Evaluation of heat-sealed ultracentrifuge tubes in the isolation of plasma lipoproteins

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Summary An initial step in the measurement of the concentration of lipids in plasma lipoproteins by the Lipid Research Centers' protocol is the separation of very low density lipoproteins from low density and high density lipoproteins by ultracentrifugation. We compared the performance of heat-sealed centrifuge tubes to the conventionally capped centrifuge tubes required by the protocol in terms of the separation and recovery of cholesterol and triglyceride. No significant differences in either parameter were found, however the heat-sealed tube required much less time to prepare and eliminated the need for periodic replacement of relatively expensive cap parts. We recommend the use of heat-sealed tubes for the routine separation of plasma lipoproteins.—**Cole, T. G., and D. W. Gibson.** Evaluation of heat-sealed ultracentrifuge tubes in the isolation of plasma lipoproteins. *J. Lipid Res.* 1984. **25:** 312–314.

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The accurate measurement of lipoprotein cholesterol and triglyceride has aided in the diagnosis of many lipid disorders and this has contributed to the decline of cardiovascular disease in the United States. Since 1972 the Lipid Research Clinics (LRC) of the National Institutes of Health have followed rigidly standardized procedures for the collection of data concerning plasma lipids and lipoproteins. The LRC protocol for the measurement of lipoprotein lipids involves the preliminary separation of VLDL ($d < 1.006$ g/ml) from LDL and HDL ($d > 1.006$ g/ml) by ultracentrifugation of plasma at its unadjusted density which is approximately 1.006 g/ml (1). The concentrations of lipid in the resulting top (VLDL) and bot-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LRC, Lipid Research Clinics; QS, Quick Seal.

tom (LDL, HDL) fractions are then measured by automated and standardized assays. LDL lipids are determined by subtracting from the bottom fraction the concentration of lipids in HDL, which are measured directly in plasma after precipitation of non-HDL lipoproteins with heparin and manganese. In addition, agarose electrophoresis of the top and bottom fractions allows the identification of abnormal lipoproteins which may be present in plasma, such as β-VLDL, a characteristic lipoprotein of type III hyperlipoproteinemia.

The preparation of centrifuge tubes for the separation of lipoproteins by the LRC protocol is somewhat tedious and time consuming, involving the use of tubes and caps which require assembly before use and cleaning afterwards. Moreover, the caps need periodic maintenance and the occasional replacement of relatively expensive parts. A newer type of ultracentrifuge tube is available (Quick Seal, Beckman), which is readily sealed by heat and requires no caps or preliminary preparation or maintenance. We evaluated the separation of plasma lipoproteins in both the Quick Seal tubes and capped tubes.

MATERIALS AND METHODS

Ultracentrifuge tubes, caps, and associated centrifugation equipment were purchased from Beckman Instruments (Palo Alto, CA). All other materials were of reagent grade quality and were approved for use by the LRC protocol.

Plasma was obtained from fasted patients seen at the Lipid Research Clinic of Washington University in accordance with the LRC protocol (1). Duplicate 5-ml aliquots of fresh plasma were taken for ultracentrifugal separation of lipoproteins using either 2.5 × 0.5-in Ultra Clear tubes (Beckman #344088) with aluminum caps or 2.5 × 0.5-in Quick Seal Ultra Clear tubes (Beckman #344320). The samples were overlaid with 0.15 M NaCl (containing EDTA, 0.001 M) and the tubes were capped or sealed according to the manufacturer's recommendations (2). The tubes were centrifuged in the Beckman 50.3 rotor for 18 hr at 10°C at 105,000 g (40,000 rpm) in a Beckman Model L centrifuge. All duplicate samples were centrifuged simultaneously in the same rotor. After centrifugation the tubes were cut using a tube slicer (Nuclear Supply Co., Washington, DC) as follows. For capped tubes, the set screw was removed from the fill hole and the hole was covered with a finger tip as the tube was sliced 3 cm from the bottom. The material in the top of the tube was removed through the fill hole by using a Pasteur pipet and transferred to a 5-ml volumetric flask. Any adherent lipoprotein in the top was washed into the flask with 0.15 M NaCl. The material in the bottom of the tube was transferred to another 5-ml volumetric flask

TABLE 1. Concentrations of lipids in the ultracentrifugally separated fractions of plasma

