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AGGLUTINATION

60.1 INTRODUCTION

Agglutination is one of the antigen and specific antibody reactions which takes place when the two are mixed in-vitro in laboratory in the presence of electrolytes at a suitable temperature and pH. Agglutination word comes from the Latin “agglutinare”, meaning "to glue,” it is also clumping of particles. Examples of agglutination in biology are clumping of cells such as bacteria (Widal test) or red blood cells (Blood grouping) in the presence of specific antibody. The antibody binds multiple antigen particles and joins them, creating a large lattice like complex which we can see with naked eye.

Agglutination reaction used for diagnosis of diseases in lab either uses the particulate or soluble antigens. Example of agglutination reaction using particulate antigens is Salmonella typhi bacteria to detect specific antibody in serum from patient suffering from typhoid fever (Widal test). Example of agglutination reaction is latex agglutination and other particle agglutination tests. The soluble antigen is first made particulate by coating it on inert particles like red cells, latex particles, gelatin particles and micro beads. These particles support or carry the soluble antigens to make the reaction visible to naked eye.

Agglutination assays have good sensitivity, do not require sophisticated equipment, are easy to perform, require no wash procedures and are cost effective. The lattice network formed during agglutination reaction can be visualised macroscopically or microscopically as per the directions of the manufacturer.

OBJECTIVES

After reading this lesson, you will be able to:

- define agglutination

- discuss the process of agglutination
- describe the various methods of agglutination
- read the result of agglutination reaction with naked eye and under the microscope
- describe the various applications of agglutination

60.2 DEFINITION OF AGGLUTINATION

Large antigens, carrying many epitopes, easily sedimented particles such as animal cells, erythrocytes, or bacteria when mixed with specific antibodies, at appropriate temperature and ionic strength solution result in cross-linking the particles, forming a lattice like structure seen as clumps with naked eye. This reaction which is sensitive and specific is termed agglutination. Agglutination is a serological reaction like precipitation reaction; only difference is that antigen is large and particulate in case of agglutination. Most common example of agglutination is the testing for blood group.

You will see during practical laboratory exercise that agglutination is more sensitive than precipitation. However, you can make precipitation reaction also very sensitive by attaching/coating soluble antigens to large, inert carriers, such as erythrocytes or latex beads so that precipitation reaction now becomes an agglutination reaction.

60.3 HISTORY

Two bacteriologists namely, Herbert Edward Durham and Max von Gruber discovered specific agglutination in 1896. This reaction was named as Gruber-Durham reaction to honour the discoverers. Later, Gruber named any substance that caused agglutination reaction as "agglutinin" (from the Latin).

Same year Fernand Widal (1862–1929) used agglutination for diagnosis of typhoid fever. Widal found that blood serum from a typhoid carrier caused a culture of typhoid bacteria to clump, whereas serum from a typhoid-free person did not. Widal test is the first example of a sero diagnostic test for an infectious disease.

Karl Landsteiner found another important practical application of the agglutination reaction in 1900 i. e. for blood group (ABO) typing. This marked the beginning of safe blood transfusion and the science of transfusion medicine.

60.4 PROCESS OF AGGLUTINATION

Agglutination is clumping together in suspension of cells bearing the antigen (epitopes)/ antigen bearing microorganisms, or particles in the presence of specific antibodies called "agglutinins". We can picture a lock and Key concept

to understand the specificity of agglutination reaction. An antibody is a "Y" shaped molecule. The two arms of "Y" are the Fab portion and this has the combining site and is made of the hypervariable regions of the heavy and light chains. The antigenic determinant nestles in a cleft formed by the combining site of the antibody as illustrated in the Figure 60.1. So the antigenic determinant is the "Key" which fits onto the cleft formed by the "Fab" which is the lock. If the fit is appropriate then agglutination will happen. This concept is true for all antigen (Ag) antibody (Ab) reactions. The process of agglutination involves two steps. First step is sensitization and second is lattice formation.

