [14].

In 1980, Demacker et al. proposed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood serum. Two hundred microliters of an aqueous solution of polyethylene glycol (450 g/L) was added to 1 mL of serum. The resulting mixture was maintained at room temperature for 15 min and then centrifuged for 15 min to remove sedimented VLDL and LDL. Then, the HDL cholesterol concentration was measured in 50 μL of the centrifuged liquid by a procedure using solutions of cholesterol oxidase (EC 1.1.3.6) and catalase (EC 1.11.1.6). The mean-square deviation of the relative error of the determination of HDL cholesterol concentrations of 2, 2.5, 3.3, 3.8, and 5.5 mM in serum corresponded to 2.2, 3.3, 3.4, 1.8, and 2.7%. The presence of ethylenediaminetetraacetate (up to 1 g/L) in serum resulted in a decrease in the measurements of the HDL cholesterol concentration by 0.04–0.06 mM, and the presence of lithium heparin (up to 0.5 g/L) and triglycerides (up to 5.5 mM) had no effect on the determinations. Measurements of the HDL cholesterol concentration in serum samples by the proposed procedure were in agreement with the results obtained by a procedure involving ultracentrifugation; the correlation coefficient was 0.99. In addition, measurement of the HDL cholesterol concentration in the centrifuged liquid by a spectrophotometric procedure using a solution containing 4-aminophenazone, phenol, cholesterol esterase (EC 3.1.1.13), cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7) or by a spectrophotometric procedure using Liebermann–Burchard reagent instead of a procedure with cholesterol oxidase and catalase practically did not alter the determinations of HDL cholesterol in serum [15].

In 1980, Demacker et al. compared the determination of the HDL cholesterol concentration in blood serum samples by the following procedures:

• Two procedures using VLDL and LDL sedimentation with solutions of sodium heparin and manganese chloride (Procedures 1 and 2);

• A procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride (Procedure 3);

• A procedure using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium chloride (Procedure 4);

• A procedure using VLDL and LDL sedimentation with solutions of polyethylene glycol (Procedure 5);

• A procedure using ultracentrifugation (Procedure 6).

In Procedures 1–5, the HDL cholesterol concentration remaining in the liquid after centrifugation was measured by the spectrophotometric procedure using solutions of cholesterol oxidase (EC 1.1.3.6) and catalase (EC 1.11.1.6) or the spectrophotometric procedure using a solution containing 4-aminophenazone, phenol, cholesterol esterase (EC 3.1.1.13), cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7). When the spectrophotometric procedure with cholesterol oxidase and catalase was used, the correlation coefficient between the results for the HDL cholesterol concentrations in 32 serum samples with the triglyceride concentration of 2.5 mM was estimated at 0.98 according to Procedures 1 and 6; 0.9 according to Procedures 2 and 6; 0.98 according to Procedures 3 and 6; and 0.99 according to Procedures 4 and 6, as well as 5 and 6. For the spectrophotometric procedure using a solution containing 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase, the correlation coefficient between the measurements of HDL cholesterol in 32 serum samples with a triglyceride concentration of up to 2.5 mM was 0.99 according to Procedures 1 and 6, 2 and 6, 4 and 6, and 5 and 6 and was 0.98 according to Procedures 3 and 6. When a spectrophotometric procedure using cholesterol oxidase and catalase was used, the correlation coefficient between the results obtained for HDL cholesterol in 22 serum samples with the triglyceride concentration from 2.5 to 18.5 mM corresponded to 0.96 by Procedures 1 and 6; 0.92 according to Procedures 2 and 6; 0.97 by Procedures 3 and 6; 0.95 according to Procedures 4 and 6; and 0.98 according to Procedures 5 and 6 [16].

In 1980, Tallet et al. developed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

Serum (0.1 mL) was mixed with 2 mL of a solution containing heparin (150 kIU/L), and magnesium chloride (20 mM), and albumin (10 g/L). The resulting mixture was maintained at 20°C for 15 min and then centrifuged at 4°C for 20 min to remove sedimented VLDL and LDL. The HDL cholesterol concentration in the centrifuged liquid was determined by a spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase, implemented on an automatic flow device. The absorbance of the solution of the formed colored compound was directly proportional to the HDL cholesterol concentration in the serum up to 2.5 mM. The mean-square deviation of the relative error was 2.9% for the HDL cholesterol concentration of 1.04 mM in serum and 5% for the concentration of 1.2 mM. The presence of triglycerides in serum did not affect the measurement results. The determinations of the HDL cholesterol concentration in serum samples according to the developed procedure correlated with the measurements by the procedure with VLDL and LDL sedimentation by solutions of heparin and manganese(II) (the correlation coefficient was 0.98; 100 samples) and by the procedure with VLDL and LDL sedimentation by solutions of phosphotungstic acid and magnesium(II) (the correlation coefficient was 0.98; 82 samples). However, the determinations of the HDL cholesterol concentration in serum by the developed procedure were 5% below the measurement results obtained by the procedure with VLDL and LDL sedimentation by solution of heparin and manganese(II) on average and 9% below by the procedure with VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium(II) [17].

In 1980, Brooks and Smith discussed issues related to the selectivity of oxidation of α cholesterol by the catalytic action of cholesterol oxidase (Eq. (3)) [18].

In 1980, Clark and Grooms also considered some aspects affecting the selectivity of oxidation of α cholesterol by the catalytic action of cholesterol oxidase with respect to amperometric procedures [19].

In 1981, Warnick et al. studied the use of 14 kinds of samples based on human plasma or serum as quality control samples in the determination of the HDL cholesterol concentration in plasma and serum by the following procedures:

• A procedure using VLDL and LDL sedimentation with solutions of heparin and manganese chloride (Procedure 1);

• A procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride (Procedure 2).

The mean-square deviations of the relative error of determination of the HDL cholesterol concentration in test plasma and serum samples by the above procedures are presented in Table 1. It is easy to notice

Table 1. Mean-square deviations of the relative error (RSD) of determination of HDL cholesterol in blood plasma and serum samples

Sample of blood plasma or serum	RSD of determination of HDL cholesterol, %		Sample of blood plasma or serum	RSD of determination of HDL cholesterol, %	
	Procedure 1	Procedure 2		Procedure 1	Procedure 2
1	3.64	3.42	8	3.93	3.64
2	4.17	3.37	9	21.68	6.53
3	5.91	6.07	10	18.88	6.85
4	4.59	4.61	11	20.98	10.47
5	6.57	5.59	12	17.68	17.28
6	4.48	1.95	13	6.32	11.31
7	14.18	27.0	14	3.80	8.92

that only samples 1, 2, 4, 6, and 8 could be suitable for quality control (the standard deviation of the relative error is less than 5%) in determining the HDL cholesterol concentration in plasma and serum according to Procedures 1 and 2 [20].

In 1981, Schifman et al. compared the measurements of the HDL cholesterol concentration in blood plasma and serum by the following procedures:

• Electrophoretic procedure using plates with cellulose acetate (Procedure 1).

• Enzymatic spectrophotometric procedure using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II) (Procedure 2).

The mean-square deviation of the relative error of the determination of the HDL cholesterol concentration of 130–180 mg/L (0.34–0.47 mM) in serum was 16.7 (Procedure 1) and 6.7% (Procedure 2); for the concentration of 320–390 mg/L (0.83–1.01 mM), it was 8 (Procedure 1) and 3.9% (Procedure 2). The correlation coefficient between the determinations of the HDL cholesterol concentration in 99 blood serum samples according to Procedures 1 and 2 was 0.8925. Schifman et al. concluded that the use of the electrophoretic procedure in measuring the HDL cholesterol concentration in serum samples was inappropriate [21].

In 1981, Weisweiler et al. developed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in serum for their medical studies. The procedure involved VLDL and LDL sedimentation with solutions of heparin and manganese(II) [22].

In 1981, Telesforo et al. applied a procedure for determining the HDL cholesterol concentration in plasma serum with VLDL and LDL sedimentation with solutions phosphotungstic acid and magnesium(II) in their medical studies [23].

In 1981, Izzo et al. proposed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 2,4-dichlorophenol sulfonate to form a colored compound (Product) under the catalytic action of peroxidase (EC 1.11.1.7)

$$2H_2O_2 + \text{4-aminophenazone} + \text{DCPS} \xrightarrow{\text{Peroxidase}} \text{Product} + 4H_2O \quad (8)$$

where DCPS is 2,4-dichlorophenol sulfonate.

Serum (200 μL) was mixed with 200 μL of a buffer solution (glycine–sodium hydroxide, 200 mM, pH 10) containing polyethylene glycol (200 g/L). The resulting mixture was maintained at 20–25°C for 10 min and then centrifuged at 4°C for 20 min to remove sedimented VLDL and LDL. The HDL cholesterol concentration in the centrifuged liquid was determined at 515 nm after the addition of a solution containing 4-aminophenazone, 2,4-dichlorophenol sulfonate, cholesterol esterase, cholesterol oxidase, and peroxidase. The mean-square deviation of the relative error of the determinations in serum was estimated to be 3.6% for the HDL concentration of 269 mg/L (0.7 mM), 3% for the concentration of 436 mg/L (1.13 mM), and 1.9% for the concentration of 641 mg/L (1.66 mM). The presence of triglycerides (up to

Table 2. Effect of serum components on the determination of HDL cholesterol

Serum component	Component concentration, mg/L	HDL cholesterol concentration, mg/L (mM)	Serum component	Component concentration, mg/L	HDL cholesterol concentration, mg/L (mM)
Without addition of components	0	511 (1.32)	3,4-Dihydroxy-phenylacetic acid	5	503 (1.30)
Sodium citrate	4000	517 (1.34)		10	490 (1.27)
Sodium oxalate	2000	517 (1.34)	α-Methyldopa	10	503 (1.30)
Sodium fluoride	2000	513 (1.33)		50	468 (1.21)
Sodium ethylenedi-aminetetraacetate	2000	510 (1.32)	Gentisic acid	30	504 (1.30)
Sodium heparin	2000	509 (1.32)		50	492 (1.27)
Urea	2000	513 (1.33)	Sodium metamizole	50	503 (1.30)
Creatinine	100	511 (1.32)		100	490 (1.27)
Uric acid	200	507 (1.31)	Bilirubin	50	516 (1.33)
Glucose	4000	512 (1.32)		100	518 (1.34)
Reduced glutathione	200	504 (1.30)		150	523 (1.35)
Acetylsalicylic acid	800	511 (1.32)	Ascorbic acid	20	485 (1.25)
L-Dopa	5	504 (1.30)		50	442 (1.14)
	10	495 (1.28)	–	–	–

10.68 g/L) did not affect the measurements of the HDL cholesterol concentration. The measurements of the HDL cholesterol concentration in 62 serum samples according to the proposed procedure were in agreement with the results obtained by the procedure using ultracentrifugation (the correlation coefficient was 0.957) and by the procedure involving VLDL and LDL sedimentation with solutions of heparin and manganese(II) (the correlation coefficient was 0.998) [24].

In 1981, Grillo et al. developed five enzymatic spectrophotometric procedures for determining the HDL cholesterol concentration in blood serum. The procedures were based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 2,4-dichlorophenol sulfonate to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (8)).

In all of the procedures, VLDL and LDL were sedimented from serum. In Procedure 1, 200 μL of serum was mixed with 200 μL of a polyethylene glycol (200 g/L) solution in glycine buffer (200 mM, pH 10). The resulting mixture was maintained at 20–25°C for 10 min and centrifuged for 10 min. According to Procedure 2, 500 μL of serum was mixed with 50 μL of a solution containing heparin (2000 kIU/L) and manganese chloride (505 mM). The resulting mixture was maintained at 20–25°C for 15 min and centrifuged at 4°C for 30 min. In Procedure 3, 500 μL of serum was mixed with 50 μL of a solution containing heparin (2000 kIU/L) and manganese chloride (1010 mM). The mixture was maintained in at 20–25°C for 15 min and centrifuged at 4°C for 30 min. According to Procedure 4, 500 μL of serum was mixed with 50 μL of a solution containing sodium phosphotungstate (40 g/L) and magnesium chloride (500 mM). The resulting mixture was maintained at 20–25°C for 15 min and centrifuged at 4°C for 30 min. In Procedure 5, 500 μL of serum was mixed with 50 μL of a solution containing dextran sulfate (10 g/L) and magnesium chloride (1 M). The mixture was maintained at 20–25°C for 15 min and centrifuged at 4°C for 30 min. Then, 2 mL of an aqueous solution containing phosphate buffer (150 mM), ethylenediaminetetraacetate (8 mM), 4-aminophenazone (495 μM), 2,4-dichlorophenol sulfonate (3 mM), cholesterol esterase (36 IU/L), cholesterol oxidase (27 IU/L), and peroxidase (13 kIU/L) was added to 25 (Procedure 1) or 13 μL (Procedures 2–5) of the centrifuged liquid. The resulting mixture was kept at 20–25°C for 15 min; then its absorbance was measured at 515 nm. The absorbance of the resulting mixture was directly proportional to the HDL cholesterol concentration in serum from 100 to 2000 mg/L (0.26 to

5.17 mM). For Procedure 1, the mean-square deviation of the relative error of the quantification of HDL cholesterol in blood serum of 276 mg/L (0.71 mM) was 3.1%; it was 2% for 444 mg/L (1.15 mM) and 1.8% for 646 mg/L (1.67 mM). The effect of possible serum components on the measurement results of the HDL cholesterol concentration by Procedure 1 is illustrated in Table 2. The determination of the HDL cholesterol concentration in 70 serum samples by the proposed procedures correlated with measurements by the spectrophotometric procedure using Liebermann–Burchard reagent with correlation coefficients of 0.9928 (Procedure 1), 0.9952 (Procedure 3), 0.9955 (Procedure 4), and 0.9978 (Procedure 5). The correlation coefficients between the determination of the HDL cholesterol concentration in 70 serum samples were estimated to be 0.9892 by Procedures 1 and 3; 0.9871 by Procedures 1 and 4; and 0.9945 by Procedures 1 and 5 [25].

In 1981, Levy pointed out the importance of determination of the concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol in blood plasma and serum by procedures using ultracentrifugation or by electrophoretic procedures. However, it was mentioned that procedures with ultracentrifugation were labor-intensive and rather expensive, while electrophoretic procedures enabled mainly qualitative rather than quantitative measurements [26].

In 1981, Seigler and Wu proposed an enzymatic procedure for determining the HDL cholesterol concentration in plasma and serum with VLDL and LDL sedimentation with solutions of sodium phosphotungstate and magnesium chloride. According to the first procedure, 500 μL of plasma or serum was mixed with 50 μL of a solution containing the phosphotungstic salt (40 g/L) and 50 μL of magnesium chloride (0.5 M). The mixture was centrifuged for 15 min, the centrifuged liquid was mixed with a reactive solution, and the absorbance of the new mixture was measured at a wavelength of 500 nm. The absorbance of the mixture was linearly dependent on the HDL cholesterol concentration in plasma or serum up to 1150 mg/L (2.97 mM). The addition of a solution of sodium phosphotungstate and magnesium chloride independently to plasma or serum caused more complete VLDL and LDL sedimentation. The mean-square deviation of the relative error of measurements of the HDL cholesterol concentration of 364 mg/L (0.94 mM) in serum corresponded to 4%. The determinations of LDL cholesterol in 54 serum samples by the proposed procedure were in agreement with the results obtained by the procedure involving ultracentrifugation; the correlation coefficient was 0.97 [27].

