

There are two exercises to perform.

YOU WILL ALSO BE SHOWN A VIDEO AND NEED TO EXAMINE THE RESULTS OF THE IMMUNODIFFUSION FROM PRACTICAL CLASS 6.

WORK IN PAIRS THROUGHOUT.

1. PLAQUE-FORMING CELLS

The aim of this practical is to detect lymphocytes secreting antibody specific for sheep red cells. The antibody diffuses from the lymphocyte and combines with antigen on the surface of adjacent red cells. In the presence of complement, the red cells coated with antibody will lyse, thus giving a clear plaque around each antibody-producing cell. These plaque-forming cells can either be embedded in agarose together with the red cells, or else the reaction can take place in a monolayer of cells at the bottom of a thin chamber. The latter is the technique that you will use today.

MATERIALS

1. Sheep red blood cells labelled **SRBC**.
2. Spleen cells from thymus-deficient mice (nude mice) previously injected with SRBC (labelled **nu/nu**)
3. Spleen cells from normal littermates which were also injected with SRBC (labelled **Normal**).
4. Complement labelled **CP**.
Important: Do not confuse the complement used for this test, labelled CP, with that for use in Exercise 2 which is labelled COMP.

METHOD

1. Put two drops of SRBC in each of two bijoux with a pastette.
2. Add two drops of complement (CP) to each tube. Label one tube nu and the other +.
3. Add two drops of nu/nu spleen cells to one tube (nu) and two drops of normal spleen cells to the other (+).
4. Fill the slide chambers with the well-mixed suspensions, ensuring that there are no air bubbles.
Label the slides at the ends.
5. Dip the edges of the slide into wax and place the slides on the slide trays. They will be incubated at 37°C for one hour for you.
6. Examine the slides with a light source below, keeping the slides horizontal. If you are not careful the cells in the monolayer will move!

Catalogue Number	Image
I_06.jpg	Plaque forming cells

7. Look at a plaque with a microscope and try to identify the antibody-secreting cell.

Catalogue Number	Image
I_11.jpg	Plaque forming cells

2. TITRATION OF THE HAEMOLYTIC ACTIVITY OF GUINEA PIG COMPLEMENT FOR THE COMPLEMENT FIXATION TEST (SEE PRACTICAL CLASS 8)

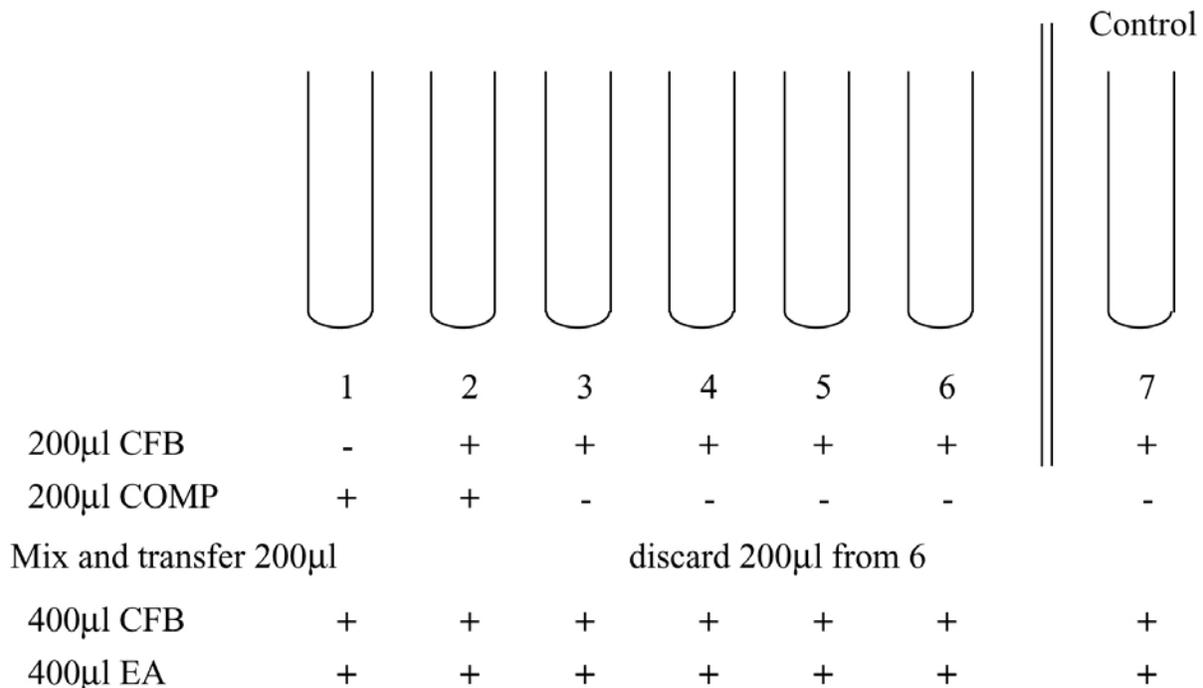
Complement binds to certain antigen-antibody complexes. If the antigen is on the surface of a cell, such as a red cell, the cell will be lysed (see the General Notes to the Immunology Practical Classes).

