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Separation and purification of hepatitis B antigens using a zonal rotor – (1)

CP-WX series preparative ultracentrifuge/P35ZT zonal rotor

Currently, most of hepatitis B vaccines have been produced by purifying hepatitis B antigens biosynthesized by gene recombinant. Although it is a conventional method to separate, purify and inactivate hepatitis B antigens in hepatitis B antigen positive human plasma, the method of separation from human plasma can be used as a model for learning the centrifugal method in order to produce vaccines. As a first step, we introduce the following method to float low-density hepatitis B antigen components in the high-density liquid layer by adding plasma.

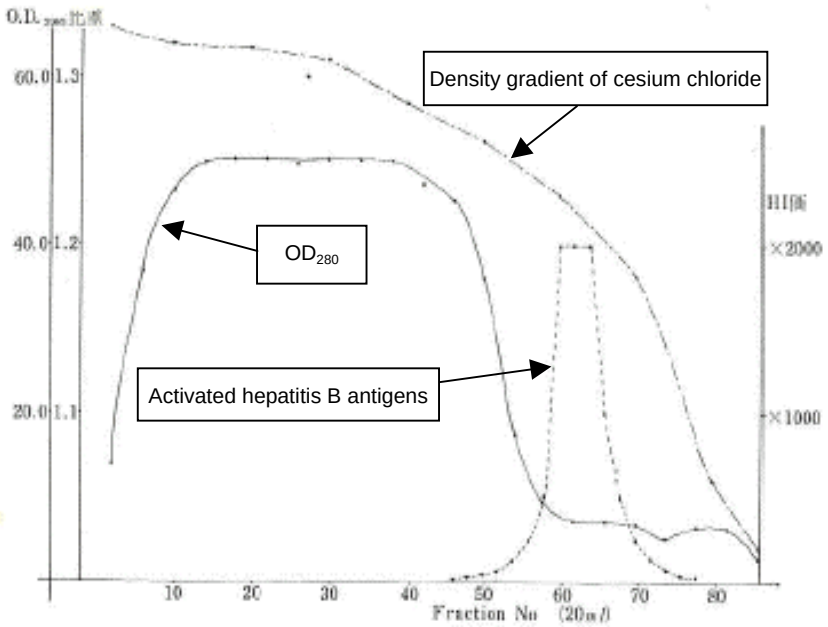
Reference: “Separation and purification of hepatitis B antigens” Takahashi, et al., Nippon Rinsho, Vol. 32, No. 12 (1974), Separate Volume

Experiment

1. Conditions for centrifugation

Centrifuge: CP-WX series preparative ultracentrifuge
Rotor: P35ZT zonal rotor
Speed: 28,000 rpm
Time: 18 hours
Density gradient solution: Pour 600 ml of the continuous density gradient solution whose cesium chloride density is 1.10 g/ml (12% (w/w)) to 1.30 g/ml (30% (w/w)) into the center of the rotor.
Sample: About 1,100 ml of human plasma whose density is prepared to 1.32 g/ml with cesium chloride

2. Result



(1) Hepatitis B antigens were floated and concentrated to 1.15 g/ml to 1.25 g/ml (20 fractions from No. 53 to 72, 400 ml).
(2) The density of serum proteins was 1.26 g/ml to 1.30 g/ml.

3. Explanation

While the buoyant density of hepatitis B antigens is 1.15 g/ml to 1.25 g/ml, the buoyant density of serum proteins to be removed is about 1.3 g/ml. Therefore, cesium chloride or potassium bromide is dissolved in the sample serum (plasma) to adjust the density to 1.32 g/ml. This sample serum is subjected to centrifugal separation in order to separate the low-density hepatitis B antigens from the high-density serum proteins by floating only the low-density hepatitis B antigens. Plenty of time is taken for centrifugation because this separation method is based on the difference of density between hepatitis B antigens and serum proteins.

Instruments

CP-WX series preparative ultracentrifuge

P35ZT zonal rotor

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