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A Rapid Micro-Scale Procedure for Determination of the Total Lipid Profile

Lily L. Wu,[1,2,5] G. Russell Warnick,[3] James T. Wu,[2] Roger R. Williams,[1] and Jean-Marc Lalouel[4]

We describe a one-day micro-scale procedure for determining the total lipid profile. Only 0.55 mL of plasma is needed for complete quantification of total cholesterol (TC), triglyceride (TG), and all lipoproteins. After precipitation with dextran sulfate and magnesium, the high-density lipoprotein (HDL) fraction was separated by centrifugation in an Eppendorf microcentrifuge. Very-low-density lipoprotein (VLDL) was separated from low-density lipoprotein (LDL) plus HDL in a Beckman TL 100 ultracentrifuge. TC, TG, and cholesterol in different lipoprotein fractions were measured enzymatically in a Baker "Encore II" automated analyzer. CVs, both within-day and day-to-day, were <3% for TG and TC, and <5% for HDL-C determinations. CVs for LDL-C and VLDL-C were <7.5% and 15%, respectively. Results by our micromethods (n = 66) agreed well with those by the conventional methods used at the Northwest Lipid Research Center, which are standardized against the Reference Methods of the Centers for Disease Control. Coefficients of correlation between the two methods were 0.98 for TC, 1.0 for TG, 0.98 for HDL-C, 0.94 for LDL-C, and 0.96 for VLDL-C. Results of electrophoresis on agarose gel and radioactivity-recovery studies indicate that our micro-centrifugation and slicing procedures result in clean separation of VLDL from other lipoproteins.

Additional Keyphrases: *cholesterol · triglycerides · lipoproteins · enzymatic methods · ultracentrifugation*

Coronary heart disease is a major cause of disability and death in the United States as well as in many other Western countries. Analyses of the total lipid profile—including measurement of total cholesterol (TC), triglycerides (TG), and the cholesterol contents of various lipoprotein fractions—are valuable, not only for assessing the risk of atherosclerosis but also for diagnosing specific familial syndromes and monitoring the treatment of lipid abnormalities (*1–4*).[6]

The conventional procedure for analysis of the total lipid profile usually takes two days and requires about 7 mL of plasma (*5, 6*). When large-scale investigations are to be undertaken—such as investigations with an epidemiological or genetic perspective—a rapid micro-scale procedure is desirable. Surveying all newly developed ultracentrifuges and their rotors, we found it is possible to use much less plasma for lipoprotein fractionations. We also realized that most modern automated analyzers, such as the Baker Encore II, require only a few microliters of plasma for cholesterol measurement. Using an Eppendorf microcentrifuge for HDL fractionation and a Beckman TL-100 ultracentrifuge to separate VLDL from other lipoproteins, we developed the rapid microprocedure for total lipid profile determination that is reported here. Only 0.55 mL of plasma is required, with no sacrifice in accuracy and precision, and results compare well with those of conventional methods used at the Northwest Lipid Research Center (NWLRC) and at the Cleveland Clinic Foundation.

Materials and Methods

Materials

Specimens. Lyophilized serum controls with both normal and abnormal concentrations of triglyceride and cholesterol were obtained from Beckman Instruments, Brea, CA 92621, and Baker Instruments Corp., Allentown, PA 18103. Quick-frozen aliquots of EDTA-treated plasma with known concentrations of TG, TC, and high-density lipoprotein cholesterol (HDL-C) were purchased from NWLRC, Seattle, WA 98104. For use in the comparison studies, we obtained aliquots of frozen plasma from NWLRC. An additional 30 patients' specimens with known TG, TC, and HDL-C values were purchased from H. Naito's laboratory at the Cleveland Clinic Foundation, Cleveland, OH 44106. The specimens were kept at 4 °C, shipped to us on ice, and analyzed within a week of receipt.

Chemicals. Reagents for cholesterol and triglyceride determinations in the Encore II automated analyzer and the precipitation reagent for HDL isolation were from Baker Instruments Corp.; 1.5-mL Eppendorf tubes were from American Scientific Product Co., McGaw Park, IL 60085-6787; ^{125}I was from Amersham Corp., Arlington Heights, IL 60005; and "Paragon" lipoprotein electrophoresis kits were from Beckman Instruments.

