Rapid Separation of Human Serum Lipoproteins Using the Thermo Scientific Sorvall MTX and MX Plus Micro-Ultracentrifuge Series

Key Words
Lipoprotein Separation, Sorvall MTX Micro-Ultracentrifuge, Sorvall MX Plus Micro-Ultracentrifuge, S150-AT Rotor, S140-AT Rotor, S120-AT2 Rotor, S120-AT3 Rotor

Introduction

Human serum lipoproteins are commonly isolated to study lipid metabolism and hyperlipidemia. The most frequently studied lipoproteins are VLDL (very low density lipoprotein), LDL (low density lipoprotein), and HDL (high density lipoprotein). VLDL particles ($\rho < 1.006$ g/mL) are formed when the liver synthesizes fats and packages them into a particle that is both hydrophobic and hydrophilic. This enables the VLDL and other lipoproteins to move freely in the bloodstream so that they may deliver lipids to various body cells. While in the bloodstream, lipoprotein lipase de-esterifies triglyceride in the VLDL creating a heavier particle. Some of these remnant particles are cleared by the liver, while the remainder is converted into an LDL particle (1.006 g/mL $< \rho <$ 1.063 g/mL). The major transporter of cholesterol in humans is LDL. HDL apolipoproteins ($\rho > 1.063$ g/mL) are formed in both the liver and the intestine. The HDL particles retrieve cholesterol from various cells and transfer it to other lipoproteins for transport back to the liver for further metabolism or excretion.

Multiple rotors and protocols are available for the rapid separation of lipoproteins from human serum with Thermo Scientific Sorvall MTX or MX Plus micro-ultracentrifuges (Table 1).

Procedures

The separation of serum lipoproteins occurs in a three-step process:

1. Separation of VLDL.
2. Separation of LDL.
3. Separation of HDL.

An exception is the method described using the Thermo Scientific S120-AT3 rotor.

Figure 1. Thermo Scientific Sorvall MX Plus Micro-Ultracentrifuge.

PROTOCOL 1: Using the Thermo Scientific S150-AT, S140-AT or S120-AT2 Rotor
(See Figure 2 for schematic representation.)

1. Add Fat Red 7B to all samples for easier interpretation of results.
2. Layer 300 µL of Solution A onto 600 µL of serum.
3. For VLDL separation, perform centrifugation with the appropriate parameters in Table 1 (Acc. 5, Dec. 7).
4. Remove the 300 µL top layer containing the VLDL fraction from the very bottom layer from step 3 and mix the remainder with 300 µL of Solution B.

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Thermo Scientific Rotor	Speed (rpm)	RCF (x g)	Temp (°C)	VLDL Separation Time (min.)	LDL Separation Time (min.)	HDL Separation Time (min.)
S150-AT	150,000	899,744	16	60	60	60
S140-AT	140,000	1,048,600	16	50	80	140
S120-AT2	120,000	649,826	8	85	125	210
S120-AT3*	120,000	649,826	10		90	

Table 1. Thermo Scientific Micro-Ultracentrifuge Rotors and Protocols for Lipoprotein Separation.

*Due to the small volume of sample in this rotor, the separation protocol is a single-step process, 90 minutes in length, and will sediment LDL and HDL, or float LDL, depending on density.

