

Triple Lipid Screening Test: A Homogeneous Sequential Assay for HDL-Cholesterol, Total Cholesterol, and Triglycerides

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Background: The analysis of lipids in serum lipoprotein fractions is useful in assessing the risk for coronary artery disease, but it typically involves performing multiple tests. An automated single-tube assay, referred to as the triple lipid screening (TLS) test, can be used for measuring HDL-cholesterol (HDL-C), total cholesterol, and triglycerides (TGs) with no specimen pretreatment.

Methods: The first part of the assay is based on a homogeneous assay for HDL-C that uses either an anti-apolipoprotein B antibody (TLS-A test) or a polyanion (TLS-B test) that blocks the enzymatic measurement of cholesterol on the non-HDL fraction. After the addition of deoxycholate, which solubilizes the unreacted cholesterol from the non-HDL fraction, the remaining cholesterol in the sample is subsequently measured enzymatically. Using the same enzyme detection system as the cholesterol assay, TGs are measured in the last step, after the addition of the enzymes for the TG assay.

Results: The TLS assay (y) had acceptable analytic performance and compared favorably with standard tests (x) for each analyte: for HDL-C, TLS-A = $0.99x + 0.19$ ($R = 0.980$); TLS-B = $1.00x - 0.15$ ($R = 0.974$); for total cholesterol, TLS-A = $1.03x + 0.12$ ($R = 0.997$); TLS-B = $1.07x - 0.30$ ($R = 0.965$); and for TGs, TLS-A = $1.02x + 0.02$ ($R = 0.988$); TLS-B = $1.04x - 0.28$ ($R = 0.980$).

Conclusions: The TLS test is a single-tube homogeneous assay for the analysis of all of the major serum lipoprotein fractions and can be used as a simple screening test for the detection of hyperlipidemia.

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Cholesterol is transported in the blood on various types of lipoprotein particles that differ in their density and in their role in the pathogenesis of atherosclerosis (1). The current guidelines for the laboratory evaluation of hyperlipidemia recommend measuring the cholesterol content of the serum lipoprotein fractions for assessing the risk of coronary artery disease (2). HDL-cholesterol (HDL-C),¹ a negative risk factor for coronary artery disease (3–5), and LDL-cholesterol (LDL-C), a positive risk factor (1, 2), are the most commonly measured lipoprotein cholesterol fractions. Serum triglyceride (TG) concentrations are also often determined in the analysis of serum lipoproteins because they can be used for estimating LDL-C (6), and because of the role of TGs as a positive risk factor for coronary artery disease (7). Most laboratories analyze serum lipoproteins by performing three separate tests, namely tests for total cholesterol (TC), HDL-C, and TGs.

Because of the relatively high frequency of hyperlipidemia in the general population and because of the recommendation that all adults undergo periodic cholesterol screening (2), any procedure that would simplify and reduce costs for the analysis of serum lipoproteins would be advantageous. The recent development of homogeneous assays for lipoprotein cholesterol fractions that do not require any specimen preprocessing was a major advance in simplifying lipoprotein lipid analysis (8, 9). There have also been several reports of single-tube assays that involve the sequential enzymatic measurement of TC and TGs (10–12). These assays use a common enzyme detection system, but they sequentially measure TC and TGs by taking advantage of the lipid substrate specificity of the enzymes used in each part of the assay (10–12). We recently described a sequential test for HDL-C and TC (13), referred to as the dual HDL/TC test. Although these sequential test designs reduce the number of tests needed,

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¹ Nonstandard abbreviations: HDL-C and LDL-C, HDL- and LDL-cholesterol; TG, triglyceride; TC, total cholesterol; TLS, triple lipid screening; and apo, apolipoprotein.

none of them provides all of the necessary results for performing a complete analysis of the major lipoprotein cholesterol fractions.

In this report, we describe a new sequential test, the triple-lipid screening (TLS) test, that can be used for measuring HDL-C, TC, and TGs. The TLS test begins with a homogeneous HDL-C assay that uses either an antibody (TLS-A) or a polyanion (TLS-B) to block the measurement of cholesterol from the non-HDL fraction. After the addition of a detergent, TC and TGs are sequentially measured. The TLS test is performed in a single tube with no specimen pretreatment, can be readily automated, and compares favorably with standard lipoprotein lipid assays.