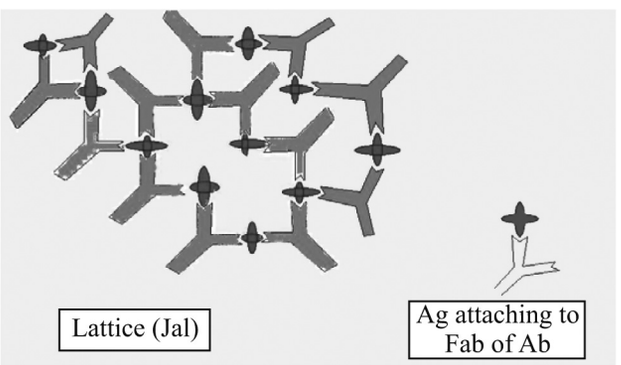

Fig. 60.1

60.4.1 Sensitization

It is attachment of specific antibody to corresponding antigen. pH, temperature and time of incubation influence the reaction. IgM antibodies react best at 4 to 22 degrees C and IgG antibodies react best at 37 degrees C. Time of incubation can range from 15 to 60 minutes.

60.4.2 Lattice formation:

Lattice is just like a "Jaal". It is formed by cross linking between sensitized particles. It takes more time than sensitization and we may be able to see the result with naked eyes. IgM best at this type of reaction because of large size but IgG antibodies may need enhancement.

INTEXT QUESTIONS 60.1

1. Agglutination means .....
2. Most common example of agglutination is testing for .....

3. Agglutination is clumping together of antigen with their specific antibodies called .....
4. Attachment of specific antibody to antigen is .....

60.4.3 Methods of enhancing agglutination

These include centrifugation (Bridges distance); treatment with enzymes (Reduces Zeta Potential); colloids (Albumin, reduces zeta potential) and use of anti- human globulin. Electrokinetic potential in colloidal systems is termed as Zeta potential. It is actually the degree of repulsion between adjacent, similarly charged particles in a solution. A high zeta potential confers so it will resist agglutination. So, reducing Zeta potential will favour agglutination.

60.4.4 Grading agglutination reactions

Grading may be macroscopic or microscopic. Follow the criteria given in the package insert of the commercial kit. Example of blood grouping used in blood banks is given below.

60.4.5 Macroscopic

Example blood grouping:

4+ One solid aggregate or clump of cells.

3+ Several large aggregates, clear background.

2+ Small to medium sized aggregates, clear background.

1+ Small aggregate, turbid reddish background.

+W Tiny aggregates, turbid reddish background.

MF Mixed Field – Any degree of agglutination in a sea of un-agglutinated cells.

Hem - Hemolysis is interpreted as a positive reaction and may be graded as complete or partial. Both hemolysis and agglutination may be recorded on the same tube.

Ø Negative - no agglutination, smooth reddish background.

60.4.6 Microscopic

+ Positive - aggregates of at least 3-5 cells.

Ø Negative - no agglutination.

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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

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60.7.2 Post-zone phenomenon:

This refers to the reaction wherein excess of antigen results in no lattice formation and a false negative agglutination reaction. Antigen excess is also the probable cause of false-negative antigen-antibody agglutination/precipitation reaction.

INTEXT QUESTIONS 60.3

1. Electrokinetic potential in colloidal system is termed as .....
2. Reduction of ..... favours agglutination
3. In rapid agglutination test the intensity of agglutination indicates .....in the serum
4. In slow agglutination test, a positive results is by presence of .....
5. In Micro agglutination, positive reaction is indicated by formation of .....
6. False negative reaction because of high antibody is termed as .....
7. False negative reaction because of high antigen is term as .....

60.8 APPLICATIONS OF AGGLUTINATION

We hope that by now you understand what agglutination is and how it happens and how we read the results. Let us now try to understand what the importance of agglutination reaction in medicine is. Agglutination testing helps you to find either antigens or antibodies in a sample. The sample can be any body fluids such as urine, blood, saliva, and cerebrospinal fluid (CSF) in which either we want to detect antigen or antibody, the sample can also be bacterial culture which we want to type to know whether it is pathogenic or not. The first step is to collect the sample. Blood is collected by venipuncture following standard work precautions; CSF is collected by the clinician by lumbar puncture and urine is collected by patient using the clean catch method. In blood agglutination test, a sample of blood is collected from the patient vein by a method called venipuncture.