In 1981, Bachorik et al. assessed the the determination of total and HDL cholesterol in blood plasma samples obtained in 12 clinical diagnostic laboratories. The total cholesterol concentration in plasma was measured by spectrophotometric procedures using Liebermann–Burchard reagent or a solution of iron(III) chloride. For the determination of HDL cholesterol, a procedure with VLDL and LDL sedimentation with solutions of heparin and manganese chloride were used. The mean-square deviation of the relative error of the determination of total cholesterol of 2000 mg/L (5.17 mM) in plasma was 2.1% by procedures with Liebermann–Burchard reagent and 2.5% by procedures involving iron(III) chloride [28].

In 1981, Yamaguchi et al. developed an enzymatic spectrophotometric procedure for determining the concentration of β cholesterol in blood serum with preliminary removal of α cholesterol. To remove α cholesterol from serum, the following reactions were used:

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide and phenol under the catalytic action of peroxidase (EC 1.11.1.7).

The concentration of β cholesterol remaining in serum was measured by the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

One milliliter of a phosphate buffer solution (100 mM; pH 7) containing phenol (400 mg/L), surfactant Triton X-100 (0.1%), cholesterol oxidase (1 kIU/L), and peroxidase (40 kIU/L) was added to 25 μL of serum. The resulting mixture was maintained at a temperature of 37°C for 20 min. Then, 1 mL of a solution containing 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase was added to the mixture. The resulting mixture was kept at 37°C for 15 min; its absorbance was then measured at a wavelength of 500 nm. The mean-square deviation of the relative error of determination of the β cholesterol concentration in serum of 1025 mg/L (2.65 mM) was 3.1%. The presence of α cholesterol (up to 3000 mg/L (7.76 mM)), bilirubin, and ascorbic acid in blood serum did not affect the measurements of β

cholesterol. The determinations of β cholesterol in serum samples by the proposed procedure were in agreement with the results obtained by the procedure, in that the concentration of β cholesterol was calculated as the difference between measured concentrations of total cholesterol and α cholesterol; the correlation coefficient was 0.98 [29].

In 1981, Clark et al. proposed an enzymatic amperometric procedure for determining the total cholesterol concentration in blood plasma and serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3)).

Twenty-five microliters of a solution containing cholesterol esterase (40 kIU/L) and cholesterol oxidase (60 kIU/L) and 10 μL of plasma or serum were added at 50°C to a buffer solution (pH 7.25) containing Na_2HPO_4 (7.05 g/L), NaH_2PO_4 (1.5 g/L), sodium chloride (2.85 g/L), sodium benzoate (0.9 g/L), sodium ethylenediaminetetraacetate (0.6 g/L), sodium azide (0.45 g/L), and sodium cholate (1.65 g/L). The emerging hydrogen peroxide diffused through membranes made of polycarbonate and cellulose acetate films to an electrochemical sensor equipped with a platinum anode and a silver cathode, the voltage between which was 700 mV. The sensor output signal (electric current) was proportional to the total cholesterol concentration in plasma and serum. The membrane protected the electrochemical sensor against the penetration of bilirubin, uric acid, and ascorbic acid, which ensured the selectivity of measurements. The measurements of the total cholesterol concentration in 105 serum samples and 105 plasma samples by the proposed procedure correlated with measurements by the spectrophotometric procedure using Liebermann–Burchard reagent; the correlation coefficient was 0.9994 for serum and 0.9997 for plasma [30].

In 1981, Sklov et al. reported of the determination of total cholesterol in blood serum samples taken from 4000 children. The total cholesterol concentration in serum was measured by the spectrophotometric procedures using Liebermann–Burchard reagent and implemented in an automatic flow device [31].

Wilson et al. studied the relationship of concentrations of VLDL cholesterol and triglycerides in the blood plasma of patients in 1981. Overall, the the study were consistent with data obtained by Friedewald et al. in 1972 [32].

In 1981, Avigad and Robertson developed an enzymatic spectrophotometric procedure for determining the concentration of total cholesterol, α cholesterol, and β cholesterol in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Decomposition of hydrogen peroxide upon the catalytic action of NADH peroxidase (EC 1.11.1.1).

$$2H_2O_2 + NADH + H^+ \xrightarrow{\text{NADH peroxidase}} 2H_2O + NAD^+ \quad (9)$$

where NADH and NAD^+ are, respectively, reduced and oxidized nicotinamide adenine dinucleotide.

Twenty-five microliters of serum and 10 μL of a solution of cholesterol oxidase (25 kIU/L) were added to 855 mL of phosphate buffer (100 mM, pH 6.6) containing sodium azide (5 mM), sodium acetate (50 mM), ethylenediaminetetraacetate (10 mM), surfactant Triton X-100 (0.5%), and albumin (1 g/L). The resulting mixture was maintained at a temperature of 37°C for 5 min. Next, 100 μL of a solution of reduced nicotinamide adenine dinucleotide (0.28 mM) was added, and, 5 min later, the absorbance of the mixture (A_0) was measured at 340 nm (which is indicative of the transition of reduced nicotinamide adenine dinucleotide into oxidized nicotinamide adenine dinucleotide). Next, 10 μL of a solution of NADH peroxidase (40 kIU/L) was added; after 5–6 min, the absorbance of the resulting solution (A_1) was measured at 340 nm. Then, 10 μL of a solution of cholesterol esterase (50 kIU/L) was added, and the absorbance of the resulting solution (A_2) was determined at 340 nm after 7–8 min. The difference in absorbances $\Delta A_\alpha = A_0 - A_1$ is proportional to the concentration of α cholesterol; the difference $\Delta A_\beta = A_1 - A_2$ is proportional to the concentration of β cholesterol; and the difference $\Delta A = A_0 - A_2$ is proportional to the total cholesterol concentration in blood serum. The measurements of the total cholesterol concentration in 63 serum samples according to the developed procedure were in agreement with those obtained by

Table 3. Determination of the total cholesterol concentration in five serum samples

Procedure	Total cholesterol concentration, mM				
	Serum 1	Serum 2	Serum 3	Serum 4	Serum 5
1	3.39	4.68	6.11	7.35	8.59
	3.39	4.73	6.15	7.57	8.85
2	3.43	4.72	6.15	7.47	8.79

the spectrophotometric procedure using Liebermann–Burchard reagent; the correlation coefficient was 0.984 [33].

In 1981, Avigad and Robertson proposed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood serum. The procedure was also based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Decomposition of hydrogen peroxide upon the catalytic action of NADH-peroxidase (EC 1.11.1.1) (Eq. (9)).

Serum was mixed with solutions of heparin and manganese chloride and then centrifuged to remove sedimented VLDL and LDL. One hundred microliters of the centrifuged liquid and 10 μL of a solution of cholesterol oxidase (25 kIU/L) were added to 780 mL of phosphate buffer (100 mM, pH 6.6) containing sodium azide (5 mM), sodium acetate (50 mM), ethylenediaminetetraacetate (10 mM), surfactant Triton X-100 (0.5%), and albumin (1 g/L). The resulting mixture was kept at a temperature of 37°C for 5 min. Next, 100 μL of a solution of reduced nicotinamide adenine dinucleotide (0.28 mM) was added, and the absorbance of the mixture (A_0) was measured at 340 nm5 min later. Then, 10 μL of a NADH peroxidase solution (40 kME/L) was added, and 10 μL of a solution of cholesterol esterase (50 kIU/L) was poured into the mixture after 5–6 min. After 7–8 min, the absorbance of the resulting mixture was measured (A_1) at a wavelength of 340 nm. The difference of absorbances $\Delta A = A_0 - A_1$ was directly proportional to the HDL cholesterol concentration in serum [33].

In 1982, Schaffer et al. compared the determination of the total cholesterol concentration in five blood serum samples obtained by two chromatography–mass spectrometric procedures (Table 3) [34].

In 1982, Demacker et al. studied the properties of samples intended for quality control of measurements of the HDL cholesterol concentration in serum. In the study, they used a spectrophotometric procedure for determining the HDL cholesterol concentration in serum with VLDL and LDL sedimentation with solutions of heparin and manganese(II), followed by the treatment of centrifuged liquid or ultrafiltrate by solutions of cholesterol oxidase (EC 1.1.3.6) and catalase (EC 1.11.1.6) [35].

In 1982, Grillo and Murador assessed the effect of bilirubin on the determination of the HDL cholesterol concentration in blood serum by the spectrophotometric procedure based on the following reactions [36]:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 2,4-dichlorophenol sulfonate to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (8)).

In 1982, Moshides pointed out the need to use surfactants (for example, Triton X-100) in the reaction of hydrolysis of β cholesterol, yielding α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (reaction (5)) in procedures for determining the concentrations of total cholesterol, HDL cholesterol, and LDL cholesterol in serum and plasma [37].

In 1982, Kohlmeier and Schlierf investigated a change in the HDL cholesterol concentration in blood plasma upon storage in the frozen state. In the study, a spectrophotometric procedure was used. It involved LDL and VLDL sedimentation with solutions of phosphotungstic acid and magnesium chloride, followed by the treatment of the centrifuged liquid with solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase [38].

Table 4. Effect of serum components on the determination of total cholesterol

Serum component	Component concentration	Change in the measurement results of total cholesterol concentration, mg/L (mM)	Serum component	Component concentration	Change in the measurements of total cholesterol concentration, mg/L (mM)
1	2	3	4	5	6
L-Dopa	6 mg/L	−11.4 (−0.03)	*p*-Aminosalicylic acid	230 mg/L	+0.4 (+0.0)
Uric acid	150 mg/L	−37.6 (−0.10)	Dextran	10 g/L	+82.4 (+0.21)
Ascorbic acid	30 mg/L	+13.3 (+0.03)	Gentisic acid	5 mg/L	+5.0 (+0.01)
Tyrosine	240 mg/L	+21.0 (+0.05)	Isoniazid	4 mg/L	+9.8 (+0.03)
Phospholipids	4 g/L	−12.9 (−0.03)	Iodide	2 mM	−8.9 (−0.02)
Hemoglobin	500 mg/L	−37.5 (−0.10)	Glutathione	10 mg/L	+20.4 (+0.05)
Proteins	100 g/L	−183.0 (−0.47)	Chlorothiazide	30 mg/L	+9.0 (+0.02)
Salicylic acid	350 mg/L	+2.4 (+0.01)	6-Mercaptopurine	15 mg/L	+13.5 (+0.03)
Glucose	6 g/L	+6.2 (+0.02)	Sulfathiazole	60 mg/L	+26.9 (+0.07)
Urea	1 g/L	−25.5 (−0.07)	Bilirubin	200 mg/L	+9.8 (+0.03)
Triglycerides	8 g/L	+362.8 (+0.94)	Heparin	8 kIU/L	+22.9 (+0.06)
Ethyl alcohol	3 g/L	−69.1 (−0.18)	pH	6.8	+16.8 (+0.04)
Acetylsalicylic acid	300 mg/L	−10.8 (−0.03)		8.8	−10.1 (−0.03)
Acetaminophen	50 mg/L	−65.1 (−0.17)	–	–	–

In 1982, Rubiés-Prat et al. determined the concentrations of total cholesterol and HDL cholesterol in blood serum samples of patients suffering from liver disease. The total cholesterol concentration in serum was measured by an enzymatic procedure, and the HDL cholesterol concentration was measured by the procedure with VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium(II) [39].

In 1982, Katan et al. studied the characteristics of the spectrophotometric procedure for determining the total cholesterol concentration in blood serum, using Liebermann–Burchard reagent. The mean-square deviation of the relative error of measurements of the total cholesterol concentration of 2.6–10.3 mM in serum did not exceed 1–2%. The relative systematic error of the determination of total cholesterol from 5.2 to 7.8 mM in serum was 1%; it was 2% for the concentration from 7.8 to 10.3 mM [40].

In 1982, Dappen et al. developed an enzymatic reflectance photometry procedure using test strips for the determination of the total cholesterol concentration in serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide and a triarylimidazol dye to form a colored compound (Product) under the catalytic action of peroxidase (EC 1.11.1.7)

$$H_2O_2 + \text{Triarylimisazol dye} \xrightarrow{\text{Peroxidase}} \text{Product} + 2H_2O.$$

The test strips were composed of a transparent substrate coated with a layer of gelatin (pH 6.25) and a porous polymeric layer containing barium sulfate, cellulose acetate, Triton X-100, KH_2PO_4, a triarylimidazol dye, cholesterol esterase, cholesterol oxidase, and peroxidase. Ten microliters of serum was applied to the porous layer. The test strip was placed into a specialized measuring device at 37°C; after 5 min, it was measured at a wavelength of 540 nm by the reflectance of its substrate. The device output signal was linearly dependent on the total cholesterol concentration in blood serum up to 5500 mg/L (14.22 mM). The mean-square deviation of the relative error of the total cholesterol quantification in blood serum of 1030 mg/L (2.66 mM) was 2.3%; it was 1.2% for the concentration of 1680 mg/L (4.34 mM) and 1.74%

for 3640 mg/L (9.41 mM). The effect of blood serum components on the measurement of the total cholesterol concentration (2480 mg/L (6.41 mM)) is demonstrated in Table 4. The measurements of the total cholesterol concentration in 120 serum samples according to the developed procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent; the correlation coefficient was 0.974 [41].

In 1982, Nanji used a spectrophotometric procedure for determining the total cholesterol concentration in plasma his medical studies. The procedure involved the use of Liebermann–Burchard reagent and VLDL and LDL sedimentation with solutions of heparin and manganese(II) [42].

In 1982, Warnick et al. proposed two spectrophotometric procedures for determining the HDL cholesterol concentration in blood plasma and serum. One hundred microliters of a solution containing dextran sulfate (10 g/L) and magnesium chloride (500 M) was added to 1 mL of plasma or serum. The resulting mixture was maintained at room temperature for 10 min and then centrifuged at 4°C for 10 min to remove sedimented VLDL and LDL. The HDL cholesterol concentration in the centrifuged liquid was determined by Procedure 1 with the use of Liebermann–Burchard reagent and by Procedure 2 with the use of solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase. The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in plasma and serum by Procedure 1 was 2.9 and 2.6%; it was 3.6 and 3.9% by Procedure 2. The correlation coefficient between the measurements of the HDL cholesterol concentration in 42 blood serum samples according to Procedures 1 and 2 was 0.989. In addition, the measurements of the HDL cholesterol concentration in 199 plasma samples according to Procedure 1 were in agreement with the results obtained by the spectrophotometric procedure using VLDL and LDL sedimentation with solutions of heparin and manganese(II), followed by treatment of the centrifuged liquid by Liebermann–Burchard reagent; the correlation coefficient was 0.98 [43].

In 1982, Benizen et al. developed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration and the sum concentration of VLDL and LDL cholesterol in blood plasma and serum. They used a separation column 65 × 8 mm, packed with heparin–agarose, which let HDL pass but retained VLDL and LDL. Plasma or serum (0.5 mL) passed through the column and was collected in vessel 1. Next, the column was washed with 2 mL of sodium chloride (70–150 mM), which were added to the content of the vessel. Then, 3 mL of sodium chloride solution (1 M) was passed through the column at a flow rate of 1 mL/min; the eluate was collected in vessel 2. The solutions from vessels 1 and 2 (each 200 μL in volume) were treated with 1.8 mL of a solution containing enzymes, and spectrophotometric measurements of, respectively, the HDL cholesterol concentration and the sum concentration of VLDL and LDL cholesterol in plasma or serum, were performed. The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in serum of 445 mg/L (1.15 mM) was 2.33%; it was 3.7% for the total concentration of VLDL and LDL cholesterol of 847 mg/L (2.19 mM). The time required for the separation of HDL, VLDL, and LDL was 30–40 min. The measurements of the HDL cholesterol concentration and the sum concentration of VLDL and LDL cholesterol in 58 samples of plasma and serum by the proposed procedure were in agreement with the results obtained by the procedure involving ultracentrifugation; the correlation coefficient was 0.91. Moreover, the correlation coefficient between the results obtained for HDL cholesterol in 22 serum samples using the developed procedure and by the procedure with the sedimentation of LDL and VLDL with solutions of phosphotungstic acid and magnesium(II) was estimated at 0.99 [44].