Materials and Methods

REAGENTS

Direct HDL-C (immunoinhibition based; EZ-HDL), direct LDL-C (EZ-LDL), and GPO-Trinder TG reagent sets, and deoxycholate, glycerol standards, ATP, lipase, glycerol kinase, glycerol phosphate oxidase, ascorbic acid, and a

lipid linearity set were obtained from Sigma. A polyanion-based, homogeneous assay for HDL-C (Liquid N-geneous HDL) was obtained from Genzyme. TG (glycerol blanked) and TC reagents for the Hitachi 917 were obtained from Roche. Reagents for a dextran precipitation-based method for HDL-C were purchased from Polymedco. Unconjugated bilirubin was obtained from Pfanstiehl Laboratories. Purified human HDL ($d = 1.125\text{--}1.21$ kg/L) and LDL ($d = 1.009\text{--}1.063$ kg/L) were obtained from EDTA-plasma by density gradient ultracentrifugation (14). Isolated lipoproteins were >95% pure, as judged by agarose gel electrophoresis (Helena Laboratories).

PROCEDURE FOR THE TLS TEST

The Cobas FARA II analyzer (Roche) was used to perform the TLS, direct LDL-C, and HDL-C tests by the Polymedco method. A description of the reagents and assay conditions for the two versions of the TLS test are shown in Table 1. The first part of the TLS-A test uses the R1 (antibody reagent) and R2 (cholesterol reagent) compo-

Table 1. Description of the TLS assay.

	Volume, μL	Time, min
TLS-A reagents		
Step 1A: Blocking of non-HDL		
Add sample (prediluted 1:10)	12.5	
Add R1: anti-human β -lipoprotein antibody, POD ^a (2400 U/L), 4AA (0.9 mmol/L), Good's buffer (30 mmol/L, pH 7.0)	225	4
Step 1B: HDL-C assay		
Add R2: CHE (7200 U/L), CO (7200 U/L), FDAOS (1.1 mmol/L), Good's buffer (30 mmol/L, pH 7.0)	75	5
Step 2: TC assay		
Add R3: 100 mmol/L deoxycholate	15	5
Step 3: TG assay		
Add R4: Lip (1 250 000 U/L), GK (6250 U/L), GPO (12 500 U/L), ATP (2.0 mmol/L), Good's buffer (30 mmol/L, pH 7.0)	50	5
TLS-B reagents		
Step 1A: Blocking of non-HDL		
Add sample	2.4	
Add R1: polyanion (0.1%), 4-AA (0.1%), ascorbic acid oxidase (5500 U/L), MES buffer (30 mmol/L, pH 6.5)	240	4
Step 1B: HDL-C assay		
Add R2: CHE (1500 U/L), CO (2000 U/L), POD (5500 U/L), HDL-selective detergent (1.0%), DSBmT (0.05%), MES buffer (30 mmol/L, pH 6.5)	80	5
Step 2: TC assay		
Add R3: 100 mmol/L deoxycholate	15	5
Step 3: TG assay		
Add R4: Lip (1 250 000 U/L), GK (6250 U/L), GPO (12 500 U/L), ATP (2.0 mmol/L), MES buffer (30 mmol/L, pH 6.5)	50	5
TLS reaction conditions		
Absorbance	600 nm (TLS-A)	
	540 nm (TLS-B)	
Temperature	37 °C	
Read interval	60 s	
Reaction type	Endpoint	

^a POD, peroxidase (EC 1.11.1.7); 4AA, 4-aminoantipyrine; CHE, cholesterol esterase (EC 3.1.1.13); CO, cholesterol oxidase (EC 1.1.3.6); FDAOS, *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt; Lip, lipase (EC 3.1.1.3); GK, glycerol kinase (EC 2.7.1.30); GPO, glycerol-3-phosphate oxidase (EC 1.1.3.21); DSBmT, *N,N*-bis(4-sulfobutyl)-*m*-toluidine-disodium.

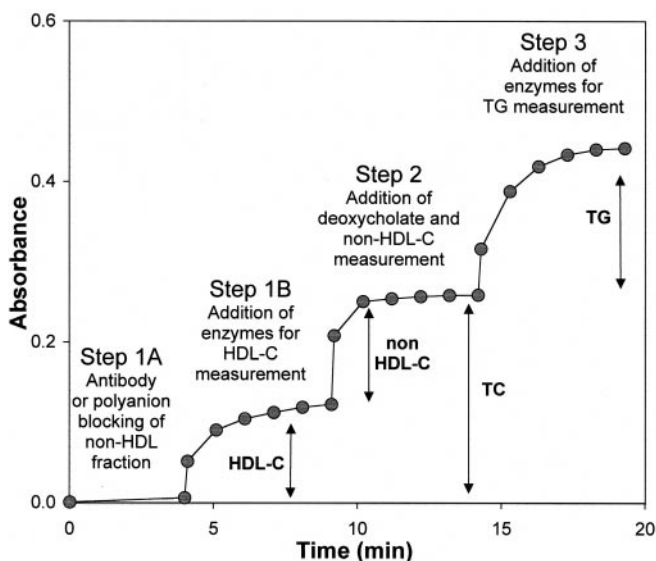


Fig. 1. Absorbance profile of the TLS test.

