

himac APPLICATION

No. 89 JULY 1999

Subject Separation of lipoprotein from human serum with the preparative micro ultracentrifuge

Model CS-GX series preparative micro ultracentrifuges

Separation of lipoprotein from human serum using the S80AT3 angle rotor

Lipoproteins that are included in serum have a close relation to metabolism of lipid, and they are noticed in the research field on hyperlipemia. This time, lipoproteins were separated gradually from human serum with the preparative micro ultracentrifuge and the 6PC thick tubes (actual capacity: 5.3 ml). It is possible to carry out centrifugation under the conditions similar to those of the large ultracentrifuge because serum can be centrifuged up to 3.4 ml per tube in the S80AT3 angle rotor. The specific gravity liquid was prepared according to the reference.

1. Instruments used

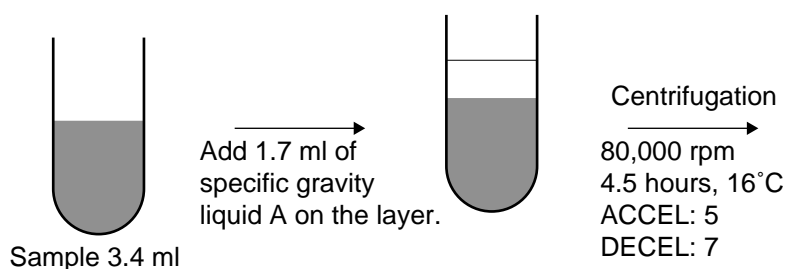
Centrifuge: Hitachi CS-GX preparative micro ultracentrifuge

Rotor: S80AT3 angle rotor

Tube: 6PC thick tubes

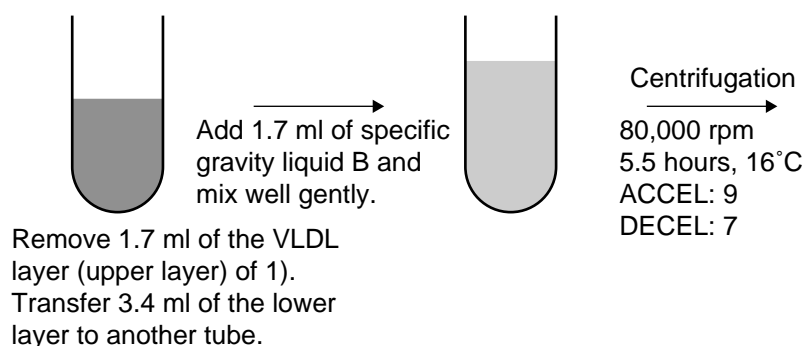
2. Procedures and results

1) Separation of VLDL, including chylomicron ($\rho < 1.006 \text{ g/cm}^3$)



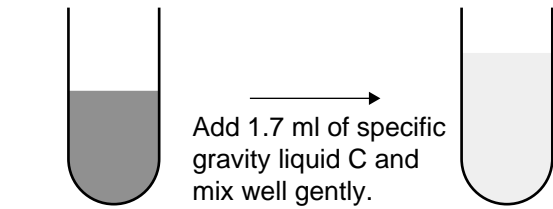
Upper layer: VLDL

2) Separation of LDL, including IDL ($1.006 \text{ g/cm}^3 < \rho < 1.063 \text{ g/cm}^3$)



Upper layer: LDL
(including IDL)

3) Separation of HDL ($1.063 \text{ g/cm}^3 < \rho < 1.21 \text{ g/cm}^3$)



Remove 1.7 ml of the LDL layer (upper layer) of 2).
Transfer 3.4 ml of the lower layer to another tube.

Centrifugation
80,000 rpm
8 hours, 16°C
ACCEL: 9
DECEL: 7



Upper layer: HDL

Lower layer: albumin, etc.

Specific gravity liquid A*¹: 1.006 g/cm^3
Specific gravity liquid B*²: 1.182 g/cm^3
Specific gravity liquid C*³: 1.478 g/cm^3

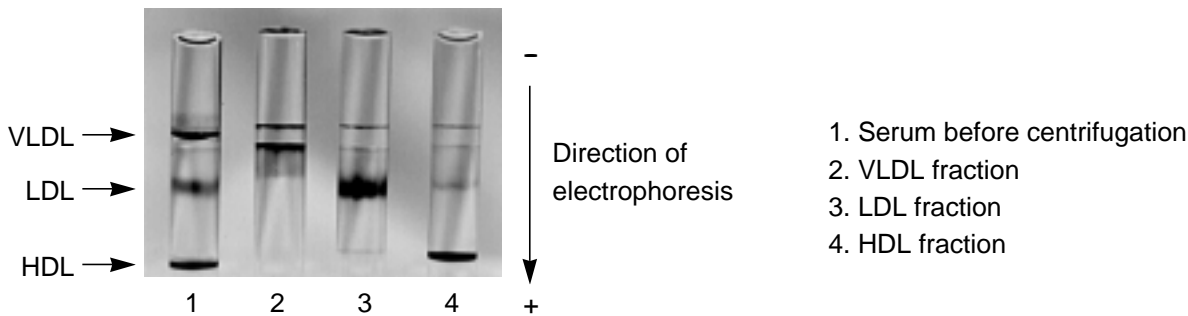


Fig. 1 Result of electrophoresis*⁴

*1: Specific gravity liquid A:

Put 11.40 g of NaCl and 0.1g of EDTA-Na₂ in a 1,000 ml measuring flask. Add 500 ml of distilled water and 1 ml of 1N NaOH, and mix them well until they are dissolved. Add distilled water up to 1,000 ml and then add additional 3 ml of distilled water. (NaCl: 0.195 mol)

*2: Specific gravity liquid B:

Add 24.98 g of NaBr to 100 ml of the specific gravity liquid A. (NaCl: 0.195 mol, NaBr: 2.44 mol)

*3: Specific gravity liquid C:

Add 78.32 g of NaBr to 100 ml of the specific gravity liquid A. (NaCl: 0.195 mol, NaBr: 7.65 mol)

*4: PAG disk electrophoresis for lipoprotein: Lipophor kit (JOKOH CO., LTD.)

For further information, please contact Hitachi Koki Scientific Instruments Group.

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