Micromethod for Rapid Separation of Lymphocytes from Peripheral Blood

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Introduction

In histocompatibility typing, especially in cadaver transplants, where speed is essential, a rapid, simple and reliable micromethod for the separation of lymphocytes from peripheral blood is important. The procedure that is outlined is a modification of previously described techniques.1,2,3

Principle

The separation of lymphocytes from the other blood cells is based on the differences in their specific gravity. Centrifugation of whole blood against an isopaque-ficoll gradient results in the formation of three separate layers: the top one contains the slowly sedimenting cells (lymphocytes, platelets and some monocytes); the intermediate one, the granulocytes; the bottom layer contains the erythrocytes, aggregated by the ficoll.

Reagents

1. Venous blood is mixed with either 5 percent disodium EDTA, in the proportion of 0.04 ml per 1 ml of blood, or with heparin (1000 U per ml), in the proportion of 0.025 ml per 1 ml of blood. Sodium citrate or ACD solution can also be used as anticoagulants.

2. Isopaque (sodium diatrizoate). A 33.9 percent solution is made in distilled water.

3. Ficoll (sucrose polymer, m.w. 400,000). A 9 percent solution is made in distilled water.

4. Isopaque-ficoll gradient. Ten parts of isopaque solution are mixed with 24 parts of ficoll solution to obtain a gradient with a specific density between 1.076 and 1.080. This mixture is kept at 4°C but is warmed to 37°C prior to use.

5. Tris buffer, pH 7.3. Precisely 6.85 g of TRIZMA HCL and 0.8g of TRIZMA BASE are dissolved in distilled water and the volume made up to one liter.

6. Ammonium chloride (0.83 percent)

7. Tris-ammonium chloride solution, pH 6.2-6.4. One part of tris buffer is mixed with nine parts of the ammonium chloride solution.

8. Approximately 5 percent calf serum in N Hank’s solution.

Procedure

Ten Beckman micro-tubes are half filled with isopaque-ficoll gradient warmed to 37°C. Two ml of anticoagulated blood are layered upon the gradient to fill the tubes and centrifuged for 2% minutes at full speed (12,000 g) in a Beckman Spinco microfuge.

Presented at the Applied Seminar on Chemical Hematology, November, 1970.

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MICROMETHOD FOR RAPID SEPARATION OF LYMPHOCYTES FROM 2 ML OF PERIPHERAL BLOOD

Beckman tube
- Blood (anticoagulated) x 10 tubes
- Isopaque-ficoll gradient (warmed to 37°C)

2 1/2 minutes centrifugation at full speed (17,000 rpm) in Beckman Spinco microfuge

Plasma layer
Interface
Gradient layer
Erythrocyte layer

30 second centrifugation at full speed

Supernatants are discarded, buttons resuspended in warmed (37°C) Tris-ammonium chloride solution and allowed to stand for 1 minute to lyse the erythrocytes

30 second centrifugation at full speed

Supernatants discarded, buttons resuspended in N Hank's

30 second centrifugation at full speed

Supernatants are discarded. The 4 lymphocyte buttons are combined by resuspending them in 0.3 ml of 5% calf serum in N Hank's.

Lymphocyte counts are made and adjusted to the desired cell concentration.

Figure 1. Micromethod for rapid separation of lymphocytes from 2 ml of peripheral blood.
The lymphocytes are harvested above the interface between the plasma and the gradient and are distributed into four Beckman tubes. These aliquots are centrifuged at full speed for 30 seconds, and the supernatant, which contains platelets, is discarded. The lymphocyte button is resuspended in tris buffer-ammonium chloride solution (also warmed to 37°C), and is allowed to stand for one minute in order to lyse the contaminating erythrocytes. After a 30 second centrifugation at full speed, the supernatant is discarded and the button is resuspended in N Hank's solution. After another 30 second centrifugation at full speed, the supernatant is discarded, and the four lymphocyte buttons are combined and resuspended in an appropriate medium (N Hank's, 5 percent calf serum in N Hank's, AB serum, etc.). The resulting lymphocyte suspension is adjusted to the desired concentration (e.g., 2000 per mm$^3$ for HL-A lymphocyte typing).

**Results**

In less than ten minutes, from one ml of blood, an average of $3 \times 10^5$ lymphocytes (98 percent viable cells) are obtained, which are ready to be used as an antigen for histocompatibility typing when resuspended in an appropriate medium (Hank's, 5 percent calf serum-Hank's, AB serum, etc.). In parallel studies, the lymphocytes prepared by the described technique showed the same antigenicity and high purity as the lymphocytes obtained by using the more time consuming standard methods.

**Discussion**

Because of its rapidity and simplicity, this microtechnique for lymphocyte separation is a method of choice for emergency tissue typing of cadaver donors. Since it requires only one or two of blood to prepare a lymphocyte antigen, it becomes also the elective method for typing young children for genetical studies.

**References**