Serum was analyzed by the TLS-A test, and the change in absorbance was monitored during the individual steps of the assay.

nents of the EZ-HDL-C reagent set, whereas the TLS-B test uses the R1 (polyanion reagent) and R2 (cholesterol reagent) components from the N-geneous HDL-C reagent set. Randomly collected (fasting and nonfasting) serum samples were used to develop and evaluate the TLS tests. For the TLS-A assay, the serum sample was diluted 10-fold with phosphate-buffered saline by the analyzer before the first step of the assay.

We calibrated both the HDL-C and TC parts of the assay for cholesterol, using either the Sigma EZ-HDL calibrator for the TLS-A test or the Genzyme HDL calibrator for the TLS-B test. TC was calibrated by taking the factor (i.e., the slope of the calibration curve) generated in step 1B (HDL-C) of the assay and applying it to the absorbance data generated during step 2 (TC). The TG assay was calibrated with glycerol standards (Sigma). The enzyme reactions were monitored colorimetrically with peroxidase (4-aminoantipyrine) and *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt (TLS-A), or *N,N*-bis(4-sulfobutyl)-*m*-toluidine-disodium (TLS-B) supplied in the homogeneous HDL-C reagent sets. Absorbance values were converted to concentrations by an endpoint calculation, with the absorbance at the beginning of each step as the baseline and the absorbance at 5 min from the start of the step as the final point. LDL-C was calculated using the Friedewald equation (6).

ANALYTICAL EVALUATION OF THE TLS TEST

The specificity of the TLS test for lipoprotein cholesterol fractions was assessed by analyzing samples supplemented with either purified HDL or LDL. Interassay precision was calculated from 20 replicate results obtained during a single analysis of a serum pool. Interassay

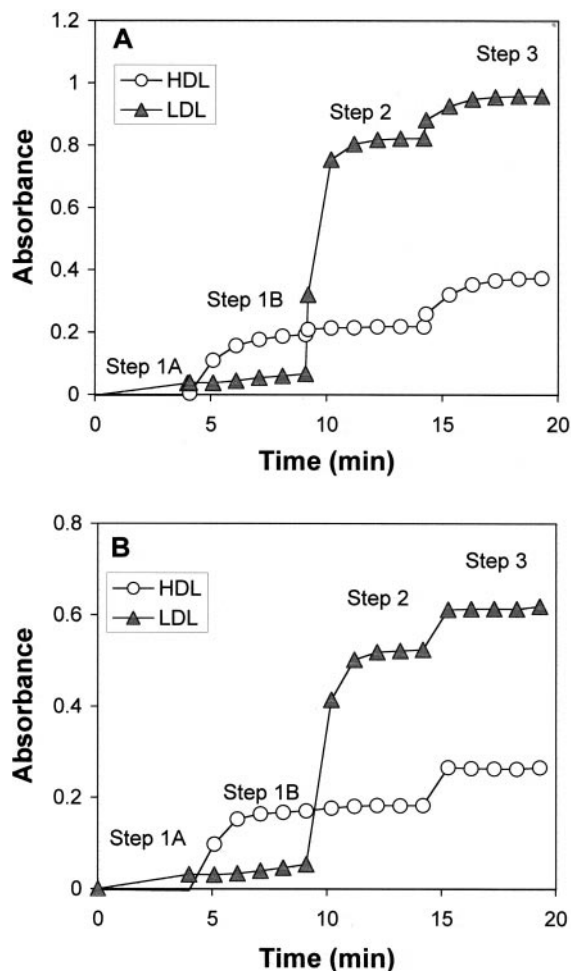


Fig. 2. Lipoprotein specificity of the TLS tests.

(A), analysis of purified HDL (○; final concentration, 1.6 mmol/L) and purified LDL (▲; final concentration, 6.6 mmol/L), diluted in phosphate-buffered saline, by the TLS-A test. (B), analysis of purified HDL (○; final concentration, 1.1 mmol/L) and purified LDL (▲; final concentration, 4.1 mmol/L), diluted in phosphate-buffered saline, by the TLS-B test.

precision was calculated based on the analysis of a frozen serum pool measured in 10 separate analytical runs (2 runs per day for 5 days) using a different calibration for each run. Interference in the TLS assay by hemolysis was tested after the addition of a lysate from washed human red blood cells. Interference in the TLS assay by bilirubin and ascorbic acid was tested after the addition of either unconjugated bilirubin or a fresh solution of ascorbic acid. The interfering substances were added to a serum pool in eight different concentrations and assayed in triplicate; deviations >10% compared with the unadulterated samples were considered significant. Linearity for the TLS tests was assessed using a lipid linearity set (Sigma) at seven different concentrations assayed in triplicate. Deviations >10% from the linear regression line were considered significant. Standard tests for HDL-C (Polymedco), TC (Roche), TGs (Roche), and direct LDL-C (Sigma) were compared with the results of the TLS test by Deming regression analysis and Bland-Altman plots. A different

Table 2. Precision, linearity, and interference of the TLS assay.

