

himac APPLICATION

No. 57 April 1997

Subject Separation of lipoprotein

Model High performance preparative ultracentrifuge CP100 α

Rapid separation of lipoprotein from human serum by fixed-angle rotor P100AT2 (VLDL : 2h, LDL : 3h, HDL : 4h)

Human serum lipoprotein is thought to have something to do with atherosclerosis or hyperlipidemia and recently the association is investigated in various ways. We tried to separate lipoprotein rapidly from human serum by high performance preparative ultracentrifuge CP100 α with maximum rotating speed of 100,000rpm (maximum centrifugal force of 803,000 X g).

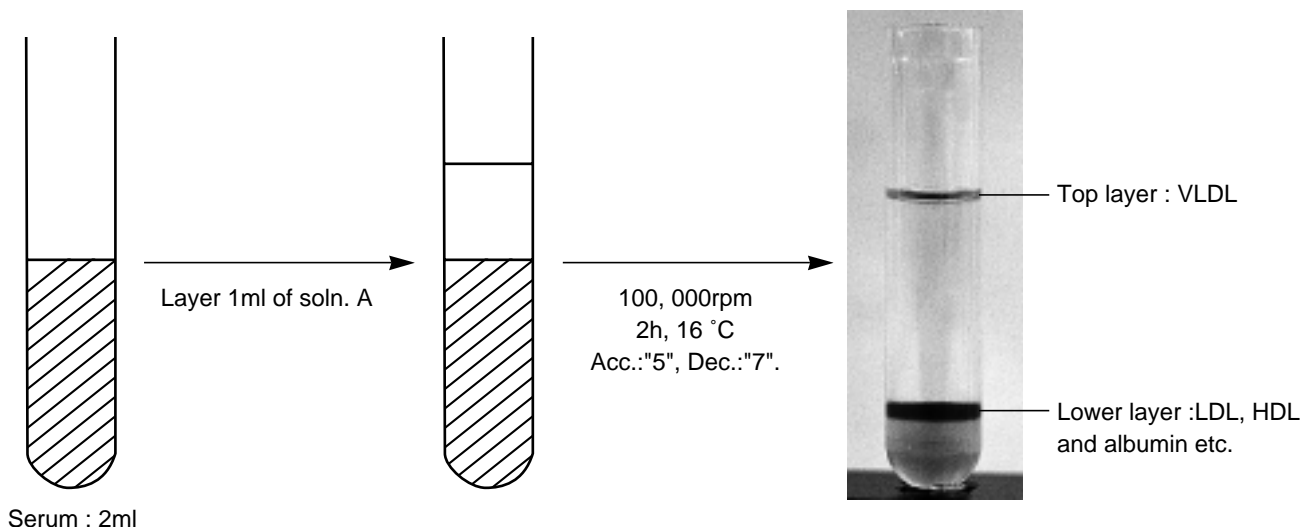
1. Models used

Centrifuge : Hitachi high performance preparative ultracentrifuge CP100 α
Rotor : P100AT2 (Fixed-angle rotor)
Tube : 4.7PC thick-walled tube (Single use only)
Cap : B - Ti lid (Be sure to use)

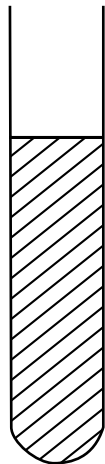
2. Methods and results

Fat Red 7B is added for recognition of the results easily.¹⁾

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



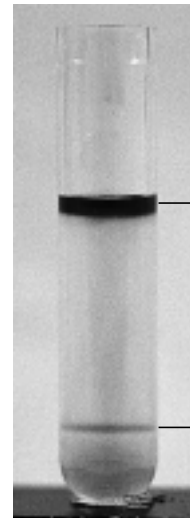
2) Separation of LDL (including IDL) ($1.006\text{g/cm}^3 < \rho < 1.063\text{g/cm}^3$)



New tube

Add 1ml of soln. B to
2ml of lower layer of 1),
mix well gently and
centrifuge.

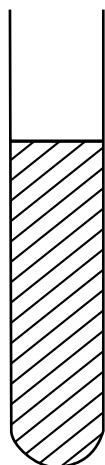
100, 000rpm
3h, 16 °C
Acc.: "9", Dec.: "7".



Top layer : LDL

Lower layer : HDL
and albumin etc.

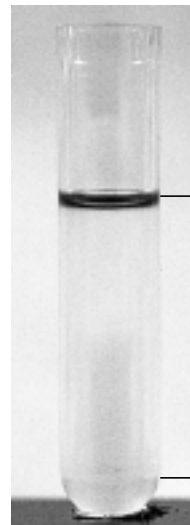
3) Separation of HDL ($1.063\text{g/cm}^3 < \rho < 1.21\text{g/cm}^3$)



New tube

Add 1ml of soln. C to
2ml of lower layer of 2),
mix well gently and
centrifuge.

100, 000rpm
4h, 16 °C
Acc.: "9", Dec.: "7".



Top layer : HDL

Lower layer :
Albumin etc.

Soln. A (1.006g/cm^3) : Dissolve 11.4g of NaCl and 0.1g of EDTA-Na₂ with 500ml of distilled water (DW) and add 1ml of 1N NaOH. Then add DW up to 1000ml and add 3ml of DW. (NaCl : 0.195mol)

Soln. B (1.182g/cm^3) : Dissolve 24.98g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 2.44mol)

Soln. C (1.478g/cm^3) : Dissolve 78.32g of NaBr to 100ml of soln.A. (NaCl : 0.195mol, NaBr : 7.65mol)

Reference

1) Bronzert T. J. and Brewer H. B. *Clin. Chem.*, 23, 2089 (1977).

HITACHI

(Export office)

Nissei Sangyo Co., Ltd.

Head Office :

1 - 24 - 14, Nishishinbashi, Minato-ku, Tokyo, 105 Japan

Tel : 81 - 3 - 3504 - 7281

Fax : 81 - 3 - 3504 - 7302

(Manufacturer)

Hitachi Koki Co., Ltd.

Scientific Instruments Div.

1060, Takeda, Hitachinaka - city, Ibaraki - pref., 312 Japan

Tel : 81 - 29 - 276 - 7384

Fax : 81 - 29 - 276 - 7475