In 1982, Murai et al. developed an enzymatic procedure for determining the HDL cholesterol concentration in serum in examination of their patients. The procedure involved VLDL and LDL sedimentation with solutions of heparin and calcium chloride [45].

In 1982, Webb et al. proposed a chromatographic procedure for determining the total cholesterol concentration in blood serum. Fifty microliters of serum was added to 2 mL of a solution of potassium hydroxide in ethanol. The resulting mixture was maintained in a water bath at 75°C for 30 min to transform β cholesterol into α cholesterol. After cooling to room temperature, 2 mL of distilled water and 5 mL of hexane were added to the mixture. The formed mixture was stirred for 15 min and centrifuged for 5 min. Next, 3 mL were of the top (hexane) layer were collected; the solvent was evaporated under nitrogen. The precipitate was dissolved in 0.85 mL of isopropanol, 50 μL of which were injected into a liquid chromatograph. A mixture of acetonitrile (50%) and isopropyl alcohol (50%) served as an eluent. The eluent flow rate was 2 mL/min. A spectrophotometric detector operating at a wavelength of 205 nm recorded the peaks of separated compounds. The retention time of corresponded to 4.23 min for vitamins A and D; 4.53 min for ergosterol; 4.67 min for desmosterol; 4.77 min for 7-dehydrocholesterol; 5.1 min for lanosterol; 5.22 min for fucosterol; 5.6 min for α cholesterol; and 5.88 min for campesterol. The peak area of α cholesterol was directly proportional to the total cholesterol concentration in serum up to 5000 mg/L

(12.93 mM). The mean-square deviation of the relative error of determination of total cholesterol at a concentration of 1040, 1360, 1440, 1920, 2100, and 2910 mg/L (2.69, 3.52, 3.72, 4.97, 5.43, and 7.53 mM) in serum was 3, 2.6, 3.1, 1.6, 2.3, and 1.7%. The results of measurements of the total cholesterol concentration in eight serum samples according to the developed procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent; the correlation coefficient was 0.998 [46].

In 1982, Rehak and Young developed an enzymatic calorimetric procedure for determining the total cholesterol concentration and α cholesterol in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Decomposition of hydrogen peroxide form water and oxygen under the catalytic action of catalase (EC 1.11.1.6) (Eq. (4)).

When determining α cholesterol, 0.1 mL of serum was diluted 12 times with a phosphate buffer solution containing sodium cholate, cholesterol oxidase, and catalase. Then, a change in the temperature of the resulting mixture that was directly proportional to the concentration of α cholesterol in serum up to 4400 mg/L (11.38 mM) was measured. In determining the concentration of total cholesterol, 0.1 mL of serum was diluted 22-fold with a phosphate buffer solution containing sodium cholate, cholesterol esterase, cholesterol oxidase, and catalase, and a change in the temperature of the mixtures was measured. The change in mixture temperature was directly proportional to the total cholesterol concentration in serum up to 5500 mg/L (14.22 mM). The determinations of total cholesterol in serum samples by the proposed procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent (the correlation coefficient was 0.995; 39 samples) and by spectrophotometric procedures implemented in an automated flow device (the correlation coefficient was 0.997; 84 samples) [47].

In 1983, Srinivasan et al. determined the concentrations of total cholesterol and HDL, VLDL, and LDL cholesterol in blood serum samples of children. In the study, they applied the spectrophotometric procedure using Liebermann-Burchard reagent, the procedure with LDL and VLDL sedimentation with solutions of heparin and calcium chloride, and the electrophoretic procedure using ultracentrifugation [48]. In 1983, Folsom et al. determined the concentrations of total cholesterol and HDL cholesterol in blood plasma and serum. The total cholesterol concentration in plasma and serum was measured by the spectrophotometric procedure using Liebermann–Burchard reagent, and the HDL cholesterol concentration was determined by the procedure with VLDL and LDL sedimentation with solutions of heparin and manganese chloride. Folsom et al. found that the total cholesterol concentration in serum was on average 2.1% higher than its plasma concentration, while the HDL cholesterol concentration in plasma and serum did not differ [49].

In 1983, Warnick et al. reported on the determination of total and HDL cholesterol in blood plasma and serum samples obtained in 10 clinical diagnostic laboratories. All of the laboratories used enzymatic procedures to measure the total cholesterol concentration in plasma and serum. In determining the concentration of HDL cholesterol, five laboratories used procedures with VLDL and LDL sedimentation with solution of heparin and manganese(II); three laboratories applied procedures involving VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II); and two laboratories dealt with procedures with VLDL and LDL sedimentation with solution of phosphotungstic acid and magnesium(II) [50].

In 1983, Demacker and Jansen investigated a change in the HDL cholesterol concentration in blood serum upon storage in the frozen state (–20°C). In their study, the HDL cholesterol concentration in the serum samples was determined by the procedure involving VLDL and LDL sedimentation with a solution of polyethylene glycol [51].

In 1983, Brown et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips for the determination of the total cholesterol concentration in serum. One hundred microliters of serum was diluted nine times, and 30 μL of the formed solution was applied to the surface of a test strip including cholesterol esterase (EC 3.1.1.13), cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7). The test strip was placed into a specialized measuring device, and after 135 s, its reflectance was measured at a wavelength of 600 nm. The output signal of the device was linearly dependent on the total cholesterol concentration in blood serum from 500 to 4500 mg/L (from 1.29 to 11.64 mM).

The mean-square deviation of the relative error of determination of the total cholesterol concentration of 1617.5 mg/L (4.18 mM) in serum corresponded to 3.6%, and for the concentration of 2620.5 mg/L (6.78 mM), it was 3.11%. The determinations of total cholesterol in 100 serum samples by the studied procedure were in agreement with the results obtained by the enzymatic spectrophotometric procedure proposed by Allain et al. in 1974; the correlation coefficient was 0.98 [52].

In 1983, Perier et al. determined the concentration of total cholesterol, HDL cholesterol, and LDL cholesterol in blood serum of patients suffering from alcoholism. The total cholesterol concentration in plasma and serum was measured by a spectrophotometric procedure using iron(III) chloride, and the concentrations and HDL and LDL cholesterol were determined by the procedure involving the sedimentation of LDL with Concanavalin A, followed by the application of solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase [53].

In 1983, Nanji and Frohlich used a spectrophotometric procedure for determining the total cholesterol concentration in blood plasma during medical research [54].

In 1983, Jain et al. determined the concentrations of total cholesterol and HDL, VLDL, and LDL cholesterol in blood serum during their medical studies. The total cholesterol concentration in serum was measured by an enzymatic procedure; the HDL cholesterol concentration, by the procedure with VLDL and LDL sedimentation with solutions of heparin and manganese chloride; and the total concentration VLDL and LDL cholesterol, by a turbidimetric procedure with the treatment of blood serum with solutions of heparin and calcium chloride [55].

In 1983, Siedel et al. developed an enzymatic spectrophotometric procedure for determining the total cholesterol concentration in blood serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

At 20–25 or 37°C, serum was mixed with a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (250 IU/L), and peroxidase (200 IU/L). After 10 min (at temperature of 20–25°C) or 5 min (at 37°C), the absorbance of the resulting mixture was measured at a wavelength of 546 nm. The dependence of the absorbance of the mixture on the total cholesterol concentration in serum was linear up to 10000 mg/L (25.86 mM). The determinations of total cholesterol in 20 serum samples by the developed procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent, proposed by Duncan et al. in 1980; the correlation coefficient was 0.9904 [56].

In 1983, Clark et al. developed a procedure for determining the HDL cholesterol concentration in blood serum. To sediment VLDL and LDL, 1 mL of serum was mixed with 80 μL of a solution of phosphotungstic acid and 20 μL of a solution of magnesium chloride at pH 7.4 [57].

In 1983, Deeg and Ziegenhorn proposed four kinetic enzymatic spectrophotometric procedures for determining the total cholesterol concentration in blood serum. The procedures were based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

In Procedure 1, 5 μL of serum was mixed at 25 or 37°C with 50 μL of a solution of sodium chloride (9 g/L) and 500 μL of a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (1 kIU/L), and peroxidase (200 IU/L). After 65 s, a change in absorbance of the resulting mixture was recorded for 120 s (at a temperature of 25°C) or 30 s for 30 s (at 37°C) at a wavelength of 500 nm. The change in absorbance was linearly dependent on the total cholesterol concentration in serum up to 20.7 mM. The presence of triglycerides did not affect the measurements of the total cholesterol concentration. The determinations of total cholesterol in

Table 5. Effect of serum components on the determination of total cholesterol

Serum component	Component concentration, mg/L	Change in the determination of total cholesterol, %	
		Procedure 2	Procedure 4
Bilirubin	50	+1	+1
	100	–2	–1
Hemoglobin	2500	–	+2
	10000	–	+3
α-Methyldopa	200	0	+2
	500	–4	–3
	1000	–8	–14
Sodium metamizole	200	–4	–1
	1000	–1	0
	2000	–2	–3
Ascorbic acid	1000	–1	–2
	2000	–5	–8

73 serum samples by Procedure 1 (at 25°C) were in agreement with the results obtained by the enzymatic procedure developed by Siedel et al. in 1983; the correlation coefficient was 0.994. In Procedure 2, 2.5 μL of serum was mixed at 25 or 37°C with 500 μL of a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (1 kIU/L), and peroxidase (200 IU/L). After 125 s, a change in absorbance of the resulting mixture was measured at a wavelength of 492 nm. The change in absorbance of the mixture on the total cholesterol concentration in serum was linearly dependent up to 25.9 mM. The mean-square deviation of the relative error of determination of the total cholesterol concentration in serum of 5.24 mM was 2.8%. The presence of triglycerides in serum did not affect the measurements of the total cholesterol concentration. The data on the effect of bilirubin, α-methyldopa, sodium metamizole, and ascorbic acid on the determinations of the total cholesterol concentration in serum are presented in Table 5. The determinations of the total cholesterol concentration in 73 serum samples by Procedure 2 (at 25°C) were in agreement with the results obtained by the enzymatic procedure developed by Siedel et al.; the correlation coefficient was 0.995. According to Procedure 3, 3 μL of serum was mixed at 25 or 37°C with 30 μL of distilled water and 300 μL of a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (1 kIU/L), and peroxidase (200 IU/L). After 90 s, a change in absorbance of the resulting mixture was recorded for 120 s (at temperature of 25°C) or after 50 s for 30 s (at 37°C) at a wavelength of 500 nm. The change in absorbance was linearly dependent on the total cholesterol concentration in serum up to 25.9 mM. The mean-square deviation of the relative error of determination of total cholesterol concentration in serum of 2.61 mM was 2.6%. The presence of triglycerides in serum did not affect the determination of the total cholesterol concentration. The determinations of total cholesterol in 73 serum samples by Procedure 3 (at 25°C) were in agreement with the results obtained by the enzymatic procedure developed by Siedel et al.; the correlation coefficient was 0.996. In Procedure 4, 5 μL of serum was mixed at 25 or 37°C with 50 μL of distilled water and 350 μL of a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (1 kIU/L), and peroxidase (200 IU/L). A change in the absorbance of the mixture was measured at 500 nm. The change in absorbance was linearly dependent on the total cholesterol concentration in serum up to 20.7 mM. The mean-square deviation of the relative error of determination of the total cholesterol concentration in serum of 5.21 mM was 3.1%. The presence of triglycerides in serum had no effect on the measurement results for the total cholesterol concentration. The effects of bilirubin, hemoglobin, α-methyldopa, sodium metamizole, and ascorbic acid on the determinations of the total cholesterol concentration in serum are presented in Table 5. The determinations of total cholesterol in 73 serum samples by Procedure 4 (at 37°C) were in agreement with the results obtained by the enzymatic procedure developed by Siedel et al.; the correlation coefficient was 0.993 [58].

In 1983, Demacker et al. compared determinations of the total cholesterol concentration in blood serum of by 20 spectrophotometric procedures using solutions of cholesterol esterase (EC 3.1.1.13) and cholesterol oxidase (EC 1.1.3.6) and by the spectrophotometric procedure using Liebermann–Burchard reagent. The discrepancy between the measurements of the total cholesterol concentration in 12 samples of blood serum by these procedures was in the range of –9.9 to +10.7% [59].

In 1983, Assmann et al. proposed an enzymatic procedure for determining the HDL cholesterol concentration in blood serum. According to the first procedure, 0.5 mL of serum was mixed with 1 mL of a solution (pH 2.5) containing phosphotungstic acid (1.6 g/L) and magnesium chloride (25 mM). The resulting mixture was maintained at room temperature for 10 min and then centrifuged for 2 min to remove sedimented VLDL and LDL. The HDL cholesterol concentration was determined in the centrifuged liquid by the procedure developed by Siedel et al. in 1983. The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in serum of 0.995 mM was at a level of 3.3%. The determinations of HDL cholesterol in 90 serum samples by the proposed procedure were in agreement with the results obtained by the procedure involving ultracentrifugation; the correlation coefficient was 0.989 [60].

In 1984, Rehak et al. compared the measurements of the HDL cholesterol concentration in blood serum by the following enzymatic spectrophotometric procedures:

• A procedure using VLDL and LDL sedimentation with a solutions of phosphotungstic acid (Procedure 1);

• A procedure using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II) (Procedure 2).

For Procedure 1, the mean-square deviation of the relative error of quantification of HDL cholesterol in blood serum of 209 mg/L (0.54 mM) was 9.6%; it was 7% for the concentration of 356 mg/L (0.92 mM) and 10% for 381 mg/L (0.99 mM). In Procedure 2, the mean-square deviation of the relative error of determination of HDL cholesterol in blood serum of 228 mg/L (0.59 mM) was 9.2%; it was 7.8% for the concentration of 322 mg/L (0.83 mM) and 9.8% for 379 mg/L (0.98 mM). The correlation coefficient between the measurements of the HDL cholesterol concentration in 113 blood serum samples according to Procedures 1 and 2 was at a level of 0.944. The presence of bilirubin (up to 170 mg/L), hemoglobin (up to 4.3 g/L), and triglycerides (up to 5.43 g/L) only slightly affected the determination of the HDL cholesterol concentration by Procedure 1 [61].

In 1984, Wiebe and Bernert compared the measurements of the total cholesterol concentration in the blood serum samples by three enzymatic spectrophotometric procedures: The procedures were based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

According to Procedure 1, serum was mixed with a Tris–HCl buffer solution (100 mM; pH 7.7) containing magnesium aspartate (50 mM), 4-aminophenazone (1 mM), phenol (6 mM), 3,4-dichlorophenol (4 mM), surfactants based on hydroxypolyethoxy-*n*-alkanes (0.3%), sodium cholate (10 mM), cholesterol esterase (400 IU/L), cholesterol oxidase (250 IU/L), and peroxidase (200 IU/L). In Procedure 2, serum was mixed with a buffer solution based on 1,4-piperazinediethanesulfonic acid (50 mM; pH 6.9) containing potassium chloride (100 mM), 4-aminophenazone (1 mM), phenol (40 mM), surfactant Triton X-100 (0.1%), sodium cholate (3 mM), cholesterol esterase (250 IU/L), cholesterol oxidase (250 kIU/L), and peroxidase (12.5 kIU/L). In Procedure 3, serum was mixed with a solution containing 4-aminophenazone (2.2 mM), phenol (32 mM), sodium cholate (10 mM), cholesterol esterase (66 IU/L), cholesterol oxidase (120 IU/L), and peroxidase (41.8 kIU/L). The measurements of the total cholesterol concentration in eight blood samples according to these procedures are listed in Table 6 [62].