	TLS-A			TLS-B		
	HDL-C	TC	TG	HDL-C	TC	TG
Intraassay precision (n = 20)						
Mean, mmol/L	0.88	2.03	1.05	0.66	1.94	4.72
CV, %	1.1	0.77	6.3	4.7	0.99	6.7
Mean, mmol/L	1.34	10.62	4.54	1.97	3.60	1.40
CV, %	3.0	0.51	3.4	1.8	0.95	6.6
Interassay precision (n = 10)						
Mean, mmol/L	1.03	5.43	3.82	0.54	3.82	5.24
CV, %	1.4	2.0	3.0	5.0	2.4	5.7
Mean, mmol/L	2.39	7.46	1.46	1.87	6.49	2.60
CV, %	1.1	1.6	4.7	3.2	3.4	5.1
Upper limit linearity						
Mean, mmol/L	11.8	21.1	13.9	11.8	21.1	13.9
Lower limit of interference						
Bilirubin, $\mu\text{mol/L}$	880	880	880	880	880	880
Hemoglobin, g/L	10	10	10	5.0	5.0	5.0
Ascorbic acid, mg/L	1000	1000	1000	1000	1000	1000

set of serum samples was used in the comparison study for the TLS-A and TLS-B tests.

Results

PRINCIPLE OF THE TLS ASSAY

The reaction profile of serum for the TLS-A test is shown in Fig. 1. Three stepwise increases in absorbance were observed, which correspond to the sequential measurement of HDL-C, TC, and TG. In step 1A, an anti-apolipoprotein B (apoB) antibody was added, which bonded to the surface of the apoB-containing lipoproteins (LDL, VLDL, and chylomicrons). After the addition of cholesterol esterase and cholesterol oxidase (step 1B), only cholesterol from HDL particles was detected, because the enzymes were sterically blocked by the antibody from reacting with cholesterol on the apoB-containing lipoproteins. The residual or unreacted cholesterol on the apoB-containing lipoproteins (non-HDL-C) was then measured

after the addition of deoxycholate in step 2. Deoxycholate disrupted the antibody-apoB lipoprotein complex and allowed the subsequent enzymatic measurement of the unreacted cholesterol in the sample. The sum of the cholesterol measured in step 1B (HDL-C) and step 2 (non-HDL-C) was equal to TC. In step 3, TGs were measured after the addition of the enzymes for the GPO-Trinder reaction. The enzymatic reaction of TGs was monitored with the same peroxidase-based detection system that was used for cholesterol in steps 1B and 2. The reaction for each step of the assay was essentially complete after 5 min at 37 °C, based on the reaction profile of serum.

The TLS-B test of serum also produces three stepwise increases in absorbance (data not shown) and was similar in principle to the TLS-A test, except for the procedure used to block the non-HDL fraction. In step 1A, a polyanion instead of an antibody was used to sterically block

Table 3. Regression analysis of TLS assays.^a

x	y	n	Mean		SD		Slope	Intercept	$S_{y,x}$	R
			x	y	x	y				
Std ^b HDL-C	TLS-A HDL-C	57	1.06	1.25	0.45	0.45	0.99	0.19	0.064	0.980
Std HDL-C	TLS-B HDL-C	65	1.39	1.24	0.38	0.38	1.00	-0.15	0.062	0.974
Std TC	TLS-A TC	57	5.00	5.27	1.65	1.70	1.03	0.12	0.095	0.997
Std TC	TLS-B TC	65	4.80	4.86	1.15	1.23	1.07	-0.30	0.224	0.965
Std TG	TLS-A TG	57	1.65	1.70	1.06	1.08	1.02	0.02	0.116	0.988
Std TG	TLS-B TG	65	1.43	1.21	0.91	0.95	1.04	-0.28	0.131	0.980
Std cLDL-C	TLS-A cLDL-C	57	3.60	3.68	1.47	1.56	1.06	-0.14	0.109	0.995
Std cLDL-C	TLS-B cLDL-C	65	3.13	3.38	1.06	1.15	1.09	-0.01	0.193	0.970
Std dLDL-C	TLS-A cLDL-C	57	3.00	3.24	1.36	1.52	1.12	-0.11	0.185	0.984
Std dLDL-C	TLS-B cLDL-C	46	3.38	3.14	1.17	1.11	0.95	-0.06	0.256	0.951

^a The results of the TLS tests were compared with the standard tests by Deming regression analysis. SD refers to the standard deviation of the sample populations. All concentrations are mmol/L.