Applications of agglutination in clinical medicine include:

60.8.1 Typing blood cells of the recipient and donor for blood transfusion

60.8.2 To identify and type bacterial cultures

60.8.3 To detect the presence of specific antibody and quantitate the amount of antibody in patient's serum

60.8.4 Latex agglutination

60.8.5 Haemagglutination.

Let us discuss these applications one by one.

60.8.1 Typing blood cells of the recipient and donor for blood transfusion: ABO and Rh blood grouping

Principle and application

Agglutination of red cells with known anti sera (antibody) indicates presence of the corresponding antigen on the red cells. Serum can also be tested with known red cells (antigen) to determine the presence or absence of specific antibodies. ABO blood groups are classified as A, B, AB, or O depending on the presence or absence of the A or B antigens on the red cells and presence or absence of the corresponding anti-A or anti-B antibodies. However, individuals are classified as Rh (D) positive or negative depending on the presence or absence of Rh (D) antigen only. There are various methods of performing this test, such as slide, test tube, microplate or Column Agglutination Technique. Hence it is a process to determine presence or absence of ABO & Rh (D) antigens on donor/ patient red cells and presence or absence of corresponding antibodies in serum. We describe here the conventional slide, test tube and Column Agglutination Technique.

Materials required

- EDTA blood 2 ml. from donor and patient in EDTA bottle;
- Commercial blood grouping anti sera (Anti-A, -B, -D, -D blend)
- Reagent (pooled) red cells (O-cell, A-cell, B-cell), prepared in-house.
- Anti-A/Anti-B/Anti-D cards and Reverse Diluent Cards
- Test tubes 10 × 75 mm
- Normal saline (0.9 % NaCl)
- Test tube rack
- Pasteur pipette/Automated pipettes
- Table top centrifuge
- 370C dry incubator
- Incubator, Centrifuge
- Gloves
- Glass slides/applicator sticks

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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.
- Rock the slide in clock-wise/anti-clock-wise direction to see agglutination.
- 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

- Add 10 µl of reagent red cell suspension in the identified microcolumn along the wall
- Add 40 µl of serum in reverse diluents cassette.
- Centrifuge the cassette X 5 min in column agglutination centrifuge
- Read and grade the reaction as per manufacturer's instructions.

Procedural notes

- All the test tubes must be properly labeled.
- It is important to follow the manufacturer's instruction for the specific antisera in use.
- Do not perform the test at the temperature higher than room temperature (22--24°C).
- Observe agglutination against a well lighted back ground.
- Remember that contaminated blood specimens, reagents or supplies may interfere with the test results.

60.8.2 To identify and type bacterial cultures

When we isolate bacteria from a sample say blood of a patient suffering from fever, it is important to know whether the bacteria isolated are Salmonella typhi or Acinetobacter or any other bacteria. To identify the bacteria we carry out biochemical reactions which will suggest/to some extent the bacteria we are dealing with. However, to know the exact identity of bacteria we have to type the bacteria using the Salmonella typhi "O antigen" specific and "H antigen specific" sera available commercially. For this identification a drop of suspension of bacterial isolate in normal saline is mixed with a drop of Salmonella typhi "O antigen" specific and "H antigen specific" sera on a glass slide. Mix the two with nichrome loop or a wooden stick and look for macroscopic agglutination with naked eye. Positive agglutination with Salmonella typhi "O antigen" specific and "H antigen specific" sera means the isolated organism is Salmonella typhi and patient is suffering from typhoid fever. Same way using genus specific, species specific, strain specific commercially available antisera against many pathogens (e. g. Pneumococci, Meningococci and others), the bacteria isolated from patient samples can be identified.

60.8.3 To detect the presence of specific antibody and quantitate the amount of antibody in patient's serum

One such example is the slide and tube Widal test. The agglutinins against '0' (somatic) and 'H' (flagellar) antigens of Salmonella typhi, paratyphi A and

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paratyphi B are estimated qualitatively (slide test) and quantitatively (Tube test) employing killed suspension of appropriate organisms.