Methods

Blood collection. We followed the guidelines described in the Laboratory Operations Manual of the Lipid Research Clinics (*5*) for blood drawing, sample preparation, and storage. Blood samples, obtained by venipuncture from patients after a 12- to 16-h fast, were drawn into a chilled tube containing EDTA. Plasma was separated within 3 h (preferably within 1 h) and assayed immediately or stored at −70 °C.

Determination of cholesterol and triglyceride. We used Baker reagents for these determinations, following the manufacturer's protocol for the Encore II automated analyzer. Although only 3 μL of plasma is needed for each cholesterol or triglyceride determination and 12 μL for each HDL-C determination, at least 50 μL of plasma must be loaded into the sample cup. Cholesterol and triglyceride measurements were calibrated with Baker standards. Bak-

[1] Cardiovascular Genetics Research Clinic, Department of Internal Medicine, and [2] Department of Pathology, University of Utah, Salt Lake City, UT 84132.
[3] Northwest Lipid Research Center, University of Washington, Seattle, WA 98104.
[4] Howard Hughes Medical Institute and Department of Human Genetics, School of Medicine, University of Utah, Salt Lake City, UT 84132.
[5] Address for correspondence: Cardiovascular Genetics Research, Rm. 161, 410 Chipeta Way, Research Park, Salt Lake City, UT 84108.
[6] Nonstandard abbreviations: NWLRC, Northwest Lipid Research Center; TC, total cholesterol; TG, triglyceride; HDL-C, LDL-C, VLDL-C, high-, low-, and very-low-density lipoprotein cholesterol; and CDC, Centers for Disease Control.
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er's lipid controls, Beckman's above-normal lipid control, and three NWLRC lipid controls with known TG, TC, and HDL concentrations were routinely assayed with each batch of lipid analyses.

Determination of HDL. To obtain the HDL fraction, we precipitated the LDL and VLDL lipoproteins with Baker's precipitation reagent containing 50-kDa dextran sulfate and magnesium chloride, according to the manufacturer's instructions, except we slightly modified it to accommodate our small sample aliquot: we mixed 0.25 mL of plasma with 20 μL of precipitation reagent in a 1.5-mL Eppendorf tube. After vortex-mixing for 20 s, we allowed the mixture to stand at room temperature for 10 min before centrifugation at maximum speed (8800 × *g*) for 10 min. The resulting clear supernates were transferred to the Encore II sample cup with a sharp-tipped transfer pipette for cholesterol measurement. HDL-C measurements were calibrated with standards from Diagnostic Medical Associates, Inc., Arlington, TX 76011. In the event of incomplete precipitation—as usually occurs with specimens containing an increased concentration of triglycerides—turbidity or floating white particles are visible in the supernate after centrifugation. We routinely added an additional 0.25 mL of isotonic saline and 20 μL of precipitating reagent to the tube and repeated the centrifugation after thoroughly mixing the tube contents.

Determination of VLDL-C and LDL-C. For separation of VLDL from LDL plus HDL, our microprocedure requires only 0.2 mL of plasma for fractionation, with no density adjustment. We used Beckman TL-100 ultracentrifuge, a TLA-100 fixed-angle rotor (20-tube capacity), and thick-wall polycarbonate tubes (7 × 20 mm, 200-μL capacity) for lipoprotein fractionation. Centrifugation conditions were as follows: 4 h, 157 000 × *g*, 20 °C, with no interruption. After this centrifugation, the tubes were allowed to stand in an upright position for about 10 min, then sliced with a Beckman "CentriTube" slicer to separate VLDL (the top fraction) from the bottom fraction, which contains LDL plus HDL. The tube was sliced at its midpoint. Thus 36% of the total volume would be in the VLDL (top) fraction. Each fraction was thoroughly mixed, transferred to a sample cup in the Encore II automated analyzer, and cholesterol was measured according to Baker's protocol for total cholesterol determination. LDL-C was calculated as the difference between cholesterol in the the bottom fraction and the HDL-C by precipitation. VLDL-C was calculated as the difference between the total cholesterol and the cholesterol in the bottom LDL-plus-HDL fraction. Comparison of this calculated VLDL-C value with the VLDL-C recovered directly in the top fraction serves as a quality-control check on the adequacy of lipoprotein recovery.