^b Std, standard test; cLDL-C, calculated LDL-C; dLDL-C, direct LDL-C.

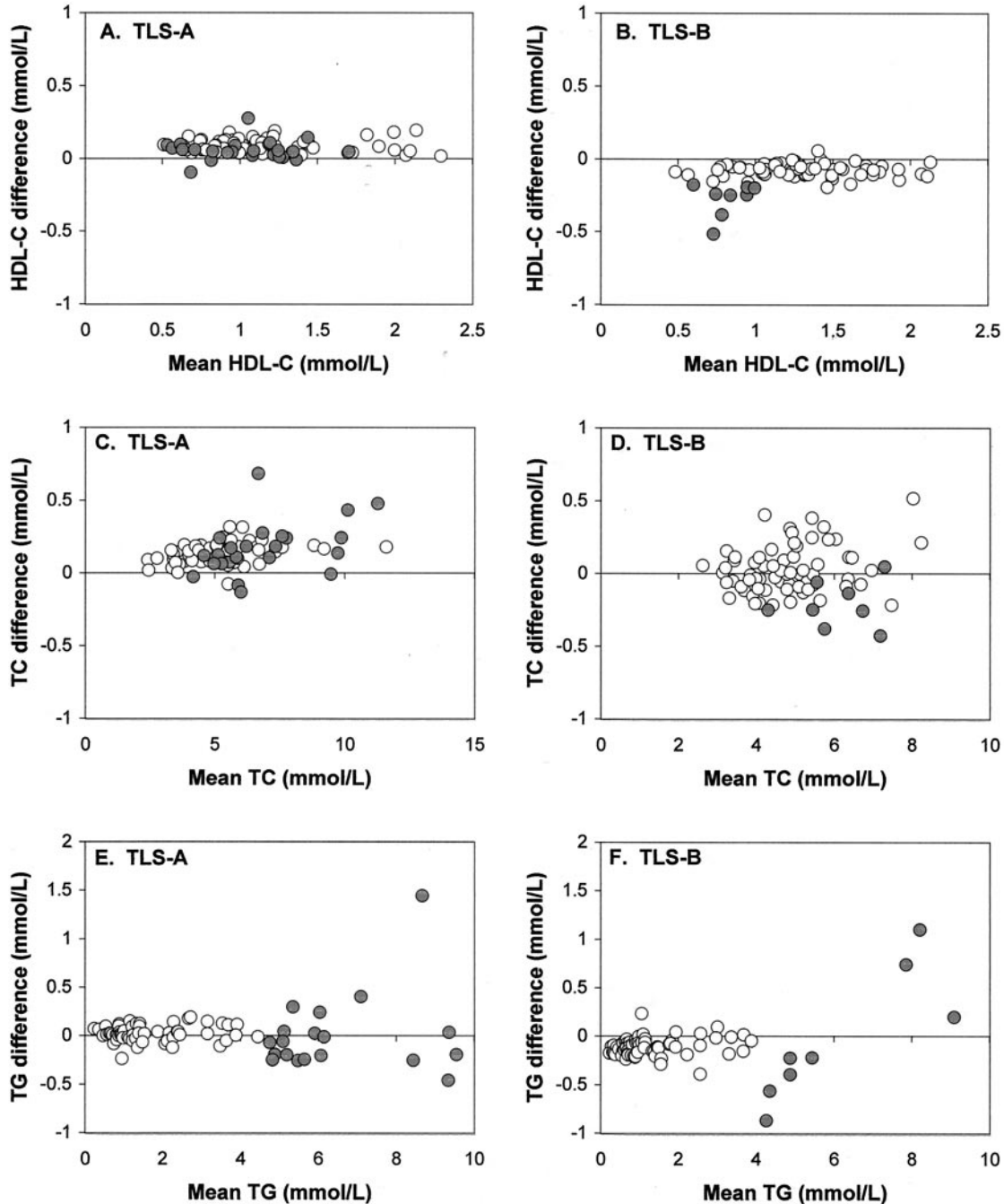


Fig. 3. Comparison of TLS test to standard tests.

