**Improved Fractionation of Lipoproteins Separated in Microultracentrifuge Tubes,** *Maria Pfeuffer* (Inst. für Physiol. und Biochem. der Ernährung, Bundesanstalt für Milchforschung, P.O. Box 6069, D-24121 Kiel, Germany)

Lipoprotein separation in an air-driven microultracentrifuge (Airfuge; Beckman, Palo Alto, CA) in tubes of up to 200 μL volume was first described in 1977 (*1*). This type of microtube can also be used in a refrigerated benchtop ultracentrifuge (TL-100; Beckman) or, with appropriate adapters, in any other ultracentrifuge. Separation on a microscale is particularly suited for studies in children and small experimental animals, for routine analysis, and for studies that require repetitive assays, such as in the postprandial state. However, recovery and reproducibility have often been unsatisfactory.

We prefer slicing of fractions over aspiration, as do some other laboratories (*2–4*). However, we cut the tubes while they are frozen, rinse the tube sections with a small amount of isotonic saline, and weigh the lipoprotein fractions obtained. For good temperature control we place the Airfuge in a cold room and use precooled compressed air (air drying and cooling system from Kaeser, Coburg, Germany).

To separate very-low-density lipoprotein (VLDL) from low- and high-density lipoprotein (LDL + HDL), we pipette 100 μL of plasma plus 75 μL of isotonic saline into a cellulose propionate tube (Beckman no. 341288) and gently mix by repeated withdrawal into and expulsion from the tip of the pipet. The samples are centrifuged for 3 h at 130 000*g* in the A-100 (18°) fixed-angle Airfuge rotor and are then frozen at −18°C.

The frozen tubes are sliced 8.9 mm from the bottom with a tube slicer (Beckman no. 924116), such that the lipid is divided into two fractions, at a volume ratio of ~1:2.5. The top fraction is quickly pushed into a preweighed vial; the bottom fraction, after thawing, is transferred to another vial. The top and bottom fragments of the tube are rinsed with 50 μL of saline each, the rinse liquids are combined with the respective lipoprotein fractions, and the vials are weighed again. The upper tube fragment holds the rinse liquid by capillary force.

VLDL or LDL + HDL cholesterol concentrations in plasma ($c'$) are calculated from the respective concentrations in the Airfuge fractions ($c$) by the equation

$$c' = c \cdot x/y$$

with $x$ (in μL) being the volume of the appropriate fraction and $y$ (in μL) being the original volume of plasma pipetted into the tube. Volumes are calculated by correcting the weights of the respective fractions including rinse liquid by a factor of 1.010 (top) or 1.019 kg/L (bottom). These factors were previously determined with a density-measuring cell (DMA 602; Paar, Graz, Austria) in nine samples.

Routine recovery of cholesterol in lipoprotein fractions, as measured in 44 human plasma samples by a CHOD-PAP enzymatic assay (*5*) in a centrifugal analyzer (Cobas Bio; Hoffmann-La Roche, Basel, Switzerland), averaged 96% (range 89.1–101.2%). When lipoproteins of a normolipidemic plasma sample were separated by our Airfuge micromethod and by conventional preparative ultracentrifugation (*6*), both methods yielded similar results (Table 1). When different normo- and hyperlipidemic plasma samples were used for comparison, the between-method correlation for VLDL cholesterol was $r = 0.967$; for LDL + HDL it was 0.995 (n = 8). Fractions obtained with both techniques were checked by agarose and polyacrylamide gel electrophoresis and shown to be of equal purity.

**Table 1. Plasma and lipoprotein cholesterol after separation by Airfuge or by preparative ultracentrifugation.**

| | Airfuge | | Preparative | |
|---|---|---|---|---|
| | Conc, mmol/L | CV, % | Conc, mmol/L | CV, % |
| VLDL | 0.493 ± 0.036 | 7.4 | 0.449 ± 0.039 | 8.8 |
| LDL + HDL | 4.725 ± 0.060 | 1.3 | 4.773 ± 0.086 | 1.8 |
| Total | 5.251 | | 5.251 | |

Concentration values are means ± SD, n = 8.

For high-quality separation of hyperlipidemic plasma, it may be wise to use a smaller plasma volume (e.g., 50 μL) and more saline (to a total volume of 175 μL). With the use of appropriate density solutions, the technique may be modified for isolation of HDL. Freezing of fractions is convenient in that slicing may be done at any time after the ultracentrifuge run; it also minimizes the loss of liquid during the slicing procedure. Nonetheless, one must still determine the exact volume by weighing, to improve overall recovery and reproducibility.

References

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**Detection and Identification of Amphetamine and Methamphetamine in Urine by GC/MS,** *Dorothea E. Blandford and Paul R. E. Desjardins*[1] (Health Sci. Centre, Dept. of Clin. Chem., 820 Sherbrook St., MS-543, Winnipeg, Manitoba Canada R3A 1R9; [1]author for correspondence: fax 204-787-3846)

Analysis of urine for drugs of abuse in many clinical laboratories involves screening with thin-layer chromatog-