

There are three exercises to perform. Work in pairs for the first one and work individually for the blood grouping.

1. COMPLEMENT FIXATION TEST

This test is explained in detail in your General Notes to the Immunology Practical Classes. Briefly, serum from a patient suspected of having been infected with an agent such as syphilis is allowed to react with the antigen. If antibody is present in the patient's serum, the resulting antigen-antibody complex will consume complement, which will then not be available to lyse the indicator red cell (coated with antibody) which is added after the first phase of this test.

The amount of complement used in the test must be standardised (see **Practical 7**). As complement is labile, more than one MHD must be used to ensure that there is enough complement present to lyse the standard amount of antibody-coated red cells used as an indicator.

MATERIALS

1. Test serum from a patient diluted 1:10 (labelled **SERUM**); this has been heated at 56°C to inactivate complement.
2. Preparation of microbial antigen labelled **Ag**.
3. Red cells coated with antibody labelled **EA**.
4. Complement fixation buffer labelled **CFB**.
5. Guinea pig complement diluted to give 3 MHD labelled **COMP**.

METHOD

Use the pipettor as usual for aliquoting solutions.

1. Make a two-fold dilution series in CFB of the diluted test serum over seven tubes with a unit volume of 200µl as shown below. Remember to discard the residual 200µl from tube 7.
2. Add 200µl of 1:10 serum to tube 8. This will constitute the serum control, that is, the tube which contains antibody, but no antigen.
3. Add 200µl of complement to each tube.
4. Add 200µl of antigen to tubes 1 →7 and tube 9

	1	2	3	4	5	6	7	8	9	10
200µl CFB	-	+	+	+	+	+	+	-	-	-
200µl serum mix and transfer discard from the 7th tube	+	+	-	-	-	-	-	+	-	-
200µl COMP	+	+	+	+	+	+	+	+	+	+
200µl antigen	+	+	+	+	+	+	+	-	+	-

5. **Adjust the volumes of the control tubes (8 →10) with CFB.**
6. Mix and INCUBATE for 30 minutes at 37°C to allow fixation of complement.
7. Remove tubes from the waterbath and resuspend EA. Add 400µl EA to all 10 tubes.
8. Incubate at 37°C for a further 30 minutes.

RESULTS

1. Record the pattern of haemolysis.
2. What is the complement fixation titre of antibody in the serum?
3. Why use 3 MHD of complement in this assay?
4. A series of control tubes have been included in your experiment (tubes 8-10).
5. What do they control for and why?
6. Why might a patient's serum give a false positive?
7. Do the controls indicate that the reaction was specific?
8. How could this test be used to diagnose whether this patient has contracted syphilis?

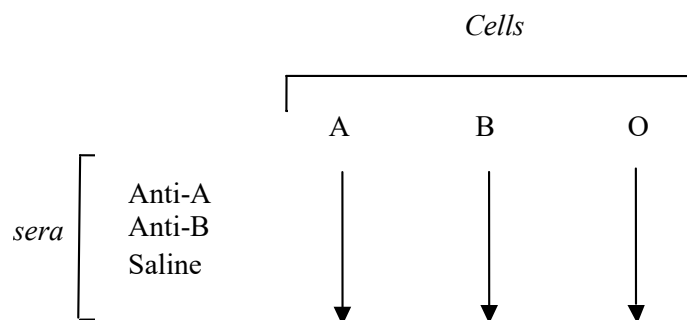
Catalogue Number	Image
I_07.jpg	Complement fixation test

2. DETERMINATION OF ABO BLOOD GROUPS

The ABO blood groups may be determined by agglutination of red cells carrying the antigens with specific anti-A and anti-B sera. Blood grouping may be confirmed by testing for anti-A and anti-B in the serum. If the antigen is absent from the red cells, the corresponding antibody will be present in the serum.

MATERIALS

1. Known **A**, **B**, and **O** red cells: 5% suspensions
2. **Anti-A** and **anti-B** sera
3. Saline



METHOD

1. Set out your plate as shown
2. Place one drop of antiserum in the wells across the plate
3. Add one drop of the appropriate cell suspension down the columns
4. Leave undisturbed for at least 45 minutes until the cells have settled. Read the agglutination patterns and confirm the result by tapping the side of the plate.

RESULTS

Record the results on your diagram with a + for agglutination and a - for no agglutination.

Catalogue Number	Image
I_09.jpg	Blood groups

After recording the results the plate can be agitated again. Non-agglutinated cells will resuspend and agglutinated cells will remain in small clumps and settle to the bottom.

Catalogue Number	Image
I_10.jpg	Blood groups(agitated)

3. DIRECT ANTIGLOBULIN TEST (THE DIRECT COOMBS TEST)

Haemolytic disease of the newborn (HDN) is a foetal anaemia which results from the presence of maternal antibody reactive with paternally-inherited blood group antigens on the foetal red blood cells. This usually involves the blood group antigen Rhesus-D.

Although IgM anti-Rhesus antibodies agglutinate red blood cells and fix complement, the IgG anti-Rhesus antibodies do not. [In haemolytic disease of the newborn--HDN--the antibodies are always IgG. Why?] This is a test for non-agglutinating Rh anti-D antibody on the red cells of a neonate suffering from HDN, haemolytic disease of the newborn.

MATERIALS

1. 5% suspension of *washed* red cells from a normal child (**N**)
2. 5% suspension of *unwashed* red cells from a neonate with HDN (**U**)
3. 5% suspension of *washed* red cells from the same neonate with HDN (**W**)
4. Rabbit anti-human globulin produced by injecting human immunoglobulins into a rabbit (**Ab**)
5. Normal rabbit serum (**RS**)

METHOD

1. Use the template here to record your results.

	N	U	W
Ab			
RS			

2. Place one drop of Ab and RS in the appropriate circles on your Test Card.
3. Add one drop each of the red cell suspensions (N, U, W) to the appropriate circles.
4. Wait for 10 minutes and then rock the card gently as demonstrated.
5. Record the agglutination results on your chart as in experiment 2

RESULTS

1. Draw a sketch to represent the mechanism of the antiglobulin reaction.
2. How are red blood cells washed? What is removed by washing the cells?
3. Why is no agglutination seen with unwashed red cells (U) from the neonate with haemolytic disease?
4. If these antibodies do not cause lysis with complement, why does their presence on the foetus's red cells lead to increased red cell breakdown?
5. Suggest reasons why the IgG anti-D antibodies do not agglutinate the red cells.
6. How is Rhesus-D incompatibility managed clinically?
7. What is the mechanism behind this intervention?

Catalogue Number	Image
I_08.jpg	Direct antiglobulin test

***Please disinfect your bench top.
Push the stool under the bench.***

Thank You