Widal-quantitative tube agglutination test

This test is done to detect antibody against Salmonella typhi, S paratyphi A and S paratyphi B to aid in the diagnosis of enteric fever.

Specimen: Blood sample 3-5 mL is collected in sterile dry screw capped unbreakable tubes and transported to the lab in upright position or stored in refrigerator (2-8°C) in case of delay. Sample can be centrifuged at 3000 rpm for 10 minutes at room temperature to remove particulate matter if required, before performing the test.

Material and instruments required

- S. Typhi 'o' antigen suspension.
- S. Typhi 'h' antigen suspension.
- S. Typhi 'ah' antigen suspension.
- S. Typhi 'bh' antigen suspension.
- Blood sample 3-5 ml
- Plain vacutainer/sst
- Normal saline
- Micropipettes
- Glass test tubes

Reagents: Commercially available test kits are used. The kit must be stored at 2 – 80°C. Follow the instructions given in the kit insert to perform the Widal tube agglutination test.

Test procedure

Step	Action
1.	For each serum sample under test ,arrange four rows of 4 tubes each in a rack
2.	Prepare master dilutions by taking 4 tubes in another rack. Place 7 ml of normal saline (0.85% sodium chloride) in the first tube and 3.5.ml in each of the remaining four tubes.
3.	Add 0.5.ml of serum to the first tube and mix well.
4.	Transfer 3.5.ml from the first tube to the next tube and mix well

5. Continue successive transfer of 3.5.ml quantities till the last tube is reached.
6. This will give final dilutions of 1:30,60,120,240 after the addition of equal volume of antigen
7. Transfer 0.5.ml quantities from the master dilution tubes to each tube of the corresponding vertical row in test rack .Put 0.5 ml of normal saline in each of the tubes in the last (i.e,5th row) to serve as controls.
8. To each of the 4 tubes in the first, second, third and fourth horizontal rows, add 0.5.ml of S.Typhi "O" S typhi "H",S paratyphi A (H) and S.paratyphi B(H) antigens respectively.
9. Shake the rack well to mix and incubate at 37ºC overnight (16-20 hours).
10. Note the highest dilution in which there is evidence of agglutination as observed by naked eye or a hand lens .With 'H' antigens, the pattern of agglutination is floccular – cottonwool type whereas with 'O' antigen it is granular and appears as a granular mat at the bottom of the tube
11. In addition to the pattern of sedimented organisms, the decrease in opacity of the supernatant as compared to the saline control tube must be observed and taken into account while judging the degree of agglutination.

Internal quality control

With every batch of test samples the following controls are put up

Negative Control: Saline control tubes in the last row

Positive Control: Known positive pooled serum

Interpretation

Sera from normal individuals may agglutinate these antigens in dilutions up to 1: 60. Agglutination titers of 1:120 and more are significant and rise in titers or repetition of the test after a few days will confirm the diagnosis of enteric fever. Agglutination titers of 1:240 and above are typically found in cases of enteric fever. However, follow the instructions in the kit insert for interpretation.The specific organism responsible is determined by noting the 'H' agglutinin titre.

Persons who have suffered from enteric infections in past or who had received TAB vaccine may show appearance of agglutinins in moderate titer when

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suffering from other unrelated illness. Such anamnestic appearance of agglutinins can be differentiated from true infection by demonstrating the marked rise/fall in the titer when the test is repeated after 7-10 days. A moderate rise in titer of all three 'H' agglutinins simultaneously against all 'H' antigens is suggestive of recent TAB vaccination.

Safety: All specimen used in this test should be considered potentially infectious. Standard work precautions (gloves) should be used for handling and disposal of materials during and after use. Use soap for routine hand washing.

60.8.4 Latex agglutination

As already discussed soluble antigens are coated on inert particles like latex to develop sensitive, specific easy to perform rapid tests termed the latex agglutination tests. Many latex agglutination tests are commercially available and are used to detect either specific antigens or antibody against specific bacteria to diagnose various diseases. Always follow the instructions given in the kit insert to perform the test.