Experiments Documenting the Clean Separation of VLDL

We performed several experiments to establish the optimal centrifugation and slicing conditions for cleanly separating the VLDL and HDL-plus-LDL fractions.

Direct visualization of lipoprotein bands. The visibility of the lipoprotein bands is enhanced by prestaining the plasma lipoproteins with Sudan Black before ultracentrifugation (*6*), by incubating 0.2 mL of plasma with 5 μL of Sudan Black solution (1 g/L in ethylene glycol). After centrifugation, the lipoproteins then were visible, VLDL and LDL appearing as distinct, dark-blue bands at the top and the lower middle portion of the tube, respectively, and HDL as a lighter colored band at the bottom.

Agarose gel electrophoresis. The top and bottom fractions obtained by ultracentrifugation were restored to their original 0.2-mL volume with isotonic saline, and 8- to 10-μL aliquots were applied to Beckman Paragon agarose gel and electrophoresed (room temperature, 100 V, 30 min). Lipoprotein bands were made visible by staining with Sudan Black after fixation.

Distribution of radioactivity. We aliquoted 0.2 mL of plasma containing about 4000 counts/min of ^{125}I into ultracentrifuge tubes, and then sliced the tubes, without centrifugation. The radioactivity in the top and bottom fractions was counted to determine the accuracy and reproducibility of the slicing technique. Ideally, the upper fraction should contain 36% and the bottom fraction 64% of the total count.

Analytical Procedures Performed at NWLRC

Cholesterol was quantified by an enzymatic procedure with use of a non-commercial reagent in the ABA 200 bichromatic instrument (Abbott Laboratories, North Chicago, IL 60064). The assay is calibrated with a secondary serum-based standard, a "Sercal" pool, provided by the Clinical Chemistry Standardization Section of the CDC. Triglycerides were quantified in the same analyzers with use of the Abbott "Agent" triglyceride reagent calibrated with primary standards of free glycerol. We made a blank correction for free glycerol in a separate assay, using the same reagent but without the lipase (*7*).

The 50-kDa dextran sulfate–magnesium precipitation method (*8*) was used for fractionation of HDL in 2 mL of plasma, which then was centrifuged (1500 × *g*, 30 min, 4 °C). Any turbid supernates were further treated, usually by ultrafiltration or, occasionally, by dilution.

We fractionated VLDL by ultracentrifugation of 5.0 mL of plasma without density adjustment, following the Lipid Research Clinic quantification method (*5*). For separation we used a Beckman floor-model preparative ultracentrifuge at 142 464 × *g* for 18 h, a 6.5-mL centrifuge tube, and a no. 50.3 rotor. LDL-C was calculated as the difference between cholesterol in the bottom fraction and the HDL-C by precipitation. VLDL-C was calculated as the difference between the total cholesterol and that in the bottom fraction. The NWLRC has participated in the NHLBI–CDC lipoprotein standardization program for nearly 20 years. The methods described here meet the CDC performance requirements for precision and accuracy.

Results

Precision Studies

TC and TG. We used lyophilized commercial serum control, frozen EDTA-treated plasma from NWLRC, and locally prepared frozen EDTA-treated plasma pools to study the within-day and day-to-day precision for triglyceride and cholesterol determinations (Table 1). Three NWLRC controls (PI, PII, PIII) containing 1.3-mL aliquots of pooled EDTA-treated plasma were quick-frozen, shipped to us on solid CO_2, and stored at −70 °C. These three controls with known TG, TC, and HDL-C concentrations were routinely assayed with each batch of lipid analyses. For triglyceride, the within-day and day-to-day CVs were <3% when we assayed pooled plasma from NWLRC. The CV was higher with the Baker's lyophilized serum control (4.1%). This poorer precision was ascribable to variability associated with reconstitution of the lyophilized material. The CDC requirement of CV for triglyceride is <5%. As

Table 1. Precision Studies for Total Cholesterol, Triglycerides, and HDL-Cholesterol