In 1984, De Bruijn et al. determined the mean-square deviation of the relative error of measurements of the HDL cholesterol concentration in serum by two spectrophotometric procedures with VLDL and LDL sedimentation with a solution of phosphotungstic acid. In one procedure, the HDL cholesterol concentration in the centrifuged liquid was found with the use of solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase (the mean-square deviation of the relative error of measurement was 3.3–3.5%), while another procedure used Liebermann–Burchard reagent (the mean-square deviation of the relative error of measurement was 6%) [63].

Table 6. Determination of the total cholesterol concentration in eight serum samples

Sample of blood serum	Certified total cholesterol concentration in serum, mg/L (mM)	Result of determination of total cholesterol, mg/L (mM)		
		Procedure 1	Procedure 2	Procedure 3
1	3010 (7.78)	2972 (7.69)	2739 (7.08)	2957 (7.65)
2	1619 (4.19)	1632 (4.22)	1514 (3.92)	1608 (4.16)
3	2287 (5.91)	2301 (5.95)	2140 (5.53)	2283 (5.90)
4	2503 (6.47)	2498 (6.46)	2308 (5.97)	2447 (6.33)
5	1217 (3.15)	1225 (3.17)	1136 (2.94)	1199 (3.10)
6	1890 (4.89)	1904 (4.92)	1769 (4.58)	1879 (4.86)
7	1901 (4.92)	1886 (4.88)	1760 (4.55)	1870 (4.84)
8	2859 (7.39)	2880 (7.45)	2638 (6.82)	2840 (7.35)

Table 7. Average determinations of HDL cholesterol in 96 plasma samples and 72 serum samples

Samples	Average determinations of HDL cholesterol, mg/L (mM)		
	Procedure 1	Procedure 2	Procedure 3
Plasma	509.2 (1.32)	562.4 (1.45)	520.9 (1.35)
Serum	563.3 (1.46)	597.2 (1.54)	566.0 (1.46)

In 1984, Davey et al. found underestimation of the determination of the total cholesterol concentrations in the blood serum of a patient who took mitotane (1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane) by the procedure using cholesterol esterase and cholesterol oxidase. Along with this, no decrease was observed in the measurements of the total cholesterol concentration in serum by another enzymatic procedure and by nonenzymatic procedure upon the administration of mitotane [64].

In 1984, Bachorik et al. developed an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood plasma and serum. Plasma or serum was mixed with solutions of heparin and manganese chloride and then centrifuged to remove sedimented VLDL and LDL. Then, a $NaHCO_3$ solution (1 M) was added to the centrifuged liquid, and reaction $Mn^{2+} + 2NaHCO_3 \rightarrow 2Na^+ + Mn(HCO_3)_2$ proceeded in the system. The resulting mixture was kept at room temperature for 30 min and centrifuged for 5 min to remove sedimented $Mn(HCO_3)_2$. The HDL cholesterol concentration was determined in the centrifuged liquid by the procedure using solutions of cholesterol esterase and cholesterol oxidase. Table 7 presents the average determinations of the HDL cholesterol concentrations in 96 plasma samples and 72 serum samples:

• By the procedure with LVDL and LDL sedimentation with solutions of heparin and manganese chloride, followed by the measurement of the HDL cholesterol concentration in the centrifuged liquid using Liebermann–Burchard reagent (Procedure 1);

• By the procedure with LVDL and LDL sedimentation with solutions of heparin and manganese chloride with determination of the HDL cholesterol concentration in the centrifuged liquid with the use of solutions of cholesterol esterase and cholesterol oxidase (Procedure 2);

• By the developed procedure (Procedure 3).

It is clear that the difference between the measurements of the HDL cholesterol concentration in plasma and serum by Procedures 1 and 3 is significantly smaller than the difference between the determinations by Procedures 1 and 2. This is confirmed by the better agreement of the results obtained by Procedures 1 and 3. For example, the correlation coefficients between the determinations of HDL cholesterol in 96 plasma samples by Procedures 1 and 3 was 0.944, while was 0.902 by Procedures 1 and 2. The correlation coefficients between the determination of HDL cholesterol in 72 serum samples corresponded to 0.964 for Procedures 1 and 3 and 0.96 for Procedures 1 and 2 [65].

In 1984, Demacker et al. examined 19 blood serum samples destined for use as quality control samples in determining the total cholesterol concentration in serum by 20 spectrophotometric procedures using cholesterol esterase and cholesterol oxidase. Animal or human blood serum served as the basis for the

preparation of test samples. The test results showed that, in measuring the total cholesterol concentration in serum by enzymatic spectrophotometric procedures, the use of samples based on human blood serum as a quality control samples were more preferable [66].

In 1984, Demacker et al. also compared the determination of the LDL cholesterol concentration in blood serum samples by five procedures:

- A procedure using solutions heparin and sodium citrate (Procedure 1);
- A procedure using solutions of polyvinyl sulfate and polyethylene glycol methyl ether (Procedure 2);
- A procedure using a buffer solution based on imidazole, including amphiphilic polymers (Procedure 3);
- A procedure using the formula proposed by Friedewald et al. (Procedure 4);
- A procedure with preliminary ultracentrifugation to separate CM and VLDL, followed by the application of a solution of polyethylene glycol (Procedure 5).

The correlation coefficients between the LDL cholesterol determinations in 83 serum samples by Procedures 1 and 5, 2 and 5, and 3 and 5 were estimated at 0.96; the correlation coefficient between the Procedures 4 and 5 was 0.98 [67].

In 1985, Alzofon et al. compared the measurements of the total cholesterol concentration in blood plasma withdrawn from a finger and vein of patients by an enzymatic procedure. The correlation coefficient between the measurements of the total cholesterol concentration in 40 samples of blood plasma from a vein and finger corresponded to 0.98 [68].

In 1985, Warnick et al. compared the measurements of the HDL cholesterol concentration in the blood plasma by the following six procedures:

- A procedure using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium chloride (Procedure 1);
- Two procedures using VLDL and LDL sedimentation with solutions of heparin and manganese chloride (Procedures 2 and 3);
- A procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride (Procedure 4);
- Two procedures using VLDL and LDL sedimentation with solutions of polyethylene glycol (Procedures 5 and 6).

According to Procedure 1, 2 mL of plasma were mixed with 200 μL of a solution (pH 7) containing dextran sulfate (10 g/L), magnesium chloride (0.5 M) and sodium azide–chloramphenicol–gentamycin. The mixture was kept at room temperature for 15 min and then centrifuged for 30 min. In Procedure 2, 2 mL of plasma was mixed with 80 μL of a solution containing heparin (5000 kIU/L) and sodium chloride (131.25 mM) and 100 μL of manganese chloride solution (1 M). The resulting mixture was maintained in at 4°C for 30 min and centrifuged for 30 min. According to Procedure 3, 2 mL of plasma was treated with 200 μL of a solution containing heparin (2264.15 kIU/L) and manganese chloride (1 M). The mixture was kept at room temperature for 10 min and then centrifuged for 30 min. In Procedure 4, 2 mL of plasma was mixed with 200 μL of a solution (pH 7.4) containing sodium phosphotungstate (32 g/L) and magnesium chloride (0.4 M). The resulting mixture was kept at room temperature for 5 min and then centrifuged for 10 min. According to Procedure 5, 1 mL of a solution (pH 10) containing polyethylene glycol (200 g/L) and glycine (200 mM) was added to 1 mL of plasma. The mixture was kept at room temperature for 10 min and then centrifuged for 30 min. In Procedure 6, 2 mL plasma was treated with 400 μL of a solution of polyethylene glycol (450 g/L). The resulting mixture was kept at room temperature for 15 min and then centrifuged for 30 min. The HDL cholesterol concentration in the centrifuged liquid in Procedures 1–6 was measured by spectrophotometrically using Liebermann–Burchard reagent. The correlation coefficient between the determinations of the HDL cholesterol concentration in the plasma samples was estimated at 0.995 for Procedures 1 and 2, 0.997 for Procedures 1 and 3, 0.993 for Procedures 1 and 4, and 0.985 for Procedures 1 and 5 [69].

Heuck et al. developed an enzymatic spectrophotometric procedure for determining the concentration of total cholesterol, HDL cholesterol, and LDL cholesterol in blood serum in 1985. In determining the concentration of HDL cholesterol, 10 μL of serum was mixed with 50 μL of type B antiserum. The mixture was kept at room temperature for 1 h and then centrifuged for 10 min. The HDL cholesterol concentration was determined in the centrifuged liquid by the spectrophotometric procedure using solutions of cholesterol esterase, cholesterol oxidase, and peroxidase proposed by Siedel et al. in 1983. The mean-square deviation of the relative error of determination of the HDL cholesterol concentration in serum was 5%. After the storage of serum for 8 days at 4°C, the mean-square deviation of the relative error of measurements of HDL cholesterol concentration increased to 8.2%. The determinations of the HDL choles-

terol concentration in 118 serum samples according to the developed procedure were in agreement with the results obtained by the procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride; the correlation coefficient was 0.93. In determining the concentration of LDL cholesterol, 5 μL of serum was mixed with 50 μL of type A-I+C antiserum. The resulting mixture was kept at room temperature for 1 h and then centrifuged for 10 min. The LDL cholesterol concentration was determined in the centrifuged liquid by the spectrophotometric procedure using solutions of cholesterol esterase, cholesterol oxidase, and peroxidase proposed by Siedel et al. The mean-square deviation of the relative error of determination of the LDL cholesterol concentration in serum was 3.8%. After storage of serum for 8 days at 4°C, the mean-square deviation of the relative error of measurements of LDL cholesterol concentration increased to 5.5–8.1%. The determinations of LDL cholesterol in 145 serum samples by the developed procedure were in agreement with the results obtained by the procedure involving the formula proposed by Friedewald et al.; the correlation coefficient was 0.94 [70].

In 1985, Cary et al. studied the characteristics of an enzymatic spectrofluorimetric procedure for determining the total cholesterol concentration in blood plasma and serum. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 3,4-dichloro-2-hydroxybenzenesulfonic acid to form a fluorescent compound (Product) under the catalytic action of peroxidase (EC 1.11.1.7)

$$2H_2O_2 + \text{4-aminophenazone} + \text{DCHBSA} \xrightarrow{\text{Peroxidase}} \text{Product} + 4H_2O,$$

where DCHBSA is 3,4-dichloro-2-hydroxybenzenesulfonic acid.

Plasma or serum was mixed with a solution containing 4-aminophenazone, 3,4-dichloro-2-hydroxybenzenesulfonic acid, fluorescein, cholesterol esterase, cholesterol oxidase, and peroxidase. Next, a change in the fluorescence intensity of the mixture was measured at a wavelength of 505 nm. The change in the fluorescence intensity of the mixture was linearly dependent on the total cholesterol concentration in plasma and serum up to 4000 mg/L (10.35 mM). The mean-square deviation of the relative error of determination of total cholesterol at a concentration of 760, 1350, 2430, 2530, and 3550 mg/L (1.97, 3.49, 6.28, 6.54, and 9.18 mM) in serum was 2.6, 2.8, 2.4, 1.3, and 0.9%. The presence of triglycerides in plasma or serum did not affect the measurement results for the total cholesterol concentration. The presence of bilirubin at a concentration of 10 mg/L in plasma or serum caused a decrease in the determination of total cholesterol concentration of 1000 mg/L (2.59 mM) by 20 mg/L (0.05 mM); at a concentration of 2000 mg/L (5.17 mM), by 25 mg/L (0.06 mM); and at a concentration of 4000 mg/L (10.35 mM), by 35 mg/L (0.09 mM). The determinations of total cholesterol in 217 plasma and serum samples by the proposed procedure were in agreement with the results obtained by two enzymatic spectrophotometric procedures; the correlation coefficients were 0.993 and 0.995 [71].

In 1985, Derks et al. developed a chromatographic procedure for determining the total cholesterol concentration in blood serum. A 0.6-mL portion of a solution containing 3α-hydroxy-5β-cholestane (used as an internal standard), 1 mL of ethanol, and 0.4 mL of a potassium hydroxide solution were added to 0.2 mL of serum. The resulting mixture was kept at 37°C for 3 h for the hydrolysis of β cholesterol yielding α cholesterol. After cooling to room temperature, 2 mL of distilled water and 4 mL of hexane were added to the mixture, and extraction was carried out. A portion of the hexane extract (approximately 120 μL) was evaporated at 60°C under a nitrogen flow. The emerging precipitate was dissolved in 0.1 mL of a solution containing *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (33%) and pyridine (67%), and after the occurrence of chemical interactions with 3α-hydroxy-5β-cholestane and α-cholesterol, 1 μL of the resulting mixture was injected into the gas chromatograph. The chromatograph was equipped with a glass capillary column with polymethylsiloxane (25 m × 0.3 mm) and a flame ionization detector. Nitrogen was a carrier gas. The retention time of the chemical compound corresponding to 3α-hydroxy-5β-cholestane was 17.7 min, and for the compound corresponding to α cholesterol, it was 20 min. The peak area of the chemical compound, which corresponded to α cholesterol, was proportional to the total cholesterol concentration in blood serum. The mean-square deviation of the relative error of measurement of the total cholesterol concentration in serum of 3.6136 mM was 0.5%; and for the concentration of 7.8031 mM, it was 0.35% [72].

In 1985, Wiebe and Smith compared the determination of the HDL cholesterol concentration in blood serum by six spectrophotometric procedures:

Table 8. Correlation coefficients for the measurements of HDL cholesterol in 90 blood serum samples

Procedures	Correlation coefficient between the determinations	Procedures	Correlation coefficient between the determinations	Procedures	Correlation coefficient between the determinations
1 and 2	0.9972	2 and 3	0.9546	3 and 5	0.9720
1 and 3	0.9496	2 and 4	0.9782	3 and 6	0.9417
1 and 4	0.9755	2 and 5	0.9887	4 and 5	0.9887
1 and 5	0.9878	2 and 6	0.9937	4 and 6	0.9693
1 and 6	0.9974	3 and 4	0.9865	5 and 6	0.9837

• Two procedures using VLDL and LDL sedimentation with solutions of heparin and manganese chloride (Procedures 1 and 2);

• Two procedures using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium chloride (Procedures 3 and 4);

• Procedure using VLDL and LDL sedimentation with solutions of a salt of phosphotungstic acid and magnesium chloride (Procedure 5);

• Procedure with VLDL and LDL sedimentation using a solution of polyethylene glycol (Procedure 6).

