Notes

60.5 METHODS OF AGGLUTINATION

Agglutination test can be performed using three different techniques. These include: rapid agglutination tests; slow agglutination tests in tubes; slow agglutination tests in micro titration plates.

60.5.1 The rapid agglutination tests:

This method involves mixing un-diluted patient/client serum and antigen on a glass slide or plate, rotation or agitation of the plate as per the instructions given in the kit literature, and macroscopic examination, usually after 2 minutes for the presence of agglutination. The antigen and serum are usually mixed in fixed proportion. The intensity of the agglutination indicates the concentration of antibody in the serum. Sometimes strong agglutination reactions need to be confirmed by heating the sera (56 C. for 30 minutes) to destroy non-specific agglutinins or by repeating the test with various dilutions of the serum.

60.5.2 Slow agglutination in tubes/tube agglutination:

This involves dilution of the serum and mixing with fixed amount of unstained antigen. The tubes are kept at temperature and for time (usually overnight) as per instructions in the kit literature. The positive results are visualized by the presence of a precipitate in the bottom of the tube and a clearing of the supernatant (as compared to antigen without any serum).

60.5.3 Micro-agglutination:

This test is carried out using small amounts of antigen and patient serum as per kit literature in a micro-titration plate. A large number of samples/ various dilutions can be tested at a time in one plate. The positive reaction is indicated by formation of a ragged blanket of coloured antigen covering the bottom of the U-shaped micro-titre well. The negative result appears as a button of un-reacted antigen in the well.

Note: The agglutination tests can be **"Qualitative agglutination test" -** agglutination test used to detect the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. e.g. a patient's red blood cells mixed with antibody to a blood group antigen to determine a person's blood type. "Quantitative agglutination test" - agglutination tests used to quantitate the level of antibodies to particulate antigens. Serial dilutions of a sample to be tested for antibody are mixed with fixed number of red blood cells or bacteria or other such particulate antigen and the last/highest dilution showing agglutination is the amount of antibody in the sample and is expressed as the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.

60.6 MISCELLANEOUS TYPES OF AGGLUTINATION

The agglutination of a particulate antigen by antibody raised against a different but related antigen is termed Cross agglutination; agglutination of members of a group of biologically related organisms (bacteria) or corpuscles by an agglutinin specific for that group is called "Group agglutination" and clumping of particulate elements within the blood vessels/red blood cell aggregation within the blood vessels is called "Intravascular agglutination"

INTEXT QUESTIONS 60.2

Match the following:

1. Qualitative Agglutination	(a) to measure the level of antibodies
2. Cross	(b) members of biologically related organism
3. Group	(c) to detect presence of antigen / antibody
4. Quantitative	(d) clumping within blood vessels
5. Intra vascular	(e) antibody against related antigen

60.7 PROZONE AND POST ZONE PHENOMENA

False negative antigen antibody reaction either agglutination or precipitation, can occur if antigen and antibody are not mixed in the right proportions. This can happen if either antibody is in excess (Prozone) or when antigen is in excess (Post zone).

60.7.1 Prozone phenomenon:

Some sera when tested un-diluted, do not show agglutination. The same sera when tested after making dilution show a positive agglutination/precipitation reaction. This phenomenon is called "Prozone phenomenon" in which agglutination or precipitation occurs at higher dilution ranges of serum, but is not visible at lower dilutions or when undiluted. Excessive levels of antibody result in false negative reaction as antibody excess results in formation of very small complexes which do not clump to form visible agglutination. Prozone reaction is the probable cause of false-negative result. Prozone reaction can also result from presence of blocking antibody or to nonspecific inhibitors in serum. When different antigens are located close to each other, the antibodies corresponding to each antigen may block binding by and competing with each other

Notes

Procedure

Procedure for ABO cell grouping (slide method)

- Confirm the identity of blood sample by checking registration number and name of the patient/donor.
- Label the slide with donor/patient name or number.
- Put 1 drop of Anti-A, 1 drop of Anti-B and 1 drop of anti D anti serum on left, middle and right portion of slide.
- Add a small drop of the approximately 50 % suspension of red cells/ capillary blood to each portion of the slide.
- Mix well with a applicator stick.
- Rock the slide in clock-wise/anti-clock-wise direction to see agglutination.
- Record in the register/form.
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- Record in the register/form.

Procedure for ABO cell grouping (tube method)

- Confirm the identity of blood sample by checking registration number and name of the patient/donor.
- Prepare 2 - 5% suspension of the EDTA red cell in normal saline
- Label three test tubes with patient name or number and tube contents (-A, -B and –D).
- To the test tube labeled A, add 1 drop of Anti-A anti serum. To the tube B, add 1 drop of Anti-B and to the tube labeled D add 1 drop of anti D anti serum.
- Add 1 drop of the 2-5 % suspension of red cells to each of the test tube.
- Mix well and centrifuge the test tubes for 1 min at 1000 rpm.
- Re-suspend the cells with gentle agitation and examine macroscopically for agglutination

Determination of and Rh (D) by column agglutination technology

- Identify the appropriate microtube of ABD and Reverse Diluent Cassette with the donor/patient's name/UHID number.
- Remove aluminum foil from the top of microcolumn(s).
- Prepare 2-5 % of red cell suspension of donor/patient/reagent red cells(Ac,Bc,Oc) by adding 40µl of packed washed red cells to 1000 µl of NS