Within-day variation				Day-to-day variation			
Control	n	Mean ± SD, g/L	CV, %	Control	n	Mean ± SD, g/L	CV, %
TC							
C1[a]	20	0.875 ± 0.012	1.4	PI[b]	36	2.805 ± 0.062	2.2
C2	20	1.795 ± 0.028	1.5	P II	36	2.156 ± 0.050	2.3
C3	24	3.216 ± 0.021	0.7	P III	36	1.862 ± 0.045	2.4
C4	24	5.808 ± 0.085	1.4	Beckman[c]	33	3.138 ± 0.107	3.4
				Baker[d]	30	1.290 ± 0.062	4.8
TG							
C1	20	0.683 ± 0.019	2.8	P I	36	1.895 ± 0.046	2.4
C2	20	1.558 ± 0.035	2.3	P II	36	1.372 ± 0..034	2.5
C3	20	2.994 ± 0.032	1.1	P III	36	1.074 ± 0.032	3.0
C4	20	5.808 ± 0.056	1.0	Beckman	33	3.211 ± 0.102	3.2
				Baker	30	0.633 ± 0.026	4.1
HDL-C							
C1	12	0.179 ± 0.005	2.5	P I	36	0.513 ± 0.023	4.8
C2	7	0.344 ± 0.007	2.0	P II	36	0.437 ± 0.016	3.8
C3	10	0.397 ± 0.006	1.6	P III	36	0.453 ± 0.015	3.4
C4	8	0.503 ± 0.007	1.3	Beckman	33	1.161 ± 0.045	3.9
				Baker	30	0.292 ± 0.012	4.2

[a] EDTA plasma controls stored at −70 °C. [b] Pooled control plasma from NWLRC, stored at −70 °C. [c] Beckman elevated control, lyophilized sera. [d] Baker normal control, lyophilized sera.

with triglycerides, the within-day precision we obtained for total cholesterol was excellent and the day-to-day precision with NWLRC's plasma controls (<3%). The CDC Lipid Standardization Program requires a CV of 3% for cholesterol; we obtained a slightly higher CV (4.8%) when we used Baker's lyophilized serum controls.

HDL-C. For HDL-C (Table 1), the within-day precision was excellent (CVs <2.5%), and the day-to-day precision was also acceptable (CVs <5%), both well within the suggested acceptable limit (SD 0.03 g/L) for the CDC program.

LDL-C and VLDL-C. The day-to-day precision obtained for VLDL-C and LDL-C by using three frozen plasma controls is satisfactory (Table 2), even though these values were derived from several separation steps, involving ultracentrifugation to separate VLDL from HDL plus LDL and a precipitation step to isolate HDL. The higher CVs obtained for VLDL-C may also be related to the much lower VLDL-C concentrations in plasma as compared with TC, the requirement for thorough mixing of the viscous layer of the VLDL fraction after centrifugation, and the need to withdraw aliquots accurately from this fraction for analysis. However, the standard deviation for VLDL determinations remained small (Table 2). The concentration of LDL-C is obtained by subtracting the concentration of HDL-C from that of the combined fraction of HDL-C and LDL-C, so the precision determined for LDL-C is affected by the precision of the HDL-C determination.

Table 2. Day-to-Day Variations for VLDL-C and LDL-C Determination

	Mean ± SD, g/L	CV, %
VLDL-C		
P I[a]	0.488 ± 0.006	11.8
P II	0.291 ± 0.004	12.6
P III	0.292 ± 0.004	14.4
LDL-C		
P I	1.603 ± 0.008	4.9
P II	1.294 ± 0.010	7.5
P III	0.990 ± 0.007	7.5

[a] Pooled control plasma from NWLRC, stored at −70 °C in 1.3-mL aliquots. n = 36 each.

Accuracy Studies

We assessed the accuracy of our procedure by comparison with the values obtained by NWLRC for the three controls, because their method is standardized to the CDC reference method. The results (Table 3) agreed well with the target

Table 3. Assessment of the Accuracy of TC, TG, and HDL-C Determinations by Comparison with NWLRC Method

	Concentration, g/L		
	TC	TG[b]	HDL-C
P I[a]			
Utah	2.805 ± 0.062	1.895 ± 0.046	0.513 ± 0.0023
Target value[a]	2.783 ± 0.014	1.887 ± 0.027	0.498 ± 0.0147
% difference	0.79	0.42	3.01
P II			
Utah	2.156 ± 0.050	1.372 ± 0.034	0.437 ± 0.0016
Target value[c]	2.123 ± 0.045	1.363 ± 0.039	0.457 ± 0.0052
% difference	1.55	0.66	4.37
P III			
Utah	1.862 ± 0.045	1.074 ± 0.032	0.453 ± 0.0015
Target value[c]	1.852 ± 0.016	1.045 ± 0.045	0.450 ± 0.0089
% difference	0.54	2.78	0.66

[a] Pooled control plasma from NWLRC, stored at −70 °C in 1.3-mL aliquots. [b] Values for TG here are total triglyceride concentration, including free glycerol present in plasma. Generally free glycerol amounts to about 10% of total triglyceride determined. n = 36 each. [c] Values from NWLRC; cholesterol measurements were all performed in duplicate in three separate analyses (n = 6), by the Abell–Kendall procedure according to the CDC protocol (*9*).