According to Procedure 1, 2 mL of serum was mixed with 180 μL of a solution containing heparin (2222.2 kIU/L), sodium chloride (66.7 mM), and manganese chloride (555.5 mM). In Procedure 2, 180 μL of a solution containing heparin (2222.2 kIU/L), sodium chloride (66.7 mM), and manganese chloride (1.11 mM) was added to 2 mL of serum. In Procedure 3, 2 mL of serum was treated with 200 μL of a solution containing dextran sulfate (10 g/L) and magnesium chloride (1 M). According to Procedure 4, 2 mL of serum was mixed with 200 μL of a solution containing dextran sulfate (10 g/L) and magnesium chloride (0.5 M). In Procedure 5, 250 μL of a solution containing sodium phosphotungstate and magnesium chloride (0.4 M) was added to 2 mL of serum. In Procedure 6, 1 mL of serum was treated with 1 mL of a polyethylene glycol solution (200 g/L) in a glycerol buffer solution (0.2 mM, pH 10). Mixtures were centrifuged, and the HDL cholesterol concentration was measured therein spectrophotometrically, using Liebermann–Burchard reagent. The mean-square deviation of the relative error of quantification of HDL cholesterol in blood serum of 491 mg/L (1.27 mM) by Procedure 1 was 1.19%; for the concentration of 470.5 mg/L (1.22 mM) by Procedure 2, it was 1.05%; for the concentration of 434.1 mg/L (1.12 mM) by Procedure 3, it was 1.28%; for the concentration of 453.7 mg/L (1.17 mM) by Procedure 4, it was 2.15%; for the concentration of 462.6 mg/L (1.2 mM) by Procedure 5, it was 2.03%; and for the concentration of 494.7 mg/L (1.28 mM) by Procedure 6, it was 1.37%. The correlation coefficients between the determinations of the HDL cholesterol concentration in 90 blood serum samples according to Procedures 1–6 are presented in Table 8. The highest correlation coefficient (0.9974) was observed between the results obtained by Procedures 1 and 6 [73].

In 1985, Terlingen et al. proposed samples of reference solutions intended for performing calibration in determining the total cholesterol concentration in serum by enzymatic procedures. Human blood serum was the basis for these samples. The correlation coefficient between the measurement of the total cholesterol concentration in 69 serum samples by the spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase (with the calibration obtained using the proposed reference solutions) and by the spectrophotometric procedure using Liebermann–Burchard reagent corresponded to 0.9989 [74].

In 1985, Hoffmann et al. compared the determination of the LDL cholesterol concentration in blood serum samples by the following five procedures:

• A procedure using ultracentrifugation (Procedure 1);

• An electrophoretic procedure (Procedure 2);

• A procedure using LDL sedimentation with a solution of polyvinyl sulfate (Procedure 3);

• A procedure using LDL sedimentation with a solution of heparin (Procedure 4);

• A procedure using the formula proposed by Friedewald et al. (Procedure 5).

The mean-square deviations of the relative error of measurements of the LDL cholesterol concentration in serum were at the levels of 5.5–6% (Procedure 1), 1.8–2.5% (Procedure 2), 4.3–5% (Procedure 3), and 4.3–6.1% (Procedure 4). The correlation coefficients for the determinations of the LDL cholesterol con-

centration in 49 serum samples was 0.94 for by Procedures 1 and 3, 0.95 for Procedures 1 and 2, and 0.98 for Procedures 3 and 5 [75].

In 1985, Demacker assessed the developed procedures using VLDL and LDL sedimentation with different reagents (dextran sulfate and manganese(II), polyethylene glycol, and others) for determining the HDL cholesterol concentration in blood plasma serum [76].

In 1985, Roche et al. studied the characteristics of an electrophoretic procedure for determining the HDL cholesterol concentration in blood plasma. Plasma lipoproteins were separated by means of polyacrylamide gel at a voltage of 150 V. The volume of blood plasma required for analysis was 25 μL. The separation time of lipoproteins time was estimated at 15–20 min. The HDL cholesterol concentration was found with a densitometer functioning at a wavelength of 620 nm (the determination range was from 0.45 to 15.07 mM). The determinations of HDL cholesterol in 22 plasma samples by the studied procedure were in agreement with the results obtained by the procedure involving ultracentrifugation; the correlation coefficient was 0.96 [77].

In 1985, Zoppi proposed a kinetic enzymatic spectrophotometric procedure for determining the total cholesterol concentration in blood serum after measurement of the triglyceride concentration in it using a solution of nicotinamide adenine dinucleotide. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

At a temperature of 37°C, 300–350 μL of a solution containing 4-aminophenazone, phenol, glycerol, cholesterol esterase, cholesterol oxidase, and peroxidase was automatically added to the mixture remaining in the cell after the determination of triglycerides in serum. After 100 s (after the interaction of glycerol with nicotinamide adenine dinucleotide), the change in absorbance of the resulting mixture was measured over 60 s at 505–510 nm. The change in absorbance of the resulting mixture was linearly dependent on the total cholesterol concentration in serum up to 8000 mg/L (20.69 mM). The mean-square deviation of the relative error of the determination of the total cholesterol concentration in serum was 8.1–12.5%. The presence of bilirubin (up to 184 mg/L), hemoglobin (up to 2 g/L), glucose (up to 5 g/L), uric acid (up to 200 mg/L), ascorbic acid (up to 100 mg/L), acetylsalicylic acid (up to 100 mg/L), sodium metamizole (up to 100 mg/L), dopamine (up to 20 mg/L), L-dopa (up to 20 mg/L), and α-methyldopa (up to 20 mg/L) had no significant effect on the measurements of total cholesterol concentration. The determinations of total cholesterol in serum samples by the proposed procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent (the correlation coefficient was 0.995; 82 samples) and by enzymatic spectrophotometric procedures (the correlation coefficients were 0.985–0.989; 82–100 samples) [78].

In 1986, Lippi et al. applied procedures for determining the HDL cholesterol concentration in plasma serum with VLDL and LDL sedimentation with solutions of polyethylene glycol, dextran sulfate, and magnesium chloride in his medical studies [79].

In 1986, Gorba reported that, in determining the HDL cholesterol concentration in plasma and serum by the procedure with VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride, the dilution of the centrifuged liquid ensured more accurate (on average by 10%) measurements of the HDL cholesterol concentrations compared with those obtained with the dilution of test plasma or serum [80].

In 1986, Spain and Wu studied the characteristics of eight enzymatic procedures for determining the total cholesterol concentration in blood serum. In all of the studied procedures, cholesterol esterase, cholesterol oxidase, and peroxidase were used. Procedures 1–7 were spectrophotometric, and Procedure 8 was a reflection photometry procedure using test strips. The absorbance of the resulting mixture was measured at 600 and 505 nm in Procedures 1 and 2, 600 and 540 nm in Procedure 3, 690 and 500 nm in Procedure 4, 500 nm in Procedures 5 and 6, and 520 nm in Procedure 7. Procedure 8 involved the recording of reflection by a test strip of radiation with a wavelength of 540 nm. The mean-square deviation of the relative error of the determination of the total cholesterol concentration in serum was 1.4% (Procedure 1), 0.7% (Procedure 2), 0.4% (Procedure 3), 5.3% (Procedure 4), 0.5% (Procedure 5), 0.9% (Procedure 6), 1.3% (Procedure 7), and 1.9% (Procedure 8). The presence of bilirubin at a concentration up to 25 mg/L in serum was not accompanied by a marked change in the measurement results of total cholesterol con-

centration in Procedures 1 and 5; at a concentration up to 50 mg/L, in Procedure 7; at a concentration up to 80 mg/L, in Procedure 2, at a concentration up to 240 mg/L, in Procedure 6; and at a concentration up to 400 mg/L, in Procedures 3, 4, and 8. After the preliminary treatment of blood serum with a sodium hexacyanoferrate(III) solution (18 μM), the total cholesterol concentration in serum found by Procedure 1 remained unchanged at a concentration of 50 mg/Lin the presence of bilirubin therein and at a concentration of 240 mg/L by Procedure 2 [81].

In 1986, Hainline et al. reported on samples of reference solutions produced for performing calibration in determining the total cholesterol concentration in serum [82].

Yatscoff et al. studied the effect of cyclosporine pharmaceutical preparation taken by patients on the determination of the total cholesterol concentrations in blood plasma in 1986. The data showed that there was no such effect [83].

In 1986, Lenzen et al. used an enzymatic spectrophotometric procedure to determine the total cholesterol concentration in blood serum, a procedure with VLDL and LDL sedimentation to measure the concentration of HDL cholesterol, and the formula developed by Friedewald et al. to determine the LDL cholesterol concentration in their medical studies [84].

In 1986, Demacker et al. proposed an enzymatic spectrophotometric procedure for determining the concentration of cholesterol in HDL fractions in blood serum: fraction HDL_2 with density from 1.063 to 1.125 mg/mL and fraction HDL_3 with density from 1.125 to 1.154 mg/mL. A 100-μL portion of a sodium heparin solution (6 kIU/L) and 150 μL of a manganese chloride solution (2 M) were added to 3 mL of serum. The resulting mixture was maintained at room temperature for 10 min and then centrifuged for 15 min to remove sedimented VLDL and LDL. Next, 2 mL of the centrifuged liquid was mixed with a solution of dextran sulfate. The mixture was also kept at room temperature for 10 min and then centrifuged for 15 min. The HDL_3 cholesterol concentration was measured in the centrifuged liquid, and the HDL_2 cholesterol concentrations were determined in the sediment after its dissolution in a sodium chloride solution. The concentrations of HDL_2 and HDL_3 cholesterol were determined by a spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase. The mean-square deviation of the relative error of determination of the HDL_3 cholesterol concentration in serum of 0.95 mM was 6.3%, and it was 5% for the concentration of 1.01 mM. The mean-square deviation of the relative error of measurements corresponded to 15.7% for the HDL_2 cholesterol concentration of 0.19 mM in serum and 2.2% for 0.9 mM. The determinations of HDL_2 and HDL_3 cholesterol in 88 serum samples by the proposed procedure were in agreement with the results obtained by the procedure involving ultracentrifugation [85].

In 1986, Cooper et al. compared the determinations of the total cholesterol concentration in serum samples obtained in 14 clinical diagnostic laboratories. The total cholesterol concentration was determined by a spectrophotometric procedure involving

- The treatment of 0.5 mL of serum by a potassium hydroxide solution in ethyl alcohol to hydrolyze β cholesterol to form α cholesterol;
- The extraction of total α cholesterol from the resulting mixture by hexane to increase the selectivity of measurements;
- Evaporation of the hexane extract;
- Chemical interaction of the hexane-extract precipitate after its dissolution with Liebermann–Burchard reagent (acetic anhydride, glacial acetic acid, and concentrated sulfuric acid);
- Measurement of the absorbance of the resulting mixture at 620 nm.

Eight clinical diagnostic laboratories (Group 1) applied automatic dosing of liquids, and six laboratories (Group 2) performed dosing manually. The mean-square deviation of the relative error of the determination of the total cholesterol concentration in serum for laboratories of Group 1 was 1.11–2.82%; the average value was 1.93%. The mean-square deviation of the relative error of measurements of the total cholesterol concentration in serum for laboratories of Group 2 ranged from 1.81 to 6.71%; the average value corresponded to 3.43%. The relative systematic error of determination of the total cholesterol concentration in serum for laboratories of Group 1 was 0.59–1.2%; the average value was 0.9%. The relative error of measurements of the total cholesterol concentration in serum for laboratories of Group 2 was estimated 0.72–6.71%, with an average value of 1.47% [86].

In 1986, Boerma et al. compared the determinations of total cholesterol in four serum samples obtained in 30 clinical diagnostic laboratories. Twenty-four laboratories measured the total cholesterol concentration in serum by enzymatic procedures, and six laboratories used nonenzymatic procedures involving Liebermann–Burchard reagent (Table 9). Boerma et al. also compared the determinations of

Table 9. Average determinations of total cholesterol in four serum samples

Serum sample	Certified concentration of total cholesterol, mM	Average determination of total cholesterol, mM	
		Enzymatic procedures	Nonenzymatic procedures
1	4.73	4.73	4.80
2	5.28	5.23	5.28
3	6.63	6.65	6.79
4	8.05	8.08	8.05

Table 10. Average determinations of total cholesterol in eight serum samples

Serum sample	Certified concentration of total cholesterol, mM	Average determination of total cholesterol, mM	
		Enzymatic procedures	Nonenzymatic procedures
1	3.15	3.05	3.46
2	3.46	3.40	3.78
3	3.96	3.84	4.16
4	5.23	5.15	5.54
5	6.00	5.90	6.31
6	8.18	7.89	8.29
7	8.97	8.77	9.40
8	9.29	9.02	9.63

Table 11. Average determinations of total cholesterol in serum samples

Serum sample	Certified concentration of total cholesterol, mM	Average determination of total cholesterol, mM	
		Enzymatic procedures	Nonenzymatic procedures
1	3.46	3.50	3.48
2	3.96	3.95	3.85
3	5.23	5.27	5.21
4	8.18	8.03	7.91
5	9.29	9.17	9.25

total cholesterol in eight serum samples obtained in 138 clinical diagnostic laboratories. In 115 laboratories, enzymatic procedures were used to measure the total cholesterol concentration in serum, and 23 laboratories applied nonenzymatic procedures (Table 10). When calibration was obtained in all 138 laboratories using the same reference solutions, the accuracy of the determination of the total cholesterol concentration in serum mainly increased (Tables 10 and 11) [87].

In 1986, Stokes et al. studied a change in the concentration of total and HDL cholesterol in blood serum upon storage in the frozen state (–15°C) for 18 weeks (Table 12). The total cholesterol concentration in serum was determined by spectrophotometric procedures using an iron(III) chloride implemented in an automatic flow device. The HDL cholesterol concentration was found by the procedure with VLDL and LDL sedimentation with a solution of phosphotungstic acid and magnesium chloride upon the maintenance and centrifuging of the resulting mixture at room temperature [88].

In 1986, Aitken studied the characteristics of procedures for determining the concentrations of HDL and LDL cholesterol in blood plasma and serum. The following procedures were used in the study [89]:

• A procedure for measuring the LDL cholesterol concentration using the formula developed by Friedewald et al.;

• Electrophoretic procedures using gels of polyacrylamide and agarose;

Table 12. Determination of total cholesterol and HDL cholesterol in serum samples

Factor	Measurement results, mM, upon storage of serum in the frozen state for a period of time, week						
	0	3	6	9	12	15	18
Total cholesterol concentration	5.211	5.217	5.203	5.252	5.286	5.267	5.245
HDL cholesterol concentration	1.285	1.314	1.299	1.324	1.324	1.311	1.290

• A procedure of determining the HDL cholesterol concentration with VLDL and LDL sedimentation with a solution of polyethylene glycol, followed by enzymatic determination of the HDL cholesterol concentration in the centrifuged liquid.

In 1986, Whitaker et al. proposed an enzymatic spectrophotometric procedure for determining the concentration of HDL, HDL_2, and HDL_3 cholesterol in blood serum. To determine the HDL cholesterol, 0.2 mL of serum was injected in a column packed with heparin bound to agarose gel; after elution with 1 mL of sodium chloride, the concentration HDL cholesterol in serum (C_{HDL}) was determined via processing of the eluate by an enzymatic procedure. In determining the HDL_2 and HDL_3 cholesterol concentrations, serum was mixed with a solution containing heparin and manganese chloride and a solution of dextran sulfate. The resulting mixture was maintained in at room temperature for 20 min and centrifuged at 4°C for 1 h. The HDL_3 cholesterol concentration in serum (C_{HDL3}) was found in the centrifuged liquid by an enzymatic procedure. The HDL_2 cholesterol concentration in serum (C_{HDL2}) was calculated by the equation $C_{HDL2} = C_{HDL} - C_{HDL3}$. The determinations of HDL, HDL_2, and HDL_3 cholesterol in 27 serum samples by the proposed procedure were in agreement with the results obtained by the procedure involving ultracentrifugation; the correlation coefficients were 0.92 (HDL cholesterol), 0.93 (HDL_2 cholesterol), and 0.84 (HDL_3 cholesterol) [90].