One such test is Cryptoccus antigen detection test. Cryptoccus antigen test is a simple, qualitative or semi-quantitative, test to detect polysaccharide antigens associated with Cryptococcus neoformans infection. Serum or cerebrospinal fluid (CSF) may be used as specimens. Sample of operative procedure based on a commercial kit "Remel Cryptococcus Antigen Latex Test" is given below.

Specimen:

Serum or cerebrospinal fluid (CSF).

Principle of the test:

Cryptococcus antigen test kit incorporates the use of latex particles sensitized with murine IgM monoclonal antibodies. Cryptococcal polysaccharide (CPS) antigens in patients serum or CSF interacts with sensitized latex particles producing visible agglutination.

Safety precautions:

- All specimen used in this test should be considered potentially infectious. Standard work precautions should be followed for handling and disposal of materials during and after processing the specimen.
- Use soap for routine hand washing.
- Follow Hospital Guidelines for biohazard waste disposal.

Reagents and materials

Reagents

- Test latex: Latex particles sensitized with IgM anti-CPS monoclonal antibody suspended in a buffer and preserved in 0.01% thimerosal (1 × 2.5 ml).
- Negative control: Normal human serum in a buffer, preserved in 0.1% sodium azide (1 × 0.8 ml).
- High positive control: Contains approx 50 mg/ml of C. neofomans CPS antigen preserved in 0.1% sodium azide. (1 × 0.8 ml)
- Low positive control: Contains approx 12 mg/ml of C. neoformans CPS antigen preserved in 0.1% sodium azide. (1 × 0.8 ml)
- Protease: 1 enzyme tablet contained in a vial. (reconstitutes to 3 ml)
- Specimen diluent: 10x NaCl/Glycine solution preserved in 1.0% sodium azide. (1 × 10 ml-dilute to 1x).
- Reaction cards: (12 × 6 circles)
- Dispensing pipette- 50 ul

Equipments

- Centrifuge
- Micropipette for serial dilution of specimens.
- Test tubes.
- Timer
- Boiling water bath
- Graduated cylinder
- Vortex mixer.

Procedure

A) Reagent preparation

Specimen diluent: The specimen diluent is provided in 10x concentration. Prepare a working strength solution (1x concentration) in a separate bottle by combining the entire contents of the 10x specimen diluent bottle with 90 ml of demineralized water. Mix the working strength specimen diluent and label with the expiration date on the 10x specimen diluent. Use as needed or store at 2-8°C.

Protease: Prepare the protease solution by adding 3 ml of working strength specimen diluent to the vial containing the enzyme tablet. Allow 30 minutes for

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complete reconstitution, swirling the vial at least twice during this time. Label the vial with the date of reconstitution. Protease solution can be stored at 2-8°C for 1 month from the date of reconstitution. Alternatively aliquot the solution into appropriate capped and labeled tubes and store at <=–20°C until the expiration date of the kit.

B. Sample preparation

CSF:

- Heat CSF in a boiling water bath (100°C) for 5 min.
- Allow contents to cool to room temperature.
- Vortex contents of tube before testing.

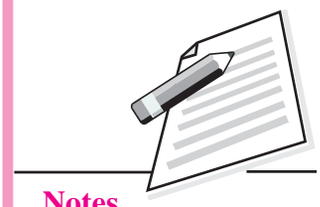
Serum:

- If necessary dilute specimen diluent (10x) to the 1x working strength.
- If necessary reconstitute the Protease with 3 ml of 1 x specimen diluent.
- Dispense 100-200 ul of serum in a tube and add an equal of Protease.
- Cap and seal the tube. Mix by vortexing.
- Place capped tube in a boiling water bath (100°C) for 10 minutes.
- Allow contents to cool to room temp and gently mix before testing.

Note: If flocculation is observed before and/or after Protease treatment centrifuge the specimen at 3000 rpm for 10 minutes at room temp. Decant the supernatant (50 µl) to avoid aspirating the pellet.