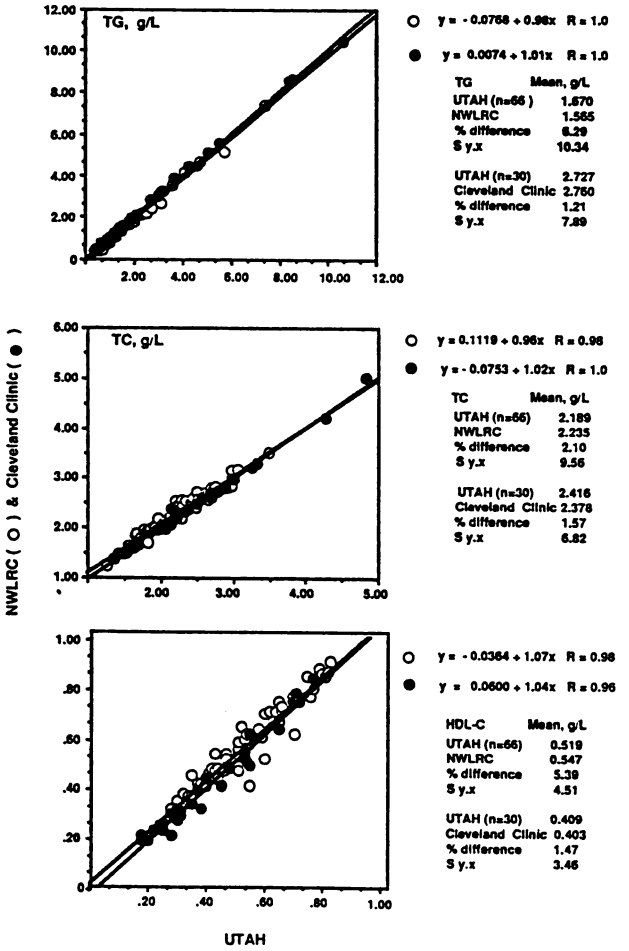

Fig. 1. Comparison of TG, TC, and HDL-C as determined by our microprocedure with results by conventional methods used at both NWLRC (○) and the Cleveland Clinic (●)

values. Biases for total cholesterol are 0.5%, 0.8%, and 1.6%, all of which meet the CDC specification for accuracy (bias within 3%). Similarly, biases for triglycerides are 0.4%, 0.7%, and 2.8%, all within the CDC specification of 5%. The accuracy of our HDL-C determination is also acceptable, being within the CDC requirement of 10%.

Comparison Studies

Besides studies on precision and accuracy, we have also compared our results for clinical specimens from NWLRC (n = 66) and the Cleveland Clinic Foundation (n = 30) for TG, TC, and HDL-C analysis (Figure 1 and Table 4); and for specimens from NWLRC for LDL-C and VLDL-C determinations (Figure 2). In both of these laboratories conventional methods are used for these analyses, which are standardized by CDC. Overall, the correlations are excellent, the coefficients of correlation (r) ranging from 0.94 to 1.0. The excellent accuracy of our procedure is indicated both in the slopes (close to 1) and in the relative differences shown in the figures. The largest relative discrepancy, 6.3% for TG between Utah and NWLRC shown in Figure 1A, results from NWLRC's subtraction of endogenous, free glycerol from the total glycerol concentration.