In 1986, Koedam et al. noted that, in determining the HDL cholesterol concentration in serum by the procedure with VLDL and LDL sedimentation with solution of phosphotungstic acid and magnesium chloride (with subsequent measurement of the HDL cholesterol concentration in the centrifuged liquid by an enzymatic procedure), the dilution of test serum twice with an ammonia acetate solution (0.1 M; pH 7–8) including albumin (50 g/L) yielded more accurate determinations of the HDL cholesterol concentration in serum as compared to its dilution with a sodium chloride solution (9 g/L) [91].

In 1986, Samman and Roberts showed experimentally that, in determining the HDL cholesterol concentration in blood plasma by the procedure with VLDL and LDL sedimentation with solution of heparin and manganese chloride, these solutions did not affect measurements of the HDL cholesterol concentration in a centrifuged liquid performed by a spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase [92].

In 1986, Warnick et al. examined an enzymatic reflectance photometry procedure for determining the HDL cholesterol concentration in blood plasma and serum. Plasma or serum were sampled in 0.5-mL portions into a tube containing dextran sulfate and magnesium(II). The centrifuged liquid was applied to a test strip bearing enzymes, and the HDL cholesterol concentration in plasma or serum was measured using a specialized measuring device. The mean-square deviation of the absolute error of measurement of the HDL cholesterol concentration in plasma or serum was 12–16 mg/L (0.03–0.04 mM). The determinations of the HDL cholesterol concentration in 120 plasma samples according to the examined procedure were in agreement with the results obtained by the spectrophotometric procedure with the precipitation of VLDL and LDL by solutions of heparin and manganese(II) with determination of the HDL cholesterol concentration in a centrifuged liquid using Liebermann–Burchard reagent and by the enzymatic spectrophotometric procedure with the precipitation of VLDL and LDL by solutions of dextran sulfate and magnesium(II) [93].

In 1986, Alzofon et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips for the determination of the total cholesterol concentration in serum. The average results of measurements of the total cholesterol concentration in serum of 251 mg/L (0.65 mM) were as follows: 233 mg/L (0.6 mM), 249 mg/L (0.64 mM), 251 mg/L (0.65 mM), and 252 mg/L (0.65 mM); the results were obtained using four specialized measuring devices. The mean-square deviation of the relative error corresponded to 4%. The determinations of total cholesterol in 17 serum samples by the procedure under study were in agreement with the results obtained by another enzymatic reflectance photometry procedure; the correlation coefficient was 0.998 [94].

Table 13. Determination of HDL cholesterol in serum samples

Serum samples	Concentration of triglycerides, mM	Determination of HDL cholesterol, mM	
		Procedure of Lippi et al.	Electrophoretic procedure
1	less than 2.0	1.48	1.52
2	2.0–5.0	1.15	1.12
3	5.1–10.0	0.79	0.80
4	10.1–25.0	0.72	0.74

Table 14. Average concentrations of total and HDL cholesterol in the blood plasma of 32 patients with myocardial infarction

Factor	Average determination, mM, after myocardial infarction, day					
	1	2	3	6	10	12
Total cholesterol concentration	5.58	5.26	5.06	5.12	5.29	5.23
HDL cholesterol concentration	1.14	1.12	1.15	1.09	1.05	1.08

Table 15. Average concentrations of total and HDL cholesterol in the blood plasma of eight female patients with myocardial infarction

Factor	Average determination, mM, after myocardial infarction, day					
	1	2	3	6	10	12
Total cholesterol concentration	6.55	5.87	5.53	5.55	5.50	5.47
Calcium concentration	2.14	2.09	2.11	2.13	2.13	2.12

In 1986, Lippi et al. developed an enzymatic procedure for determining the HDL cholesterol concentration in blood serum. One milliliter of a sodium chloride solution (0.15 M) containing polyethylene glycol (100 g/L), dextran sulfate (37.4 g/L), and magnesium chloride (2.6 M) was added to 0.1 mL of serum. The resulting mixture was maintained at room temperature for 15 min and then centrifuged for 15 min to remove sedimented VLDL and LDL; the HDL cholesterol concentration was determined in the centrifuged liquid by an enzymatic procedure. The presence of triglycerides (up to 25 mM) did not affect the determination of the HDL cholesterol concentration (Table 13). The determinations of HDL cholesterol in serum samples were in agreement with the results obtained by the electrophoretic procedure involving agarose gel (Table 13) [95].

In 1987, Speich et al. studied changes in the concentrations of total cholesterol and HDL cholesterol in blood plasma of 32 patients over 12 days after a myocardial infarction (Table.14). The total cholesterol concentration in serum was determined by an enzymatic spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase, and the HDL cholesterol concentration was measured by the procedure with the precipitation of VLDL and LDL with solutions of phosphotungstic acid and magnesium(II) [96].

In 1987, Speich also studied the relationship between the concentrations of total cholesterol and calcium in blood plasma of eight female patients over 12 days a myocardial infarction (Table 15). The total cholesterol concentration in plasma was determined by the enzymatic spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase [97]

In 1987, Huang and Kyte compared the determination of the total cholesterol concentration in blood plasma by four enzymatic spectrophotometric procedures. The procedures were based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

The mean-square deviation of the relative error of measurements of the total cholesterol concentration in plasma was 3.03% for Procedure 1, 5.17% for Procedure 2, 6.21%, for Procedure 3, and 6.34% for Procedure 4. The correlation coefficient between the measurements of the total cholesterol concentrations in 118–121 samples of blood plasma by Procedures 1–4 were from 0.898 to 0.957 [98].

Takatsu and Nishi proposed a chromatography–mass spectrometric procedure for determining the total cholesterol concentration in blood serum in 1987. Serum was mixed with a solution of [3,4-^{13}C]-labeled cholesterol. A 0.3-mL portion of an aqueous potassium hydroxide solution (8.9 M) and 2 mL of ethyl alcohol were added to the mixture. The resulting mixture was kept at 50°C for 3 h for the hydrolysis of β cholesterol yielding α cholesterol. After cooling to room temperature, 2.5 mL of distilled water and 5 mL of hexane were added to the mixture, and extraction was performed for 1 min. Then, the hexane extract was evaporated under nitrogen gas, and the precipitate was dissolved in 1 mL of methyl alcohol. The resulting solution (20 μL) was injected into a liquid chromatograph equipped with a column (4.6 mm × 25 cm) and a spectrophotometric detector operating at a radiation wavelength of 200 nm. A mixture of acetonitrile (75%) and isopropyl alcohol (25%) served as an eluent. The eluent flow rate was 1 mL/min. The retention time of [3,4-^{13}C]-labeled cholesterol and α cholesterol was estimated at 18 min. The separated [3,4-^{13}C]-labeled cholesterol and α cholesterol were collected at the chromatograph output and evaporated under nitrogen gas, and the residue, after having ben dissolved in 20 μL of ethanol, was injected into the mass spectrometer. The mass spectrometer recorded the intensity of peaks with m/z 386 (α cholesterol) and 388 [3,4-^{13}C]-labeled cholesterol), from which the total cholesterol concentration in blood serum was calculated. The mean-square deviation of the relative error of determinations of the total cholesterol concentration in serum varied from 0.3 to 0.95%. The measurements of the total cholesterol concentrations in serum samples according to the developed procedure were in agreement with the results obtained by the chromatography–mass spectrometric procedure using *N*,*O*-bis(trimethylsilyl)acetamide and gas chromatography [99].

In 1987, Von Schenck et al. compared the determinations of the total cholesterol concentration in plasma, serum, and whole blood by three reflectance photometry procedures using test strips and by a laboratory spectrophotometric procedure. According to Procedure 1 of reflection photometry, 10 μL of plasma or serum was applied on the surface of the test strip; according to Procedure 2, 30 μL of plasma, serum, or whole blood was applied; and according to Procedure 3, 20 μL of plasma or serum diluted nine times was used. The mean-square deviation of the relative error of measurements of the total cholesterol concentration in plasma, serum, or whole blood samples was 1.6–2.3% for Procedure 1, 3.4–3.7% for Procedure 2, and 2.7–3.1% for for Procedure 3. The correlation coefficient between the determination of the HDL cholesterol concentration in the plasma, serum, and whole blood samples by the laboratory procedure and Procedure 1 was 0.97 (81 samples); it was 0.92 (99 samples) by the laboratory procedure and Procedure 2 and 0.97 (106 samples) by the laboratory procedure and Procedure 3 [100].

In 1987, Callais et al. developed an enzymatic electrophoretic procedure for determining the LDL cholesterol concentration in blood serum. First, serum lipoproteins were separated electrophoretically by means of polyacrylamide gel. Then, a portion of the gel including LDL was treated with an enzyme solution. The resulting mixture was centrifuged at 5000 rpm for 10 min, and the absorbance of the centrifuged liquid was measured at 510 nm. The correlation coefficient for the determinations of the LDL cholesterol concentration in 50 serum samples by the developed procedure and by the electrophoretic procedure with the measurement of the LDL cholesterol concentration in polyacrylamide gel by means of a densitometer was 0.95 [101].

In 1987, Gillespy and Terry questioned Speich on the previously reported relationship between the concentrations of total cholesterol and calcium in blood plasma of female patients after a myocardial infarction (Table 15) [102].

Pelletler et al. proposed a chromatography–mass spectrometric procedure for determining the total cholesterol concentration in blood serum in 1987. Serum was mixed with a solution containing [3,4-^{13}C]-labeled cholesterol (used as an internal standard), potassium hydroxide, surfactant Triton X-100, and ethanol. The resulting mixture was kept at 70°C for 1 h for the hydrolysis of β cholesterol yielding α cholesterol. After cooling to room temperature, 2-mL portions of hexane and 1-mL portions of distilled water were added three times each to the mixture, and extraction was performed. Then, 1 mL of the hexane extract was evaporated under nitrogen flow, and the residue was treated with *N*,*O*-bis(trimethylsilyl)acetamide. The formed solution (1 μL) was injected into a gas chromatograph equipped with a column with polymethylsiloxane (0.31 mm × 25 m) and a mass spectrometric detector. Helium was the carrier gas; the

Table 16. Effect of ascorbic acid on the determinations of HDL cholesterol in blood serum samples

Procedure	Average decrease in the determination of HDL cholesterol in serum, %, with the concentration of ascorbic acid, mg/L		
	20	30	50
1	0.3	4.7	8.5
2	0.7	1.1	3.3
3	9.8	14.4	25.8
4	10.0	15.9	27.8
5	7.8	14.5	27.0
6	1.9	2.3	5.2

flow rate of the carrier gas was 0.8 mL/min. The mass spectrometric detector recorded compounds that corresponded to α cholesterol and [3,4-^{13}C]-labeled cholesterol. The compounds corresponded to α cholesterol was recorded at m/z 368.5, and the compound fit with [3,4-^{13}C]-labeled cholesterol, at m/z 370.5. Then, the total cholesterol concentration in the serum was calculated from the measurement results. The mean-square deviation of the relative error of determination of the total cholesterol concentration of 3.2, 3.72, 4.18, 5.15, 5.73, 7.75, and 7.9 mM in the serum was estimated at 0.32, 0.33, 0.41, 0.37, 0.46, 0.49, and 0.31% [103].

In 1987, Moshides studied the effect of ascorbic acid on the determination of the HDL cholesterol concentration in blood serum by six enzymatic spectrophotometric procedures:

• Two procedures using VLDL and LDL sedimentation with solutions of heparin and manganese(II) (Procedures 1 and 2);

• A procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium(II) (Procedure 3);

• A procedure using VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II) (Procedure 4);

• Two procedures using VLDL and LDL sedimentation with a solution of polyethylene glycol (Procedures 5 and 6).

In contrast to Procedure 1, Procedure 2 used a solution with a higher concentration of manganese(II), and, in distinction from Procedure 5, the polyethylene glycol solution in Procedure 6 had a pH level of 10. In all of these procedures, the HDL cholesterol concentration in the centrifuged liquid was determined based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 2,4,6-tribromo-3-hydroxybenzoic acid, and to form a colored compound (Product) under the catalytic action of peroxidase (EC 1.11.1.7)

$$2H_2O_2 + \text{4-aminophenazone} + \text{BHBA} \xrightarrow{\text{Peroxidase}} \text{Product} + HBr + 2H_2O, \quad (10)$$

where BHBA is 2,4,6-tribromo-3-hydroxybenzoic acid.

The experimental data on the effect of ascorbic acid on the determination of HDL cholesterol in blood serum samples by Procedures 1–6 are presented in Table 16. It is clear that the smallest effect of ascorbic acid was observed in Procedure 1 at a concentration of 20 mg/L and in Procedure 2 at a concentration of 30 and 50 mg/L [104].

In 1987, Rotterdam et al. used spectrophotometric procedures for determining the concentrations of total and HDL cholesterol in serum using Liebermann–Burchard reagent for their medical studies [105].

In 1987, Ng and Altaffer studied the characteristics of an enzymatic spectrophotometric procedure for determining the HDL cholesterol concentration in blood plasma and serum. A 0.5-mL portion of plasma or serum was sampled into a tube containing dextran sulfate and magnesium(II). The resulting mixture was maintained at room temperature for 5 min and then centrifuged for 5 min to remove sedimented VLDL and LDL. The HDL cholesterol concentration was measured in the centrifuged liquid using solutions of enzymes. The mean-square deviation of the relative error of determination of the HDL cholesterol

concentration in plasma and serum was 3.2–3.6%. The measurements of the HDL cholesterol concentration in plasma and serum samples according to the studied procedure were in agreement with the results obtained by the procedure using VLDL and LDL sedimentation with solutions of heparin and manganese(II); the correlation coefficients were 0.992 (76 plasma samples) and 0.975 (79 serum samples) [106].

In 1987, Koch et al. compared the determinations of the total cholesterol concentration in plasma, serum, and whole blood by five enzymatic procedures. Procedures 1 and 2 were spectrophotometric, and Procedures 3–5 used reflection photometry and test strips. All of the procedures were based on chemical reactions proceeding under the catalytic action of cholesterol esterase (EC 3.1.1.13), cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7). For Procedure 1, the mean-square deviations of the relative error of the quantification of total cholesterol in blood serum of 867.2, 1454.4, 1890.6, 2458.7, 3181.8, and 3372.4 mg/L (2.24, 3.76, 4.89, 6.36, 8.23, and 8.72 mM) were 4.3, 1.8, 2.5, 1.5, 1.6, and 1.9%, respectively. In Procedure 2, the mean-square deviation of the relative error of determinations of total cholesterol at concentrations of 917.8, 1436.3, 1848.2, 2397.4, 3104.4, and 3317.8 mg/L (2.37, 3.71, 4.78, 6.2, 8.03, and 8.58 mM) in serum were estimated at 3.3, 2.4, 2.2, 2.3, 2.5, and 2.8%, respectively. For Procedure 3, the mean-square deviations of the relative error of quantifications of total cholesterol in blood serum of 911.4, 1506.2, 1924.3, 2542.1, 3014.8, and 3391.4 mg/L (2.36, 3.9, 4.98, 6.57, 7.8, and 8.77 mM) were 7.1, 3.9, 5.6, 4.5, 4.1, and 3.9%, respectively. In using Procedure 4, the mean-square deviations of the relative error of determinations of total cholesterol at a concentration of 1425.2, 1765.9, 2426.3, 2916.3, and 3103.7 mg/L (3.69, 4.57, 6.28, 7.54, and 8.03 mM) in blood serum were 3.8, 2.7, 2.3, 3.1, and 3.4%, respectively. For Procedure 5, the mean-square deviations of the relative error of determinations of total cholesterol at a concentration of 991.8, 1492.2, 1889.6, 2612.6, 3229.6, and 3523.7 mg/L (2.57, 3.86, 4.89, 6.76, 8.35, and 9.11 mM) in serum were 4.3, 2.2, 2, 2, 1.3, and 1.6%, respectively. The correlation coefficient for the determinations of the total cholesterol concentration in the plasma, serum, and whole blood samples by the laboratory procedure using Liebermann–Burchard reagent and Procedure 1 was 0.98–0.99; it was 0.97–0.98 for the laboratory procedure and Procedure 2, 0.95–0.96 for the laboratory procedure and Procedure 3, 0.95–0.97 for the laboratory procedure and Procedure 4, and 0.99 for the laboratory procedure and Procedure 5 [107].