Qualitative test

- Re suspend the test latex by rapidly inverting the bottle several times. Dispence one drop of the Test latex into a sepatate test circle for each specimen and control to be tested.
- Dispense one drop of each well mixed control into a separate, test circle containing the test latex. Use the paddle end of a separate pipette to thoroughly mix each control and test latex. Discard each pipette after this step.
- Using pipettes provided in the test kit dispense one drop of each pretreated patient specimen (approximately 50 µl) into a test circle containing Test latex. With the paddle end of the pipette, thoroughly mix the specimen and Test latex, spreading over the entire area of the circle. Discard the pipette after this step.



- Place the card on a clinical rotator set to rotate at 100 to 110 rpm for 5 minutes.
- Immediately following the 5-minute rotation tilt the slide to obtain a flow pattern and carefully examine each circle for any agglutination (see results section below) and record the results.
- Compare the specimen test reaction to a negative control reaction. Record the results.

Semi-quantitative testing of positive specimen:

- If necessary dilute the 10x Specimen Diluent to the working strength.
- Obtain and mark eight tubes 1 to 8. Add 0.1 ml of specimen diluent to each tube.
- Add 0.1 ml of heat-treated CSF, or protease-treated serum contents of tube 1 and transfer 0.1 ml to tube 2. Do not mix the contents of tube 2 with the pipette.
- With a clean pipette, thoroughly mix the contents of tube 2 and deliver 0.1 ml to tube 3. Do not mix the contents of tube 3. Do not mix the contents of tube 3 with the pipette.
- Follow this method to produce serial, doubling dilutions of the specimen through tube 8. The serum dilutions which have been established are from 1:4 to 1:512 for tubes 1 to 8 respectively, For CSF 1:2 to 1:256 dilutions are made. Tube 8 can be diluted further if an endpoint is not reached.
- Test each specimen dilution following the protocol as described in the Qualitative Testing section.

Result

Qualitative test result: Any test latex clumping or cleaning, observed immediately after the 5-minute rotation step, is considered a positive result. The absence of agglutination of the test latex is considered a negative result. Test latex particles should appear as a milky suspension similar to the pattern produced by the negative control following the 5-minute rotation step.

Semi-quantitative test results: When positive specimens are examined by serial dilution the titer is the reciprocal of the last dilution which produces a positive result (agglutination).

Quality control

The High positive, Low positive and Negative controls should each be tested with every test run of patient specimens as described under qualitative testing.

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The high positive and low positive controls must agglutinate the test latex differentially.

- The High positive control must produce strong agglutination.
- The low positive control must produce weaker but clearly visible agglutination.
- The Negative control must not produce any agglutination, although trace granularity is acceptable.

60.8.5 Haemagglutination:

As the name indicates this is agglutination of RBCs as such by RBC antigen specific antibody (example of ABO blood grouping given under 8.1), or agglutination of RBCs coated with some antigen to detect specific antibody in patient's serum (example is Treponema Pallidum Haemagglutination test). The antibodies/proteins which agglutinate RBCs are called Haemagglutinins.

Materials Required

1. Adjustable multi-channel micropipette (50-300 µl)
2. Adjustable single-channel micropipette (50-200µl)
3. Adjustable single-channel micropipette (5-50µl)
4. Disposable tips
5. Reagent troughs
6. Waste discarders

Reagents and test kits

1. Micro plates (U bottom)
2. Sample diluents
3. Control cells (uncoated)
4. Test cells (sensitized with T.pallidum antigen)
5. Kit controls

Procedure

1. Approximately 30 min prior to the beginning of the test procedure; bring kit components to room temp. (15-30°C.). Mix the liquid reagents gently. Determine the total no. of specimens to be tested and no. of plates required for the assay.

