

PROTEIN A COATED SURFACE

TECHNICAL NOTE N. 25

Test for finding and identifying red cell antibodies by means of solid phase method

Protein A microplates U-shape bottom can be adapted to carry out Coombs test.

The two Coombs tests are:

- Direct Coombs test (direct antiglobulin test or DAT)
- Indirect Coombs test (indirect antiglobulin test or IAT)

The direct Coombs test is used in the diagnosis of autoimmune diseases. It detects antibodies bound to the surface of red blood cells. The red blood cells (RBCs) are washed (removing the patient's own serum) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive.

The indirect Coombs test is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood, and then the serum is incubated with RBCs of known antigenicity. If agglutination occurs, the indirect Coombs test is positive.

The two Coombs tests are based on the fact that anti-human antibodies, which are produced by immunizing non-human species with human serum, will bind to human antibodies, commonly IgG or IgM. Animal anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of RBCs, and in the appropriate test tube conditions this can lead to agglutination of RBCs.

The phenomenon of agglutination of RBCs is important here, because the resulting clumping of RBCs can be visualized; when clumping is seen the test is positive and when clumping is not seen is negative.

The clumping evaluation could be a drawback as it lacks an objective point of termination that can be readily determined automatically or visually in that the reactions that occur within the dilution series vary in intensity to the extent that weak reactions can be incorrectly interpreted as negative for example.

Through the use of a microplate U-shape bottom with Protein A it is easier to carry out a method of cross-sampling, for example.

The erythrocytes are preliminarily incubated with sera or plasma that contain antibodies against erythrocytes, and the coated or uncoated erythrocytes are washed and transferred along with an anti-human reagent to the Protein A microplate.

Erythrocytes coated with antibodies of IgG type are attached by way of anti-human IgG constituent of the reagent to the solid phase in the form a visible film.

Here as well, uncoated erythrocytes form buttons of sedimentary cells.

These two reaction patterns can be read visually or spectrophotometrically to arrive at an objective "yes" or "no"