In 1988, Kroll et al. studied the characteristics of four enzymatic procedures for determining the total cholesterol concentration in blood serum. The mean-square deviation of the relative error of measurements of the total cholesterol concentration in serum by Procedures 1 and 2 did not exceed 2.6%; it corresponded to 2.8–3.9% for Procedure 3 and was estimated at 2.4–5.3% for Procedure 4. The determinations of the total cholesterol concentration in serum according to the procedures under study were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent [108].

In 1988, Bowers pointed to the need for clinical diagnostic laboratories to obtain accurate determinations of the total cholesterol concentration in the blood of patients (with a random measurement error less than 3%, and the systematic error did not exceed 3%), especially in cases where the total cholesterol concentration in blood is in the range from 2000 to 2400 mg/L (from 5.17 to 6.21 mM) [109].

In 1988, Bowers also reported that, depending on the measured concentration values of total and LDL cholesterol in the blood, patients could be divided into three groups characterized by different degrees of risk of cardiovascular disease (Table 17). Because of this, the said indicators should be determined with high accuracy in clinical diagnostic laboratories. However, by 1985, the mean-square deviation of the relative error of measurements of the total cholesterol concentration in plasma and serum by procedures using Liebermann–Burchard reagent without prior extraction of cholesterol was 7.3%; it was 6.6% for procedures using Liebermann–Burchard reagent with prior extraction of cholesterol, 5.6% for procedures using Liebermann–Burchard reagent implemented in flow automated devices, and 2.2–13.7% for enzymatic procedures. At the same time, there was a tendency to enhance the accuracy of the determination of the total cholesterol concentration in clinical diagnostic laboratories. In particular, the mean difference between the measurement results of the total cholesterol concentration obtained in different laboratories corresponded to 23.7% in 1949, 18.5% in 1969, 11.1% in 1980, 6.4% in 1983, and 6.2% in 1986. The mean difference between the determinations of the total cholesterol concentration (by both enzymatic and nonenzymatic procedures) conducted in a separate laboratory was estimated at 4.1% in 1975, 3.8% in 1980, 3.6% and in 1985 [110].

In 1988, Råstam et al. examined an enzymatic reflectance photometry procedure for determining the total cholesterol concentration in plasma, serum, and whole blood. Plasma, serum, or whole blood was applied to the surface of a test strip containing cholesterol esterase (EC 3.1.1.13), cholesterol oxidase (EC 1.1.3.6), and peroxidase (EC 1.11.1.7). Then, the test strip was placed in a special measuring device, and the total cholesterol concentration was measured after 3 min. The mean-square deviation of the rel-

Table 17. Groups of patients with varying degree of cardiovascular risk

Degree of cardiovascular risk	Total cholesterol concentration in blood, mg/L (mM)	LDL cholesterol concentration in blood, mg/L (mM)
Low	less than 2000 (5.17)	less than 1300 (3.36)
Medium	2000–2400 (5.17–6.21)	1300–1600 (3.36–4.14)
High	more than 2400 (6.21)	more than 1600 (4.14)

ative error of measurements of the total cholesterol concentration was 3.1% in plasma and 3.2% in serum. The determinations of the total cholesterol concentration in plasma and whole blood samples according to the tested procedure were in agreement with the results obtained by the spectrophotometric procedure using Liebermann–Burchard reagent; the correlation coefficient was 0.991 for plasma and 0.973–0.979 for whole blood [111].

In 1988, Naito, based on the dependence of the degree of risk of cardiovascular diseases on the concentration of total and LDL cholesterol in blood (Table 17), stressed the importance of decreasing the error in measurements of the concentrations of total and HDL cholesterol in blood, which are often used in determining LDL cholesterol according to a formula developed by Friedewald et al. [112].

In 1988, Abu-Farsakh et al. determined the concentrations of total cholesterol and calcium in serum of 232 female patients and 123 male patients. The total cholesterol concentrations in serum samples were measured by an enzymatic procedure. In distinction from the conclusions of Speich (Table 15), Abu-Farsakh et al. actually found no relationship between these indicators. Thus, the correlation coefficient for the determinations of total cholesterol and calcium concentrations corresponded to 0.13 in the serum samples of 123 male patients, 0.039 in the serum samples of 232 female patients, and only 0.002 in the all samples [113].

In 1988, Kinter et al. proposed a chromatography–mass spectrometric procedure for determining the total cholesterol concentration in blood serum. Serum (500 μL) was mixed with 500 μL of a solution of 7,(5α)-cholestean-3β-ol (a structural isomer of cholesterol that was used as an internal standard), 600 μL of a potassium hydroxide solution (8.9 M), and 5 mL of ethanol. The resulting mixture was kept at 37°C for 3 h for the hydrolysis of β cholesterol yielding α cholesterol. Then, 10 mL of hexane was added for extraction. After evaporation of the hexane extract, the residue was dissolved in 5 mL of methanol. A 100-μL portion of the solution was evaporated again, and the residue was dissolved in a mixture of 100 μL of dichloromethane and 100 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide containing trimethylchlorosilane (10 g/L). The resulting mixture was kept at 60°C for 0.5 h to complete the chemical interaction of α cholesterol and 7,(5α)-cholesten-3β-ol and then evaporated; the residue was dissolved in 500 μL of tetradecane. The emerging solution (1 μL) was injected into a gas chromatograph equipped with a capillary column (0.32 mm × 30 m) and a mass spectrometric detector, which recorded the compounds corresponding to α cholesterol and 7,(5α)-cholesten-3β-ol at m/z 458.394. The linear range of the total cholesterol concentration in serum was from 0 to 10 000 mg/L (from 0 to 25.86 mM). The relative systematic error of determinations of the total cholesterol concentration in serum of 1423 mg/L (3.68 mM) corresponded to approximately 0.6%. The mean-square deviation of the relative error of measurements of the total cholesterol concentration of 1423 mg/L (3.68 mM) in the serum was 1.6% [114].

In 1988, Tiedink and Katan found a change in the concentration of VLDL, LDL, HDL_2, and HDL_3 cholesterol in blood serum upon storage in the frozen state (–20°C). The concentrations of these compounds were measured according to the procedure with the separation of serum lipoproteins by ultracentrifugation, followed by enzymatic determination of the cholesterol concentrations (the mean-square deviation of the relative error was 1.3% for the cholesterol concentration of 4.8 mM and 0.9% for the concentration of 8.96 mM. It was found that the storage of blood serum for 27 weeks resulted in a change in the concentrations of VLDL, LDL, HDL_2, and HDL_3 cholesterol by less than 4.1% [115].

In 1988, Peddicord and Barnes investigated the effect of ascorbic acid on the determination of the total cholesterol concentrations in serum by enzymatic procedures using solutions of peroxidase (EC 1.11.1.7). The presence of ascorbic acid in serum at a concentration of 0.5–1 mM was accompanied by a decrease in the measurements of total cholesterol by 10–25%. Note that such a significant effect of ascorbic acid on the determination results for total cholesterol concentration in serum was absent in the procedure using Liebermann–Burchard reagent [116].

In 1988, Howes et al. applied a procedure for determining the total cholesterol concentration in blood plasma in their medical studies. It was found that, by changing the position of a patient from horizontal to vertical, the total cholesterol concentration in plasma increased by an average of 19.3% [117].

In 1988, Katan et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips designed for the determination of the total cholesterol concentration in whole blood. These measurement results demonstrated a relative systematic error of determination the total cholesterol concentration in blood of 8–12%, which was caused by poor calibration of a specialized measuring device [118].

In 1988, Boerma et al. compared the measurements of the total cholesterol concentration in 200 blood serum samples by the following procedures:

• A spectrophotometric procedure using Liebermann–Burchard reagent (Procedure 1);

• A spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase (Procedure 2);

• An enzymatic reflectance photometry procedure using test strips (Procedure 3).

The correlation coefficients for the quantifications of total cholesterol in serum corresponded to 0.994 for Procedures 1 and 2 and 0.989 for Procedures 1 and 3 and Procedures 2 and 3 [119].

In 1988, Speich explained that the previously published data on the relationship of the concentrations of total cholesterol and calcium in the blood plasma (Table 15), which was criticized by Gillespy and Terry and Abu-Farsakh et al., related only to patients living in the French province of Nantes [120].

In 1988, McMillan and Warnick assessed the reliability of the determinations of total cholesterol and HDL cholesterol in blood plasma samples obtained in nine clinical diagnostic laboratories. When measuring the total cholesterol concentration in plasma of 1640–3180 mg/L (4.24–8.22 mM), 19% of the assessed results did not meet the set requirement that the relative error of determination should be less than 9%. In the measurement of the HDL cholesterol concentration of 220–830 mg/L (0.57–2.17 mM), 39% of the results did not meet the requirement that the absolute error of determination should not exceed 50 mg/L (0.13 mM) [121].

In 1988, Moshides developed an enzymatic spectrophotometric procedure for determining the concentration of α cholesterol in HDL of blood plasma. The procedure was based on the following chemical reactions:

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide, 4-aminophenazone, and 2,4,6-tribromo-3-hydroxybenzoic acid, and to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7).

Plasma (200 μL) was mixed with 200 μL of a glycine–sodium hydroxide buffer solution (200 mM, pH 10) containing polyethylene glycol (200 g/L). The resulting mixture was maintained at 20–25°C for 10 min and then centrifuged at 4°C for 20 min to remove sedimented VLDL and LDL. One milliliter of a phosphate buffer (100 mM, pH 6.35) containing 4-aminophenazone, 2,4,6-tribromo-3-hydroxybenzoic acid, and peroxidase and 50 μL of a phosphate buffer solution (100 mM; pH 6.35) containing cholesterol oxidase were added to 100 μL of the centrifuged liquid. After keeping the resulting mixture at room temperature for 10 min, its absorbance was measured at a wavelength of 515 nm (the optical path length was 1 cm). The absorbance of the mixture was linearly dependent on the concentration of α cholesterol in HDL of blood plasma up to 400 mg/L (1.03 mM). The mean-square deviation of the relative error of was 4.9% for quantifications of α cholesterol in HDL of blood plasma of 59 mg/L (0.15 mM), 2.4% for 105 mg/L (0.27 mM), and 2.2% for 149 mg/L (0.39 mM). The presence of urea (up to 2 g/L) and sodium azide (up to 5 g/L) in plasma did not change the measurements of the concentrations of α cholesterol in HDL. The presence of creatinine (up to 100 mg/L), bilirubin (up to 100 mg/L), uric acid (up to 200 mg/L), salicylate (up to 1 g/L), hemoglobin (up to 2 g/L), and glucose (up to 4 g/L) did not affect the determination of the concentrations of α cholesterol in HDL, but the presence of L-dopa (10 mg/L), α-methyldopa (50 mg/L), gentisic acid (50 mg/L), ascorbic acid (50 mg/L), and dithio-bis(nitrobenzoic) acid (600 mg/L) in plasma had a rather significant effect [122].

Howes and Krum applied procedures for determining the concentration of total cholesterol, HDL cholesterol, and LDL cholesterol in blood plasma in their medical studies in 1988. The mean-square deviation of the relative error of measurements of the total cholesterol concentration was 2.3%, and it was 5.8% for the concentration of HDL cholesterol [123].

Table 18. Determination of total cholesterol in six blood serum samples using test strips of four batches

Serum sample (total cholesterol concentration, mM)	Result of determination of total cholesterol, mM, using test strips				Maximum difference between the measurement results, mM (%)
	Batch 1	Batch 2	Batch 3	Batch 4	
1 (4.30)	4.35	4.07	4.16	4.19	0.28 (6)
2 (4.79)	5.48	5.14	5.10	5.26	0.38 (6)
3 (4.94)	4.68	4.52	4.58	4.54	0.16 (3)
4 (4.94)	4.66	4.46	4.54	4.56	0.20 (4)
5 (5.53)	5.51	5.29	5.27	5.42	0.24 (4)
6 (6.91)	7.37	7.11	7.02	7.45	0.43 (6)

In 1988, Kroll et al. analyzed the potential of two spectrophotometric procedures and two reflectance photometry procedures using test strips for the quantification of total cholesterol in blood determining the degree of cardiovascular risk [124].

In 1988, Speich et al. studied changes in the concentrations of total cholesterol and HDL cholesterol in blood plasma of patients who were in the preinfarct and postinfarct state. The total cholesterol concentration in plasma was determined by a spectrophotometric procedure using solutions of 4-aminophenazone, phenol, cholesterol esterase, cholesterol oxidase, and peroxidase, and the HDL cholesterol concentration was found by the procedure with the precipitation of VLDL and LDL with solutions of phosphotungstic acid and magnesium(II) [125].

In 1988, Boerma et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips designed for the determination of the total cholesterol concentration in plasma, serum, and whole blood. The volume of plasma, serum, or whole blood required for analysis was 30 μL. The mean-square deviation of the relative error of measurement of the total cholesterol concentration in serum of was estimated at 2.3–3.3%; in whole blood, it was 1.6–3.4%. When using test strips from different batches, the maximum divergence between the determinations of the total cholesterol concentration in the blood serum samples was 6% (Table 18) [126].

In 1988, Boyd reported that the relative systematic error of determinations of the total cholesterol concentration in blood by the spectrophotometric procedure using Liebermann–Burchard reagent implemented in an automated flow device could be as small as 1.7%, while this value for an automated enzymatic spectrophotometric procedure could be less than 1.3%. At the same time, according to the data for 1985, the measurements of the total cholesterol concentration in blood obtained in approximately a half of clinical diagnostic laboratories in the United States were characterized by a relative systematic error exceeding 5% [127].

In 1988, Kroll et al. indicated the desirability of decreasing the relative systematic error in determination of the total cholesterol concentration in blood determining the risk of developing cardiovascular diseases [128].