The TLS-A test (A, C, and E) and the TLS-B test (B, D, and F) for HDL-C (A and B), TC (C and D), and TGs (E and F) were compared with the standard tests by Bland-Altman plots. The y-axis shows the difference between the TLS test and the standard test (TLS test – standard test), and the x-axis is the mean value of the two assays. ●, serum specimens with a TG concentration >4.5 mmol/L; ○, serum specimens with <4.5 mmol/L TGs.

the enzymatic measurement of cholesterol from the non-HDL particles. In addition, the polyanion inhibition method contained a detergent that, in the presence of the polyanion, selectively solubilized HDL in step 1B. Like the TLS-A test, the addition of deoxycholate in step 2 of the TLS-B test disrupted the complex between the polyanion and the apoB-containing lipoproteins, thus allow-

ing the measurement of cholesterol on the non-HDL particles. TGs were then measured enzymatically in the final step of the assay.

LIPOPROTEIN SPECIFICITY OF THE TLS ASSAY

The change in absorbance for the two versions of the TLS test for purified HDL and LDL are shown in Fig. 2.

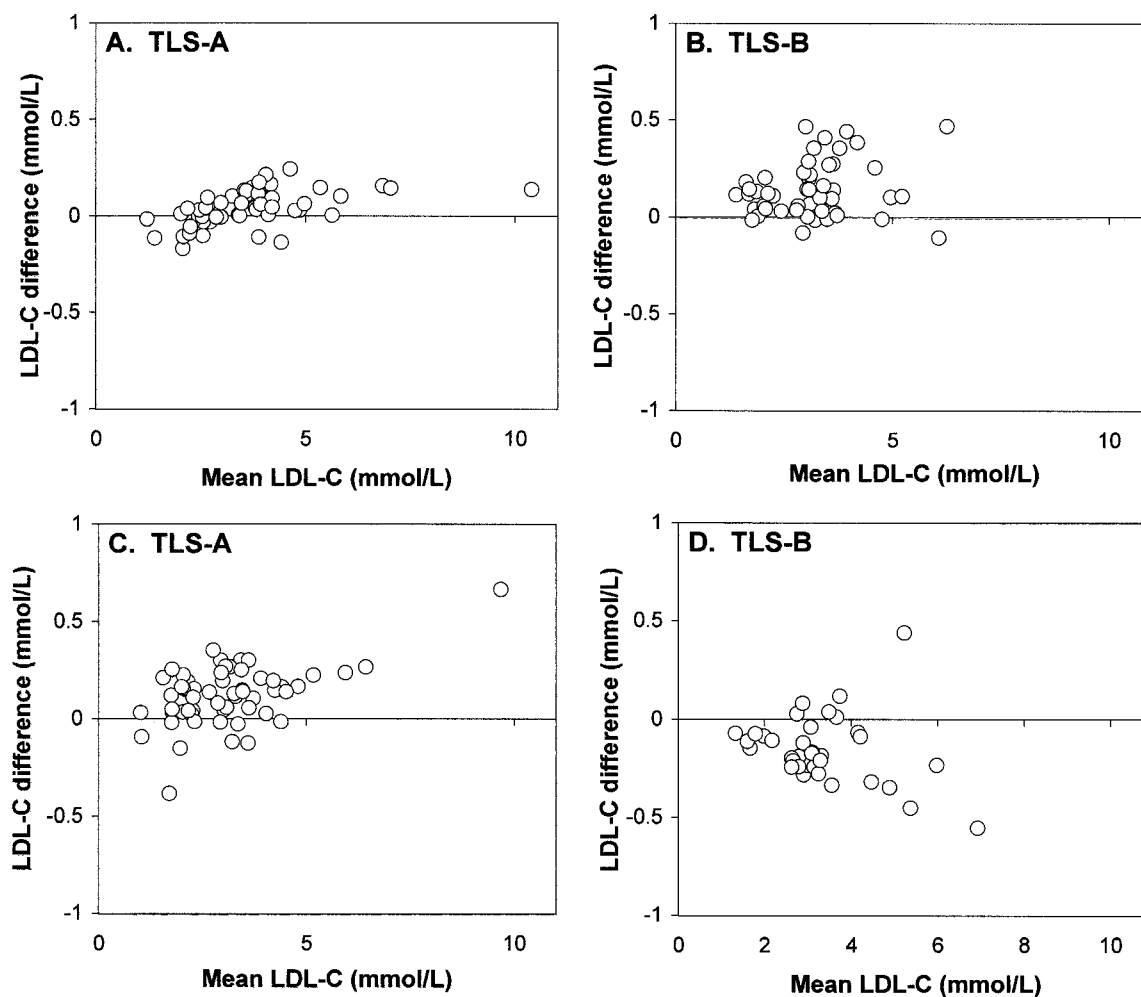


Fig. 4. Comparison of LDL-C measurement by the TLS test vs the standard test.