Fractionation of VLDL

Accurate determinations of VLDL-C and LDL-C rely on the success of VLDL separation from the combined fractions of HDL and LDL. Successful separation of these two fractions also depends on the position selected for tube slicing after the lipoproteins have been separated by ultracentrifugation. Selection of an incorrect position for slicing will result in an inaccurate estimation for both VLDL-C and LDL-C. Because in our procedure a different centrifuge, rotor, and centrifuge tube are used than in the conventional methods for VLDL fractionation, we carried out three experiments to verify that our conditions for ultracentrifugation and slicing do in fact provide clean separation of VLDL from the combined fraction of HDL plus LDL. Plasma samples containing a normal lipoprotein concentration, high VLDL, or high LDL were first ultracentrifuged, then the tubes were sliced, to separate VLDL from other lipoproteins. The individual lipoprotein fractions so separated were analyzed by agarose gel electrophoresis. Figure 3 illustrates the distribution of various prestained lipoprotein bands after ultracentrifugation. The patterns shown in Figure 3 demonstrate that the microprocedure provides a clean separation of VLDL from LDL and HDL not only for normal plasma but also for plasma containing increased concentrations of either VLDL or

Table 4. Comparison of Lipids and Lipoproteins Determinations by Utah with Those by NWLRC and Cleveland Clinic

	Mean (Utah)	Mean (ref. lab)	Slope	Intercept (y)	r	n	$S_{y.x}$	Difference, %
TC								
Utah vs NWLRC	2.189	2.235	0.96	0.1119	0.98	66	9.56	2.10
Utah vs Cleveland	2.416	2.378	1.02	−0.0753	1.00	30	6.82	1.57
TG								
Utah vs NWLRC[a]	1.670	1.565	0.98	−0.0768	1.00	66	10.34	6.29
Utah vs Cleveland	2.727	2.760	1.01	0.0074	1.00	30	7.89	1.21
HDL-C								
Utah vs NWLRC	0.519	0.547	1.07	−0.0364	0.98	66	4.51	5.39
Utah vs Cleveland	0.409	0.403	1.04	0.0600	0.96	30	3.46	1.47
LDL-C								
Utah vs NWLRC	1.298	1.354	0.96	0.1135	0.94	66	13.58	4.31
VLDL-C								
Utah vs NWLRC	0.341	0.337	0.92	0.0156	0.96	66	7.15	1.17

[a] Values from NWLRC: TG here are net triglyceride concentration, not including free glycerol present in plasma. Generally, free glycerol amounts to 10% of total triglyceride determined.

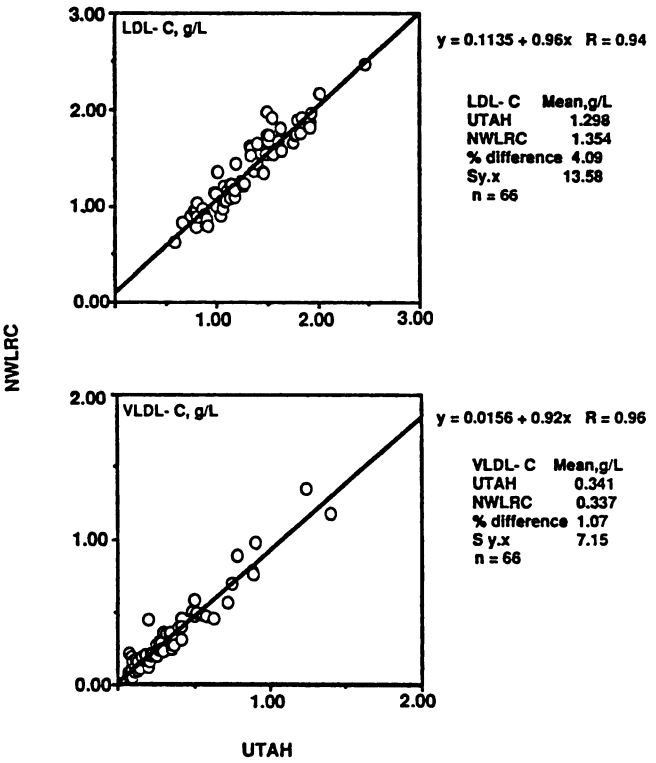

Fig. 2. Comparison of LDL-C and VLDL-C determined by our procedure (*UTAH*) with that of the conventional methods used at NWLRC

LDL. The agarose gel electrophoretic patterns (Figure 4) provide further evidence that our centrifugation conditions and slicing procedure are adequate. In the agarose gel electrophoresis, we included the original plasma, in addition to the top and bottom fractions of lipoprotein for the purpose of comparison. The double-band that appeared in lane 4*A (T)* of Figure 4 is due to the presence of an unusual lipoprotein fraction from a patient with Type III hyperlipoproteinemia. Such plasma usually has a characteristic floating β-lipoprotein band in addition to the normal

