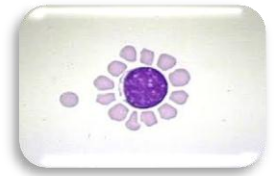


Lab. 11

T and B Rosette Forming Tests



Using rosette formation tests it is possible to differentiate between T and B lymphocytes in a lymphocyte suspension. Certain lymphocytes are capable of binding in vitro to sheep erythrocytes (SRBCs) (forming rosettes (so-called E rosettes). (Although the immunological basis of this binding is not fully understood). In suitable conditions up to 80 % of lymphocytes react in this way.

Other lymphocytes (B lymphocytes) have receptors for antigen-antibody complexes and for complement components and in suitable conditions can form rosettes with human erythrocytes coated with antibody and complement (so-called HEAC rosettes). These lymphocytes comprise up to 20 per cent of the total.

T-rosette forming test:

1. 0.5 ml. of the lymphocyte suspension (prepared in Lab. 10) was mixed with an equal volume of the (sheep RBC) SRBC suspension.
2. Centrifuged at 180G/ 5 min at room temperature.
3. The mixture then stood for 30 min. at 4°C.
4. Stain a dry preparation with leishman stain and count rosette forming cells.

The result consider positive if 3 and more SRBC found on the surface of T- cell.

B-rosette forming test:

Material required:

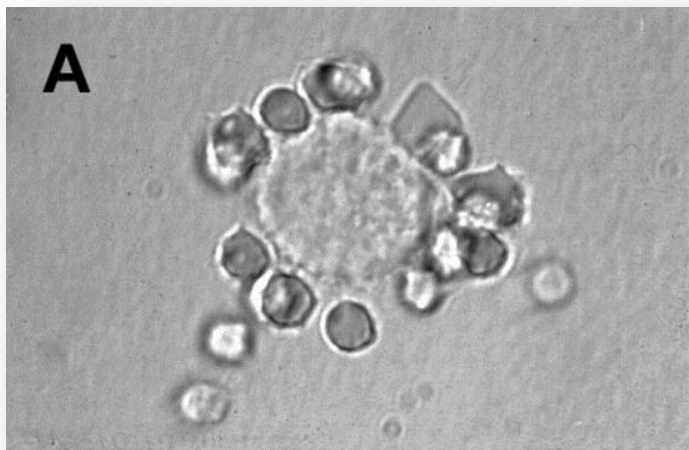
1. Mouse complement
2. Rabbit antiserum (prepared by injecting rabbit with 5% suspension of SRBC, after 1 week blood collected from rabbit by heart puncture to get rabbit antiserum).
3. Sheep RBC 5% suspension

Procedure:

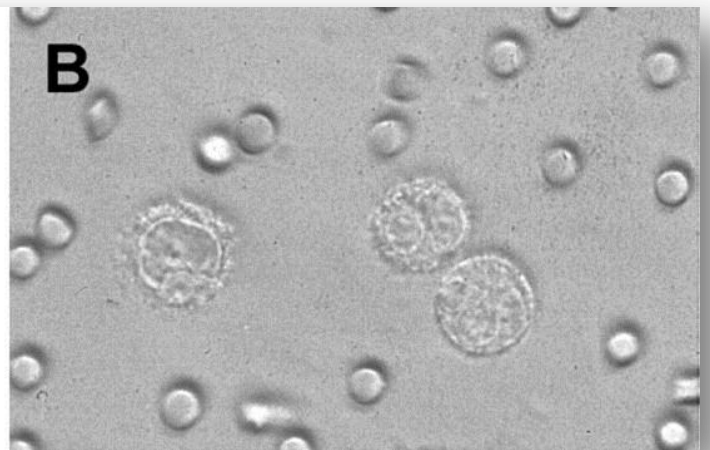
1. Mix equal quantities of Sheep RBC (Ag) + 1:200 of rabbit serum (Ab).
2. Incubate the mixture at 37 for 30 min then centrifuge 200 g for 5 min.
3. Prepare 5% of the sediment in step 2 in HBSS.
4. Mix suspension in step 3 with mouse complement, incubate the mixture at 37 for 30 min then centrifuge for 5 min /200 g, three times.

5. Prepare a suspension of the sediment in step 4 in HBSS the suspension now called **(EAC) Erythrocyte Antigen Complement**.
6. Mix equal volumes of EAC = lymphocytes suspension (prepared in Lab. 10) and leave for 1 hr in 4 °C, then centrifuge 200g/1min.
7. The same steps 3,4 in T- rosette formation test.

The result consider positive if 3 and more SRBC found on the surface of B- cell.



A: Positive result



B: Negative result

QUIZ: WITHOUT KNOWING THE PROCEDURE, CAN YOU TELL BY LOOKING TO THE ABOVE SLIDE IF THESE CELLS ARE T OR B LYMPHOCYTES?