Sample Number	Cholesterol (mg/dl)						Triglyceride (mg/dl)					
	Total Plasma	Top Fraction		Bottom Fraction		Percent[a] Recovery	Total Plasma	Top Fraction		Bottom Fraction		Percent[a] Recovery
		LRC	QS	LRC	QS	LRC QS		LRC	QS	LRC	QS	LRC QS
972	222	60	63	160	153	99.1 97.3	293	220	231	65	57	97.3 98.3
095	208	22	21	192	184	102.9 98.6	118	56	50	57	67	95.8 99.2
115	197	38	39	159	154	100.0 98.0	194	141	139	49	46	97.9 95.4
180	257	26	23	234	236	101.2 100.8	162	113	108	37	34	92.6 87.7
182	154	32	32	122	119	100.0 98.1	444	357	381	38	36	89.0 93.9
153	157	14	13	145	142	101.3 98.7	77	34	46	27	28	79.2 96.1
235	283	7	11	274	274	99.3 100.7	58	19	19	25	25	75.8 75.8
236	381	171	156[b]	189	180	94.5 88.2	916	774	701[b]	62	59	91.3 83.0
264	256	60	57	193	192	98.8 97.3	377	316	313	42	43	94.9 94.4
286	302	33	33	263	264	98.0 98.3	213	127	127	67	82	91.1 98.1
303	289	28	26	254	273	97.6 103.5	144	90	83	37	54	88.2 95.1
Mean ± SD		45± 45	43± 41	190 ± 49	197 ± 56	99.3 ± 2.2 98.1 ± 3.8		204 ± 219	200± 202	46 ± 15	48 ± 18	90.3 ± 7.1 92.5 ± 7.3
P^c		NS		NS		NS		NS		NS		NS

Duplicate 5-ml aliquots of plasma were ultracentrifuged in capped tubes (LRC) and Quick Seal tubes (QS). The concentrations of cholesterol and triglyceride in the top and bottom fractions, as well as in non-fractionated plasma, were determined by the automated Lipid Research Clinics method (1).

[a] The percent recovery was calculated by dividing the sum of each lipid in the top and bottom fraction by the concentration of that lipid in total plasma.

[b] A small portion of this fraction was lost due to spillage.

[c] Paired two-tailed t-tests; NS (not significant) = $P > 0.05$.

and the tube was washed with 0.15 M NaCl. For heat-sealed tubes, the neck of the tube was cut with wire cutters and the resultant hole was covered with a finger tip while the tube was sliced 2.5 cm from the bottom. The materials in the top and bottom of the tube were quantitatively transferred to 5-ml volumetric flasks as before. After the flasks had warmed to room temperature, the volumes were adjusted to 5.0 ml with 0.15 M NaCl. Aliquots of each flask and of fresh plasma were taken for the determination of the concentration of triglyceride and cholesterol by the automated methods of the LRC protocol (1).

Values for the concentrations of lipids in each fraction were grouped according to the method of separation and were used to compute means and standard deviations. Paired two-tailed *t*-tests were used to assess statistical significance (3).

RESULTS AND DISCUSSION

The concentrations of triglyceride and cholesterol in the plasma and in the top and bottom fractions of the ultracentrifugally separated plasma are shown in **Table 1.** The majority of the triglyceride was in the top fraction which contains the VLDL and the bulk of the cholesterol was in the bottom fraction which contains the LDL and HDL. The percent recovery was calculated by dividing the sum of the concentration of triglyceride or cholesterol in the top and bottom fractions by the appropriate concentration in the whole plasma. The recovery of cholesterol was approximately 98 to 99% by both methods, whereas the recovery of triglyceride was less efficient and showed greater variability.

No statistically significant differences were observed in the recovery of lipid between the use of the two tube types. Although both systems require an initial investment in equipment to seal the tubes, the Quick Seal tubes were much easier to prepare for use, requiring approximately one-half the time necessary to prepare and subsequently clean the capped tubes. Furthermore, unlike the Quick Seal tubes, the caps of the capped tubes need periodic replacement. Therefore, in view of the ease of use of the Quick Seal tubes, the long-term savings in replacement parts, and the equivalence to capped tubes in the separation of lipoproteins by the LRC method, we suggest the use of Quick Seal tubes for routine lipoprotein isolation.

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