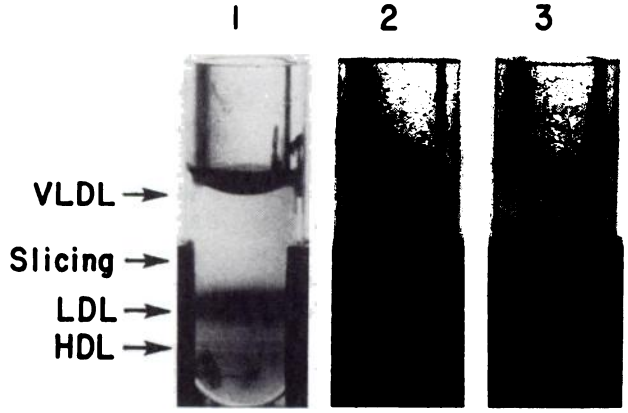

Fig. 3. Appearance of prestained lipoprotein bands after ultracentrifugation

Clean separation of various lipoproteins was obtained after plasma, prestained with Sudan Black, was centrifuged in a TL-100 ultracentrifuge for 4 h at 157 000 × *g* (20 °C). *"Slicing"* indicates the position where the tube was sliced. Plasmas in tubes *1*, *2*, and *3* contained normal concentrations of lipoproteins, increased VLDL (3.54 g/L), and increased LDL (2.26 g/L), respectively

VLDL. The blue band appearing immediately below the VLDL band is the floating β-lipoprotein band (*10*).

We routinely used ^{125}I to monitor the consistency of our slicing procedure. If slicing is performed correctly, the entire upper fraction should contain 36% and the entire bottom fraction 64% of the total radioactivity added. Adequacy of the slicing procedure was also confirmed by measuring the cholesterol concentration in the upper and bottom fractions, respectively, as well as the concentration of the original plasma. When slicing is not properly performed, the sum of the cholesterol concentrations of the two separate fractions added together (after multiplication of the cholesterol concentration of the upper fraction by 36% and the bottom fraction by 64%) will not be equal to the cholesterol concentration of the original plasma. However, our average recovery exceeded 97% (n = 38), with a CV of <2%.

Discussion

Our microprocedure differs from conventional methods in that an Encore II automated analyzer is used for cholesterol and triglycerides determinations; an Eppendorf microcentrifuge for separating HDL after precipitation of the other lipoproteins; and small centrifuge tubes, a high-speed rotor, and a refrigerated table-top TL-100 ultracentrifuge for lipoprotein separations. The relatively small volume of plasma required allows one to perform other analyses in addition to the total lipid profile on the sometimes very limited amount of specimen.

For total cholesterol and triglyceride determinations, many of the automated analyzers commonly found in clinical laboratories would be equally useful, because sensitive and specific enzymatic methods are used in them, and only a few microliters of plasma is required for total cholesterol and triglyceride determinations. Indeed, the ABA-200 used by McNamara and Schaefer (*11*) and by NWLRC (*7*) in their procedures is equally useful and results satisfy the CDC standardization criteria. Selection of the Eppendorf microfuge for obtaining the HDL fraction decreases the amount of plasma required for HDL-C measurement. We found that spinning for 10 min at maximum speed (8800 × *g*) clearly separates the HDL fraction from the precipitates. The amount of HDL isolated from a 0.25-mL plasma specimen suffices for HDL-C determination. With the Eppendorf microfuge and a rotor that can hold 40 1.5-mL centrifuge tubes, many specimens can be quickly processed.