The results of the TLS-A test (A and C) and the TLS-B test (B and D) were used to calculate LDL-C and compared by Bland-Altman plots to the calculated results based on the standard tests (A and B) or to the direct LDL-C measurement (C and D). The *y*-axis shows the difference between the TLS test and the standard test (TLS test – standard test), and the *x*-axis is the mean value of the two assays. Only results for specimens with <4.5 mmol/L TGs are shown.

Cholesterol was detected only during step 1B of the reaction when HDL was used as a sample. No additional change in absorbance was observed after the addition of deoxycholate in step 2, which indicated that the reaction of HDL-C was complete after step 1B. In contrast, when purified LDL was analyzed, a change in absorbance was observed only in step 2, which indicated that the enzymatic measurement of cholesterol on LDL was prevented in step 1B by either the antibody (Fig. 2A; TLS-A) or the polyanion (Fig. 2B; TLS-B). For both the HDL and LDL samples, TGs were detected in step 3. These results indicated that steps 1 and 2 of both versions of the TLS test were specific for measuring cholesterol from HDL and non-HDL lipoproteins, respectively.

PRECISION, LINEARITY, AND INTERFERENCE OF THE TLS ASSAY

The analytical characteristics of the TLS-A and TLS-B tests are shown in Table 2. The intra- and interassay precision

of the different steps of the TLS assays were similar to the precision of the commonly used individual lipid assays described previously (15). The TC part of the assay had the best precision, followed by the HDL-C and TG parts of the assay. Both versions of the TLS test were linear throughout the usual concentration range of the measured analytes. Interference by bilirubin, hemolysis, and ascorbic acid with the TLS tests was similar to the limits described by the manufacturers of the homogeneous HDL-C used in step 1 of the assays. Both TLS tests were relatively unaffected by bilirubin; no significant bias was observed for any step of the assays in samples containing up to 880 $\mu\text{mol/L}$ bilirubin. For hemolysis, no significant interference was observed for samples containing hemoglobin in concentrations corresponding to gross hemolysis (10 g/L hemoglobin for TLS-A; 5.0 g/L hemoglobin for TLS-B). No significant interference was observed for either assay when ascorbic acid concentrations were <1000 mg/L, which was much

higher than the concentration typically observed in human sera.

COMPARISON OF TLS ASSAY WITH STANDARD TESTS

The performance of the TLS tests was compared with the standard tests for HDL-C, TC, and TGs using serum specimens that had a wide range of lipid values. The results of the regression analysis for specimens with <4.5 mmol/L TGs are shown in Table 3, and the difference plots are shown in Fig. 3. The HDL-C measurements from both TLS assays agreed well with the results of the standard HDL-C assay (Table 3), although the TLS-A assay showed a small, fixed positive bias of 0.19 mmol/L (Fig. 3A) relative to the standard test, whereas the TLS-B test showed a small, fixed negative bias of 0.15 mmol/L (Fig. 3B). Measurement of HDL-C by the immunoinhibition method has been described previously to cause a positive bias, compared with precipitation-based methods, because of the underestimation of cholesterol on apoE-containing HDL lipoproteins by precipitation-based methods (8, 9). Specimens with a TG concentration >4.5 mmol/L (Fig. 3; ●) did appear to show a negative bias for HDL-C in the TLS-B assay, but not in the TLS-A assay. Overall, the TC measurement by both TLS assays also compared well with the standard TC assay (Table 3). There was, however, a small, fixed positive bias of 0.12 mmol/L for the TLS-A assay for TC (Fig. 3C), whereas the TLS-B assay showed increased variation relative to the standard TC test (Fig. 3D). Both tests yielded TG results comparable to the standard TG assay, but showed a poorer correlation for those specimens containing >4.5 mmol/L TGs (Fig. 3, E and F). When the TLS results from serum specimens with <4.5 mmol/L TGs were used to calculate LDL-C, they closely matched the calculated results based on the standard tests (Table 3 and Fig. 4, A and B). The calculated LDL-C results from the TLS assay also matched reasonably well with LDL-C as measured by a direct LDL-C assay (Fig. 4, C and D).