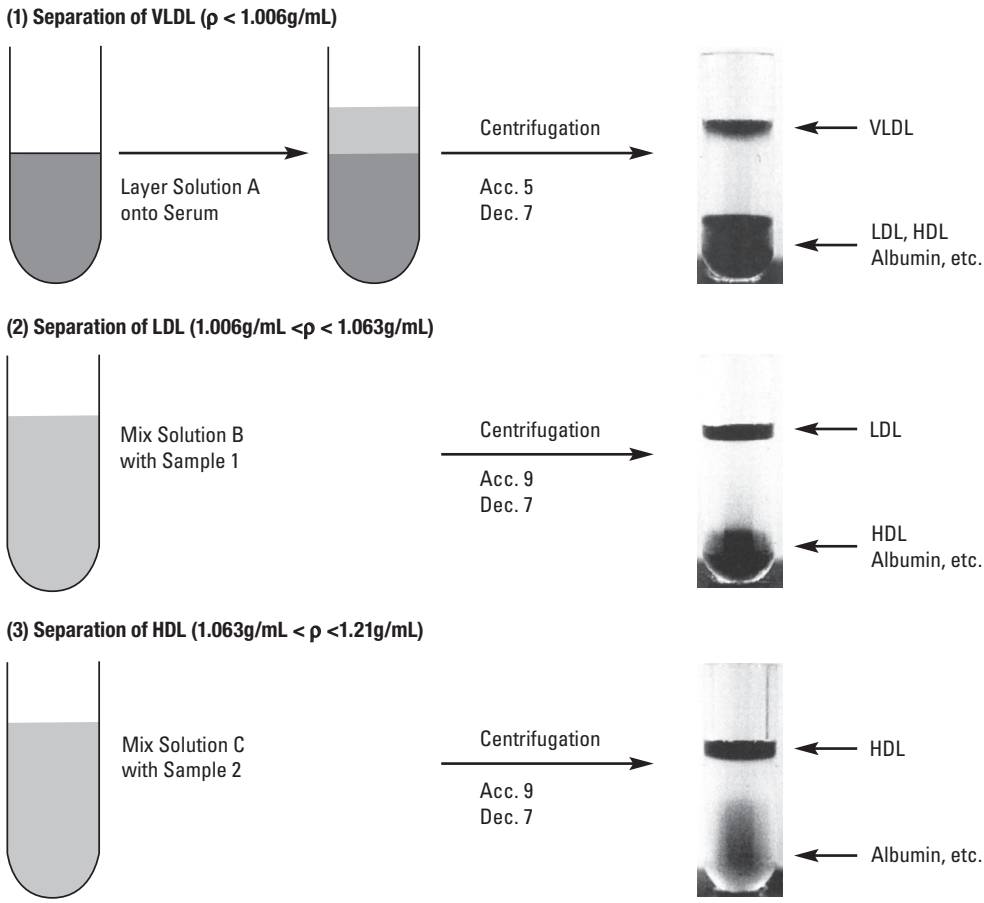

Figure 2. Separation of Serum Lipoprotein with the Thermo Scientific Small Volume Fixed Angle Rotors.

1. Solution A (ρ: 1.006 g/mL)

a. Mix 11.40 g of NaCl and 0.1 g of EDTA-2Na in a volumetric flask for 1,000 mL

b. Add 500 mL of 18 MΩ water and 1 mL of 1N NaOH

c. Mix until completely dissolved

d. Add 18 MΩ water up to 1,000 mL

e. Add an additional 3 mL of 18 MΩ water (NaCl 0.195 mol)

2. Solution B (ρ: 1.182 g/mL)
Add 24.98 g of NaBr to 100 mL of Solution A (NaCl: 0.195 mol, NaBr: 2.44 mol)

3. Solution C (ρ: 1.478 g/mL)
Add 78.32 g of NaBr to 100 mL of Solution A (NaCl: 0.195 mol, NaBr: 7.65 mol)

Table 2. Solutions for Protocol 1

5. For LDL separation, perform centrifugation with the appropriate parameters indicated in Table 1 (Acc. 9, Dec. 7).
6. Remove the 300 µL top layer containing the LDL fraction of sample 2 and mix the remainder with 300 µL of Solution C.
7. For HDL separation, perform centrifugation with the appropriate parameters indicated in Table 1 (Acc. 9, Dec. 7).
8. Remove the 300 uL top layer containing the HDL fraction.
9. All lipoprotein fractions should be stored at 4 °C.

PROTOCOL 2: Using the Thermo Scientific S120-AT3 Rotor

(See Figure 2 for schematic representation.)

PROTOCOL 2a: Sedimentation of LDL and HDL

1. Add Fat Red 7B to all samples for easier interpretation of results.
2. Mix 100 µL of 0.15M NaCl, 0.3mM EDTA-Na^2 (pH 7.4, ρ = 1.006 g/mL) and 100 µL of serum in a 0.5 mL polycarbonate thick-walled tube (PN. 45235).
3. Perform centrifugation with the following parameters: 120,000 rpm (649,826 x g) for 1.5 hr at 10 °C, Acc. 9, Dec. 7.

PROTOCOL 2b: Flotation of LDL

1. Add Fat Red 7B to all samples for easier interpretation of results.
2. Mix 100 µL of 15% (w/w) KBr (ρ = 1.12 g/mL) to 100 µL serum in a 0.5 mL tube so that average density is 1.063 g/mL.
3. Perform centrifugation with the following parameters: 120,000 rpm (649,826 x g) for 1.5 hr at 10 °C, Acc. 9, Dec. 7.

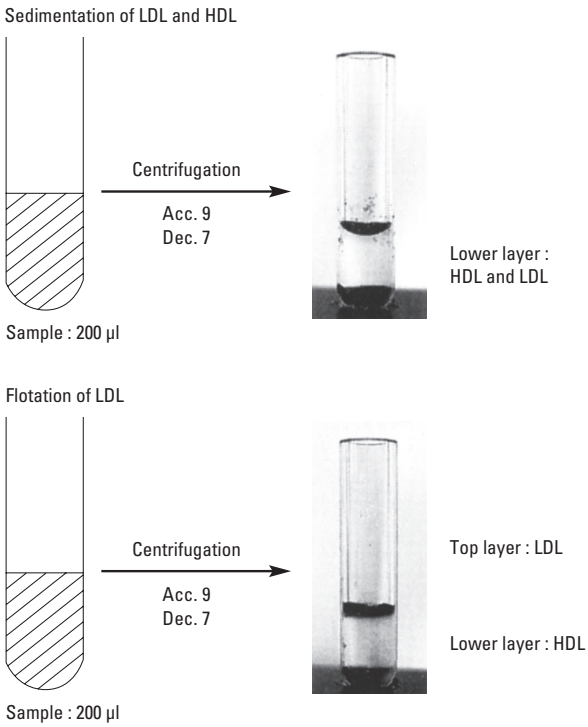

Figure 3. Separation of Lipoprotein From Human Serum using the Thermo Scientific S120-AT3 Fixed Angle Rotor.

Conclusion

Utilization of high g-forces sharply and efficiently separates small particles such as nucleic acids, lipoproteins, viruses and receptors. The separation of lipoproteins permits researchers and clinicians to study and evaluate levels of cholesterol, an indicator of cardiovascular health. Clinical laboratory testing of lipoprotein requires large numbers of samples and small sample volumes. Thermo Scientific S150-AT, S140-AT, S120-AT2, and S120-AT3 fixed angle rotors, with Thermo Scientific Sorvall MTX or MX Plus micro-ultracentrifuges can help research investigator save precious time by enabling clear, quick separation of lipoprotein.

References

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