MATERIALS

1. Complement: Guinea pig serum containing complement diluted 1:10 labelled **COMP**.
2. 1% red cells: Red blood cells coated with antibody and labelled **EA**.
3. Buffer: Complement Fixation Buffer **CFB** containing calcium and magnesium ions.

METHOD

The experiment set-up is shown in the diagram below:



1. Using the pipettor make doubling dilutions of complement in tubes 1 to 6, using a unit volume of 200µl, as shown above. [Add 200µl CFB to 2 to 7. Add 200µl of Complement to tubes 1 and 2, mix tube 2 and transfer 200µl to tube 3. Repeat the procedure up to tube 6, discarding 200µl from this tube.]
2. Replace the tip and add a further volume of 400µl of CFB to each tube. (This is added to make the volumes comparable to those in the complement fixation test which will be performed in the next class).

3. Replace the tip and resuspend the red cells coated with antibody (EA). Add 400µl of EA to each tube including tube 7 which is a control.
4. Incubate in the 37°C waterbath for 30 minutes (set this incubation up in time for the video). Please do not drip water into other peoples' tubes.

RESULTS

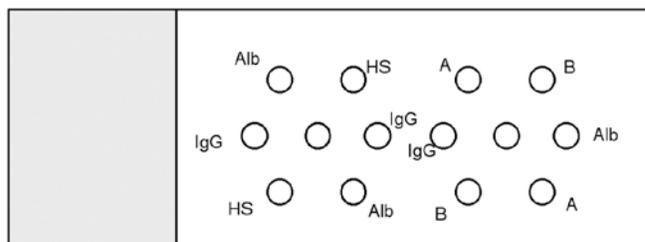
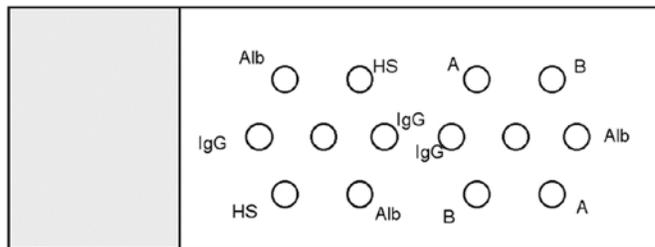
Observe the highest dilution which gives 100% lysis.
 This tube contains one minimal haemolytic dose (1 MHD). What dilution of complement contains 3 MHD?
 What other controls might be included in the test?

Catalogue Number	Image
I_03.jpg	Complement titration

3. A FILM ON CYTOTOXICITY WILL BE SHOWN

4. IMMUNODIFFUSION

Examine your agar precipitation slides from the last class. Draw what you see. Do you think the albumin and IgG are pure?



Catalogue Number	Image
I_04.jpg	Immunodiffusion

A series of experimental observations using immunodiffusion are displayed on the boards around the room. Make sure you examine these and answer any questions.

Dim and turn off the microscope light.
Disinfect your bench top. Push the stool under the bench.
Thank You.