Discussion

We found that the assay order, substrate specificity, and the detection system were important considerations in the development of the test. Although a different assay order would have been possible if only two of the three analytes were sequentially measured, only the order shown in Fig. 1 (HDL-C → TC → TGs) is compatible for sequentially determining all three analytes. The measurement of TGs in an earlier step would have disrupted the structure of the lipoproteins by the lipolysis of TGs, and the TG measurement is more effective after the addition of detergent, which solubilizes TGs from the core of the lipoprotein particles. Similarly, only HDL-C followed by TC is compatible for sequentially measuring both analytes. Deoxycholate was found suitable for disrupting both the antibody-apoB lipoprotein and the polyanion-apoB lipoprotein complexes and allowed the measurement of TC to proceed after the HDL-C step. The addition of deoxy-

cholate at the beginning of the HDL-C step would have prevented the selective measurement of cholesterol from just HDL. The analyte specificity of each part of the TLS assay was, therefore, dependent on the assay order and on the inherent substrate specificity of the lipid-modifying enzymes used in each part of the assay. A common enzyme assay detection system was used for each part of the TLS test to simplify the test and to potentially reduce reagent costs. The enzymes used in the measurement of both TC and TGs generate H₂O₂, which in the presence of peroxidase oxidizes 4-aminoantipyrine and produces a visible color. The signal from the assay detection system was relatively stable. No significant change in absorbance was observed after the completion of steps 1B and 2 (Fig. 1) for as long as 30 min after the start of the reaction (data not shown). The absorbance at the end of each step, therefore, could serve as the baseline for the endpoint calculation of the subsequent step.

Several procedures for the simultaneous or sequential measurement of various analytes, aimed at improving the efficiency of laboratory testing and reducing costs, have been reported (10–12, 16–20). Lipoprotein lipid analysis is ideally suited for this type of application because multiple lipid fractions are routinely measured on a single sample. The most recent recommendations for screening for hyperlipidemia and for monitoring cholesterol-lowering therapies emphasize the importance of measuring both LDL-C and HDL-C (2). There have been several tests developed for the sequential measurement of TC and TGs (10, 11) and one test that sequentially measures TC, TGs, and phospholipids (12). These assays, however, are not as clinically useful because of the importance of measuring not only TC and TGs, but also the cholesterol content of HDL and LDL (2). In contrast, the TLS assay provides results for HDL-C, along with TC and TGs. The TLS results from nonhypertriglyceridemic specimens (TGs <4.5 mmol/L) matched well with the standard tests (Fig. 3), particularly in the range of lipid values typically used for diagnosing hyperlipidemia (1, 2). In addition, the calculated LDL-C from the TLS tests closely matched the calculated LDL-C result from the standard tests, as well as the result from a direct LDL-C assay (Fig. 4). In an earlier report (20), we showed that the homogeneous HDL-C method used for the TLS-A test can also provide an estimate of the apoB concentration by monitoring the change in turbidity induced after the anti-apoB antibody is added in step 1A. The TLS tests, therefore, can be used to measure all of the commonly used lipid values for the screening of hyperlipidemia.

One other advantage of the TLS test compared with the current multiple test format of lipoprotein lipid analysis is that it is a single-tube assay and, therefore, potentially requires fewer disposable items, such as reaction cuvettes. More importantly, there is an overall reduction in the use of reagents. The HDL-C and TC parts of the TLS test use the same enzymes, and the TC step does not require any additional reagents except for deoxycholate (Fig. 1),

which is relatively inexpensive. Additional enzyme reagents are added for measuring TGs, but the TG reaction is monitored with the same enzyme detection system used for the HDL-C and TC parts of the assay. It is estimated that there would be a >99% reduction in reagent costs for the TC assay and an ~20% reduction for the TG assay if both tests are performed as part of the TLS test, based on the retail price of the individual components of the TC and TG assays and the cost of deoxycholate. Depending on the operations of a given laboratory, there may also be some savings in labor for the technologist performing the TLS assay, particularly when compared with precipitation-based HDL-C assays, which require a substantial amount of manual specimen preparation (8, 9).

The setting in which the TLS test may be the most useful is point-of-care sites, such as physician offices, in which multiple lipid fractions frequently are measured for screening of hyperlipidemia. Any abnormal result from the TLS test could subsequently be confirmed by individual standard lipid tests. A limitation of the TLS assay is that although the individual components of the test are commercially available, the packaging of the reagents as a single reagent set would make it easier and more practical to use the assay and would likely improve the analytical performance of the assay because of the improved standardization of the reagents and the calibrators. Another current limitation of the TLS assay is that it requires an analyzer, such as the Cobas FARA, that can add multiple reagents to a reaction cuvette. However, because the TLS assay is a single-tube and single-wavelength colorimetric assay, it could be readily adapted to a simple analyzer dedicated to the point-of-care testing of hyperlipidemia.

In conclusion, the TLS test offers a convenient way to simplify lipoprotein lipid analysis. It can be used for directly measuring HDL-C, TC, and TGs, and for calculating LDL-C; thus, the test provides results for the four commonly monitored lipoprotein lipid values. Because of the potential of cost savings, the TLS test may be particularly useful for the screening of hyperlipidemia.

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