In 1988, Dias et al. examined two procedures for determining the HDL cholesterol concentration in serum (Procedures 1 and 2) and three procedures for determining the HDL_2 and HDL_3 cholesterol concentrations in serum (Procedures 3–5). In Procedure 1, 1500 μL of serum was mixed with 500 μL of a glycine–sodium hydroxide buffer solution (200 mM; pH 10) containing polyethylene glycol (200 g/L), and the resulting mixture was centrifuged for 5 min to remove sedimented VLDL and LDL. According to Procedure 2, 500 μL of a solution containing phosphotungstic acid (310 mM) and magnesium(II) (14.2 mM) was added to 200 μL of serum, and the mixture was centrifuged for 30 min to remove sedimented VLDL and LDL. In Procedure 3, 500 μL of serum was treated with 500 μL of a glycine–sodium hydroxide buffer solution (200 mM; pH 10) containing polyethylene glycol (200 g/L), and the resulting mixture was centrifuged for 5 min to remove sedimented VLDL and LDL. Forty microliters of a glycine–sodium hydroxide buffer solution (200 mM, pH 10) containing dextran sulfate (7.2 g/L) and magnesium chloride (500 mM) was added to 360 μL of the centrifuged liquid. The resulting mixture was kept at room temperature for 30 min and centrifuged for 5 min to remove sedimented HDL_2. According to Procedure 4, 500 μL of serum was mixed with 500 μL of a glycine–sodium hydroxide buffer solution (200 mM; pH 10) containing polyethylene glycol (200 g/L), and the resulting mixture was centrifuged for 5 min to remove sedimented VLDL and LDL. Sixty microliters of a solution containing sodium chloride and potassium bromide (density, 1.351 g/mL) was added to 120 μL of the centrifuged liquid; the mixture was ultracentrifuged

Table 19. Average determinations of LDL cholesterol in serum samples

Serum samples	Total cholesterol concentration	Concentration of triglycerides	Average LDL cholesterol concentration in serum, mM, by Procedure					
			1	2	3	4	5	6
Group 1	Normal	Normal	3.58	3.68	3.60	3.59	3.81	3.63
Group 2	Normal	High	3.00	3.31	3.30	3.30	3.12	3.33
Group 3	High	Normal	5.98	6.10	6.00	6.00	6.30	6.15
Group 4	High	High	4.89	5.26	5.28	5.41	5.06	5.07

for 3.5 h for the separation of HDL_3 and HDL_2. In accordance with Procedure 5, blood serum was ultracentrifuged for the separation of VLDL, LDL, HDL_2 and HDL_3. The concentrations of HDL cholesterol in the centrifuged liquid (Procedures 1–4), HDL_3 cholesterol in the centrifuged liquid (Procedure 3), HDL_2 cholesterol in the ultracentrifuged liquid (Procedure 4), and HDL_2 and HDL_3 cholesterol in the ultracentrifuged liquid (Procedure 5) were determined by the enzymatic spectrophotometric procedure, and the results were recalculated to find the concentrations of HDL, HDL_2, and HDL_3 cholesterol in serum (C_{HDL}, C_{HDL2}, and C_{HDL3}). The HDL_2 cholesterol concentration in serum in Procedure 3 was calculated using equation $C_{HDL2} = C_{HDL} - C_{HDL3}$, while the HDL_3 cholesterol concentration by Procedure 4 was found by equation $C_{HDL3} = C_{HDL} - C_{HDL2}$. The mean-square deviation of the relative error of determination of the HDL cholesterol concentration of 1.16 mM in serum was 3.8% by Procedure 1 and 2.9% for the concentration of 1.35 mM by Procedure 2. The mean-square deviation of the relative error of determination of HDL_2 cholesterol concentration of 0.5 mM by Procedure 3 in serum corresponded to 8.6%; it was 7.5% for the concentration of 0.46 mM by Procedure 4 and 10.2% for the concentration of 0.51 mM by Procedure 5. The mean-square deviation of the relative error of measurements of the HDL_3 cholesterol concentration in serum of 0.66 mM by Procedure 3 was estimated to be 8.8%; it was 5.8% for the concentration of 0.57 mM by Procedure 4 and 8.6% for the concentration of 0.69 mM by Procedure 5. The correlation coefficient for the determinations of the HDL cholesterol concentration in 121 blood serum samples according to Procedures 1 and 2 was 0.99. The correlation coefficients between the determinations of HDL_2 cholesterol in 52 serum samples by Procedures 3 and 4 corresponded to 0.92; in 38 serum samples by Procedures 3 and 5, it was 0.91. The correlation coefficients between the determinations of HDL_3 cholesterol in 52 serum samples by Procedures 3 and 4 was estimated at 0.95; and it was 0.93 in 38 serum samples by Procedures 3 and 5 [129].

In 1988, Cocco et al. proposed a procedure for determining the HDL cholesterol concentration in blood serum similar to that developed by Lippi et al. in 1986. One milliliter of a sodium chloride solution containing polyethylene glycol, dextran sulfate, and magnesium chloride was added to 0.1 mL of serum. The resulting mixture was kept at room temperature and then centrifuged to remove sedimented VLDL and LDL. The mean-square deviation of the relative error of determinations of the HDL cholesterol concentration in serum was less than 2% [130].

In 1988, Cohn et al. used in their medical studies an enzymatic procedure for determining the total cholesterol concentration in plasma, a procedure for measuring the HDL cholesterol concentration in plasma with VLDL and LDL sedimentation with solutions of dextran sulfate and magnesium(II), and a procedure with ultracentrifugation [131].

In 1988, Rao et al. compared the determination of the LDL cholesterol concentration in 196 blood serum samples by the following procedures (Table 19):

- Five procedures in which the LDL cholesterol concentration in serum was calculated from the measurement results for total cholesterol, HDL cholesterol, and triglycerides (Procedures 1–5);
- A procedure using ultracentrifugation (Procedure 6).

In Procedures 1–5, the total cholesterol concentration in serum was determined by an enzymatic procedure, while the HDL cholesterol concentration was found by an enzymatic procedure with the precipitation of VLDL and LDL with a solution of phosphotungstic acid. According to Procedure 6, the LDL cholesterol concentration in serum was measured by an enzymatic procedure after ultracentrifugation. The correlation coefficients between the determinations of LDL cholesterol in serum samples by Procedures 1–5 and Procedure 6 corresponded to 0.93–0.937 [132].

In 1988, Sedor et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips designed for the determination of the total cholesterol concentration in plasma, serum, and whole blood. The procedure was based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. (5));

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

• Interaction of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine to form a colored compound (Product) under the catalytic action of peroxidase (EC 1.11.1.7)

$$2H_2O_2 + 3,3',5,5'\text{-tetramethylbenzidine} \xrightarrow{\text{Peroxidase}} \text{Product} + H_2O. \quad (11)$$

Plasma, serum, or whole blood (30 μL) was applied to the surface of a test strip. The top (fiberglass) layer of the test strips retained erythrocytes. The test strip was then placed into a specialized measuring device, and the reflectance of it surface of radiation at 642 nm was measured. The output signal of the device depended linearly on the total cholesterol concentration in blood plasma and serum up to 3350 mg/L (8.66 mM). The mean-square deviation of the relative error was 2.3% for determinations of the total cholesterol concentration of 1740 mg/L (4.5 mM) in plasma and 2.6% for the concentration of 1750 mg/L (4.53 mM). The mean-square deviation of the relative error of measurements of the total cholesterol concentration of 1690 mg/L (4.37 mM) in serum was 1.8%. The mean-square deviation of the relative error of determinations of the total cholesterol concentration of 1800 mg/L (4.66 mM) in whole blood corresponded to 1.8%, and it was 2.4% for the concentration of 2240 mg/L (5.79 mM). The presence of triglycerides (up to 14.7 g/L), heparin (up to 108 kIU/L), and bilirubin (up to 30 mg/L) in plasma, serum, or whole blood did not affect the measurements of the concentration of total cholesterol. The presence of bilirubin at a concentration to 200 mg/L in serum and whole blood decreased the determination results by 33%, while the presence of hemoglobin at a concentration of 8 g/L lowered the results by 25%. The measurements of the total cholesterol concentration in plasma and serum samples according to the procedure under study were in agreement with the results obtained by another enzymatic reflectance photometry procedure using test strips; the correlation coefficients were 0.95 (204 plasma samples) and 0.955 (125 serum samples) [133].

In 1989, Patsch et al. studied the characteristics of an enzymatic spectrophotometric procedure for determining the concentration of HDL, HDL_2, and HDL_3 cholesterol in blood plasma. One hundred microliters of a solution containing dextran sulfate (10 g/L) and magnesium chloride (0.5 M) was added to 1 mL of plasma. The resulting mixture was maintained at room temperature for 10 min and then centrifuged for 15 min to remove sedimented VLDL and LDL. Fifty microliters of a solution containing dextran sulfate (10 g/L) and magnesium chloride (1.5 M) was added to 0.5 mL of the centrifuged liquid. The mixture was kept at room temperature for 10 min and centrifuged for 20 min to remove sedimented HDL_2. The concentrations of HDL and HDL_3 cholesterol, respectively, and the first and second centrifuged liquids were measured by the procedure using a Tris–HCl buffer solution containing magnesium aspartate, 4-aminophenazone, phenol, 3,4-dichlorophenol, sodium cholate, cholesterol esterase, cholesterol oxidase, and peroxidase. The mean-square deviation of the relative error of determinations of the HDL cholesterol concentration of 451–822 mg/L (1.17–2.13 mM) in plasma was estimated at 2.7–3.4%. The mean-square deviation of the relative error of measurements of the HDL_3 cholesterol concentration of 267–425 mg/L (0.69–1.1 mM) in plasma corresponded to 6.9–9.0%. The mean-square deviation of the relative error of determinations of the HDL_2 cholesterol concentration of 184–398 mg/L (0.48–1.03 mM) in plasma was 7.9–12.8%. The correlation coefficients between the measurements of the concentrations of HDL, HDL_2, and HDL_3 cholesterol in 39 plasma samples by the procedure under examination and the procedure with ultracentrifugation were assessed at the level of 0.957, 0.936, and 0.825 [134].

In 1989, Sokoll and Dawson-Hughes studied the relationship of the total cholesterol concentration in the blood plasma of female patients with the concentration of ionized calcium and the total calcium content in serum [135].

Warnick et al. studied the characteristics of two enzymatic spectrophotometric procedures for determining the total cholesterol concentration in blood serum in 1989. The procedures were based on the following chemical reactions:

• Hydrolysis of β cholesterol with the formation of α cholesterol and fatty acids by the catalytic action of cholesterol esterase (EC 3.1.1.13) (Eq. 5);

• Oxidation of α cholesterol to form cholest-4-en-3-one and hydrogen peroxide under the catalytic action of cholesterol oxidase (EC 1.1.3.6) (Eq. (3));

Table 20. Determination of total cholesterol in two plasma samples in the presence of cyclosporine

Cyclosporine concentration, mg/L	Determination of total cholesterol, mM			
	Blood plasma 1		Blood plasma 2	
	Procedure 1	Procedure 2	Procedure 1	Procedure 2
0	4.65	4.56	6.59	6.41
0.625	4.62	4.59	6.57	6.35
1.25	4.67	4.55	6.59	6.39
2.5	4.63	4.54	6.56	6.39

• Interaction of hydrogen peroxide, 4-aminophenazone, and phenol to form a colored compound under the catalytic action of peroxidase (EC 1.11.1.7) (Eq. (6)).

The relative systematic error of measurement of the total cholesterol concentration in serum was 2–3% by one procedure and 1% by the other procedure. The mean-square deviation of the relative error of determinations of the total cholesterol concentration in serum corresponded to 0.5–1.5% for the first procedure and 1.0–1.8% for the second procedure. The correlation coefficients between the measurement results of the total cholesterol concentrations in serum samples by these procedures and by the spectrophotometric procedure using Liebermann–Burchard reagent were estimated at 0.998-0.999 [136].

In 1989, Rosenfeld noted that it is important for clinical diagnostic laboratories to obtain accurate determinations of the total cholesterol concentration in blood [137].

In 1989, Roche et al. determined the mean-square deviation of the relative error of measurements of the HDL cholesterol concentration in the blood serum samples by the following enzymatic procedures:

• A procedure using VLDL and LDL sedimentation with solutions of phosphotungstic acid and magnesium chloride (Procedure 1);

• A procedure with VLDL and LDL sedimentation using a solution of polyethylene glycol (Procedure 2).

The mean-square deviation of the relative error of determinations of the HDL cholesterol concentration in serum by Procedure 1 corresponded to 3.9%; by Procedure 2, it was 4.2%. A change in the phosphotungstic acid concentration from 35 to 50 g/L, in the magnesium chloride concentration from 1 to 2.5 M, or in pH from 5.2 to 6.2 had no effect on the measurement results for the HDL cholesterol concentration in serum by Procedure 1. A change in the polyethylene glycol concentration from 80 to 120 g/L or in pH from 9 to 10.5 did not affect the quantification results of HDL cholesterol in serum by Procedure 2 [138].

In 1989, Branford et al. proposed a sample for quality control of measurements of the total cholesterol concentrations in whole blood by an enzymatic reflectance photometry procedure using test strips. The lifetime of the sample corresponded to approximately two months [139].

In 1989, Kroll et al. studied samples for quality control of the determination of the total cholesterol concentrations in blood serum by four enzymatic spectrophotometric procedures (Procedures 1–4) and by an enzymatic reflectance photometry procedure using test strips (Procedure 5). The use of the samples under consideration was accompanied by the presence of the relative systematic error of measurements of the total cholesterol concentration in serum up to 0.7% (Procedure 1), 1.7% (Procedure 2), 9.4% (Procedure 3), 14.3% (Procedure 4), and 5.7% (Procedure 5) [140].

In 1989, Bachorik et al. studied the characteristics of an enzymatic reflectance photometry procedure using test strips designed for the determination of the total cholesterol concentration in blood plasma. The relative systematic error of determinations of the total cholesterol concentration in plasma was 0.8–7.8%. The mean-square random error of determinations of the total cholesterol concentration in plasma was less than 4.3%. The correlation coefficients for the determination of total cholesterol in plasma samples by the procedure under study and by four laboratory enzymatic procedures corresponded to 0.92–0.96 [141].

In 1989, Karge et al. studied the characteristics of an enzymatic procedure for determining the total cholesterol concentration and HDL cholesterol in blood serum. The HDL cholesterol concentration in the centrifuged liquid was measured after VLDL and LDL sedimentation with solutions of heparin and manganese(II). The mean-square deviation of the relative error was estimated at 1.7% for determinations of the total cholesterol concentration of 1670 or 2120 mg/L (4.32 or 5.48 mM) in serum and 1.4% for the concentration of 2780 mg/L (7.19 mM). The mean-square deviation of the relative error of measurements of the HDL cholesterol concentration in serum of 498 mg/L (1.29 mM) was 2.4% [142].

Table 21. Determination of HDL cholesterol in two plasma samples in the presence of cyclosporine

Cyclosporine concentration, mg/L	Determination of HDL cholesterol, mM			
	Blood plasma 1		Blood plasma 2	
	Procedure 1	Procedure 2	Procedure 1	Procedure 2
0	0.52	0.58	1.21	1.26
0.625	0.51	0.57	1.22	1.25
1.25	0.53	0.58	1.23	1.24
2.5	0.51	0.56	1.22	1.26

In 1989 Sgoutas and Abbott investigated the effects of cyclosporine pharmaceutical preparation on the determination of

• The total cholesterol concentration in blood plasma (Table 20) by two enzymatic spectrophotometric procedures (Procedures 1 and 2);

• The HDL cholesterol concentrations in plasma (Table 21) by two procedures using VLDL and LDL sedimentation with a solution based on phosphotungstic acid (Procedure 3) or solutions of dextran sulfate and magnesium(II) (Procedure 4).

The mean-square deviation of the relative error of measurements of the total cholesterol concentration in plasma was 3.8% by Procedure 1 and 1.8% by Procedure 2. The mean-square deviation of the relative error of determinations of the HDL cholesterol concentration in plasma by Procedures 3 and 4 was estimated at 3.6%. According to the data of Tables 20 and 21, the presence of cyclosporine in the plasma samples (up to 2.5 mg/L) had virtually no effect on the measurements of the concentrations of total cholesterol and HDL cholesterol by these procedures [143].

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