2. Put a unique plate Id on the upper-middle side of plate, if required
3. Label the plate with last three digits of donation Id on left lower side of well, identifying the positions. Include one negative control & one positive control per batch of specimens.
4. Arrange the samples according to plate map in a sampling rack.
5. Use first extra well according to number of specimens and add 190µl sample diluent.
6. Then using a fresh pipette tip for each addition, take 10µl of specimen and mix it with 190µl sample diluent in the same position in the extra well/ plate.
7. Then transfer 25ml of the diluted sample in both control & test wells.
8. After sampling put the sample in the same position in the same/different sampling rack.
9. Add 25µl negative & positive controls to their respective positions.
10. Mix the bottles of control & test cells gently to make homogenous suspension & add 1 drop (75µl) of test cells & control cells to their respective wells including positive & negative control wells.
11. Mix the contents of the well by rotating the plate slowly. Keep the plate on a smooth steady surface. Read the results after 1 hr. of incubation at room temp.

Interpretation of the test result

1. All control wells should have compact button formation. If any control well doesn't show button formation repeat the test to exclude any technical error. (Agglutination of the control cells as well as the test cells indicates the presence of anti-cell antibody and in this event the test is not valid & should be repeated after first performing absorption of the test serum. To achieve this dilute the test serum 1/4 with control cells & allow standing at room temp. After centrifuging the sample (1000 rpm/5 min.). Dilute the supernatant 1/5 in diluent. Test this dilution directly, without any further dilution, using test & control cells suspensions).
2. If there is a compact button in a test well, the result is considered non reactive.
3. If there is a characteristics ring pattern or net of the cells in the and there is a compact button formation in the control well, specimen is considered as reactive for T. pallidum.

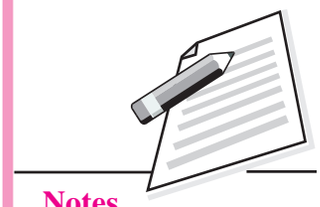
Notes

4. All positive tests should be repeated by TPHA.
5. If any sample is positive by repeat test also, identify the sample and separate out the sample and retest with RPR.

Agglutination has been commonly used to determine whether a patient had or has a bacterial infection. For example, if a patient is suspected of having typhoid fever, the patient's serum is mixed with a culture of Salmonella typhi. If an agglutination reaction occurs, shown as clumping of the bacteria, the patient either had or has an S. typhi infection. Since certain antibodies can persist in a patient's blood for years after the patent has recovered from the infection, a positive reaction does not mean that the patient currently has the infection. To determine whether a patient is currently suffering from typhoid fever, the amount or titer of the antibody will be determined at the onset of illness and two weeks later.

WHAT HAVE YOU LEARNT

- Agglutination word means to glue
- Agglutination is one of the antigen and specific antibody reactions in-vitro in laboratory in the presence of electrolytes at a suitable temperature and pH.
- Agglutination is used for diagnosis of diseases in laboratory either uses the particulate or soluble antigens.
- Agglutination involves two steps. First step is sensitization and second is lattice formation.
- Sensitization is the atachment of specific antibody to corresponding antigen.
- The degree of repulsion between adjacent similary charged particles in a soluiton is termed as zeta potential.
- Reducing Zeta potential will favour agglutination.
- Rapid agglutination, slow agglutination in tubes; slow agglutination in micro titration plates are the different techniques Agglutination
- False negative antigen antibody reaction, either agglutination or precipitation, can hppen if either antibody is in excess (prozone) or when antigen is in eecess (post zone).
- Clinically agglutination is used
  - Typing blood cells of the recipient and donor for blood transfusion
  - To identify and type bacterial cultures



TERMINAL QUESTIONS

1. Define agglutination
2. Describe the process of agglutination.
3. Explain the methods of agglutination.
4. Explain prozone and postzone phenomena.
5. List the clinical applicaiton of agglutination.
6. Describe ABO and RH blood grouping in brief

ANSWERS TO INTEXT QUESTIONS

60.1

1. To glue
2. Blood group
3. Agglutinins
4. Sensitization

60.2

1. (c)
2. (e)
3. (b)
4. (a)
5. (d)

60.3

1. Zeta potential
2. Zeta potential
3. Concentration of antibody
4. Precipitate in the bottom & clearing of supernatant
5. Ragged blanket of coloured antigen
6. Pro-zone phenomenon
7. Post-zone phenomenon