Bronzert and Brewer (*12*) used a Beckman Airfuge for lipoprotein separation. In the procedure they described, the same 175-μL portion of plasma was used twice for lipopro-

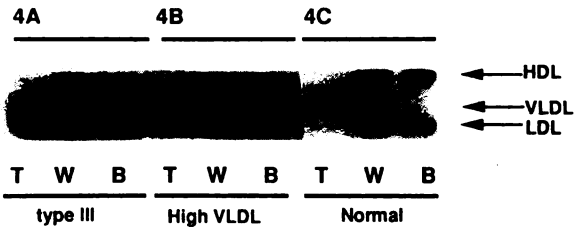

Fig. 4. Electrophoresis patterns of lipoprotein fractions obtained by ultracentrifugation

Plasma specimens containing high VLDL (*4B*) or normal concentrations of lipoproteins (*4C*), and plasma from the patient with Type III hyperlipidemia (*4A*) were first separated by ultracentrifugation. After slicing, the top fraction (*T*), the original whole plasma (*W*), and the bottom fraction (*B*) were subjected to electrophoresis on agarose gel. The bands were then made visible by staining with Sudan Black

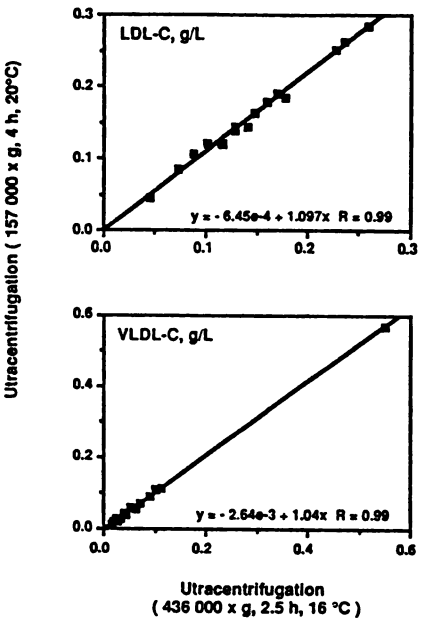

Fig. 5. Comparison of LDL-C and VLDL-C obtained by microprocedure under two different conditions during ultracentrifugation

Both LDL-C and VLDL-C obtained by shorter centrifugation time (2.5 h) correlate well with that by 4-h centrifugation

tein fractionation. This required density adjustment and two separate centrifugations. The Airfuge has no temperature control, so the tube may heat up during prolonged centrifugation. Moreover, the rotor available can only accommodate six tubes, and these tubes cannot be sliced. Aliquots must be withdrawn directly from the tube for cholesterol determination. Such direct aspiration not only can cause remixing of various lipoprotein fractions but it can also produce inaccurate results when small aliquots of specimen from a viscous lipoprotein fraction are being removed.

David et al. (*13*) and Naito (*14*) also recommended the use of the Beckman TL-100 ultracentrifuge for lipoprotein separation. They elegantly and successfully isolated every lipoprotein fraction by using salt solutions of various densities in a series of differential flotation runs. However, the TLA-100.2 rotor holds only 10 1-mL centrifuge tubes, and 10 h of centrifugation is required to complete the lipoprotein separation. Their procedure is, therefore, more of a research tool and may not be practicable for analyzing large numbers of specimens. By contrast, a TLA-100 rotor accommodates 20 tubes and requires less plasma to separate VLDL cleanly from the combined HDL-plus-LDL fraction in only 4 h. The concentration of LDL-C is then calculated as we have described. The precision and comparison studies both indicate that this rapid microprocedure is suitable for total lipid profile determinations in a routine clinical laboratory.

Recently we also found that the separation of VLDL by ultracentrifugation can be shortened to 2.5 h if a higher speed, such as 436 000 × *g*, is used. Results of VLDL-C and LDL-C obtained by the 2.5-h procedure correlate well with that of the original 4-h procedure (Figure 5). However, extra effort is required to mix the bottom LDL and HDL fraction to a homogeneous state before an aliquot is withdrawn for cholesterol determination. We prefer the 4-h procedure because, during the studies of apolipoprotein E phenotyping by isoelectrofocusing, we found that centrifugation at 157 000 × *g* provided a better recovery of apolipoprotein E in the VLDL fraction than did centrifugation at 436 000 × *g*.

The microprocedure also allows identification of type III hyperlipoproteinemia. As shown in Figure 4*A*, both increased VLDL and the floating β-lipoprotein band, characteristic of this disease (*10*), are well separated from the rest of lipoproteins in the top fraction, and no VLDL or floating β-band is found in the bottom fraction.

In summary, we have developed and validated a rapid procedure for determining total cholesterol, triglycerides, and LDL-C, HDL-C, and VLDL-C in only 0.55 mL of plasma. The total lipid profile determination can be completed in one working day. This procedure should be valuable whenever large-scale studies calling for multiple biochemical determinations need to be performed on small samples.

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