Medical Laboratory Techniques Department

Lab 7 : Agglutination

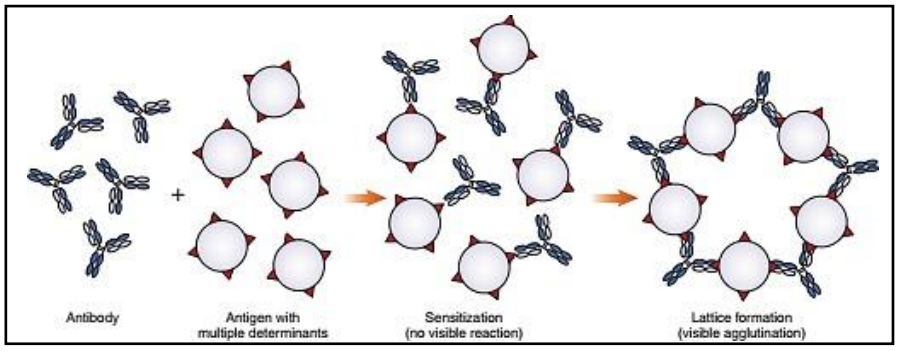
Msc. A'laa Khalaf Bediwi

Agglutination

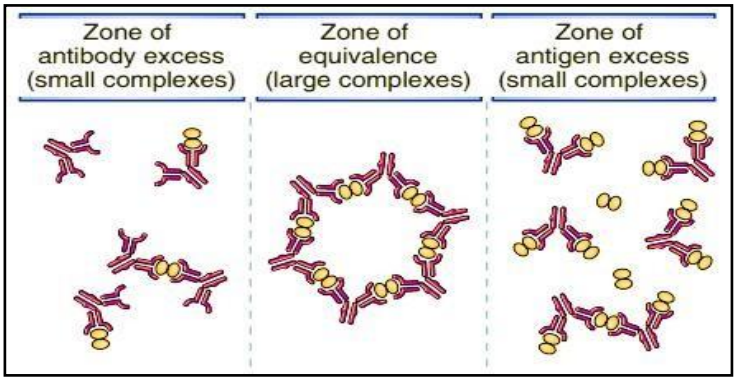
- Agglutination tests are based on the reaction of antibodies (Ab) and antigens (Ag) to forming visible clumps.
- **Agglutination is a two-step process that formation of stable lattice network:**

1- Sensitization: involves antigen-antibody combination through single antigenic determinants and is rapid, reversible.

2- Lattice formation: involves antigen-antibody complexes through multiple antigenic determinants and the formation of stable visible clumps.



- The **false-negative** agglutination can occur if antigen and antibody are not mixed in the right proportions.
- ✓ **Prozone effect:** The antibody excess results very small complexes that not form visible clump and gives false-negative agglutination.
- ✓ **Post-zone effect:** The antigen excess results no lattice formation and gives false-negative agglutination.



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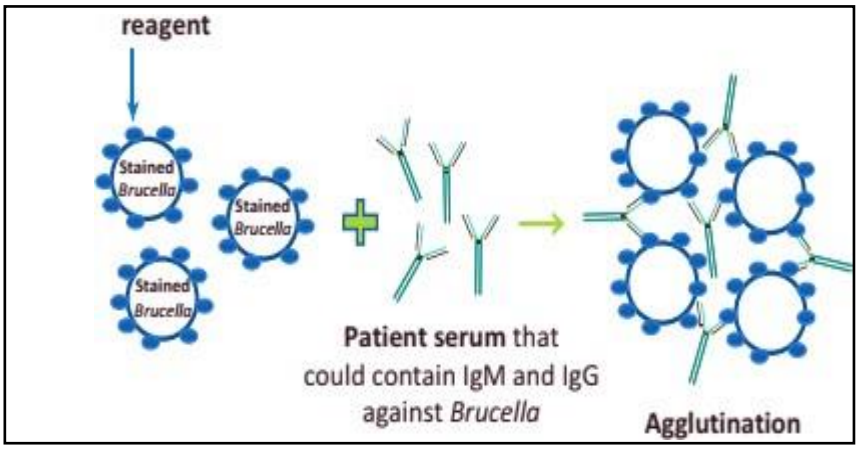
Types of agglutination test

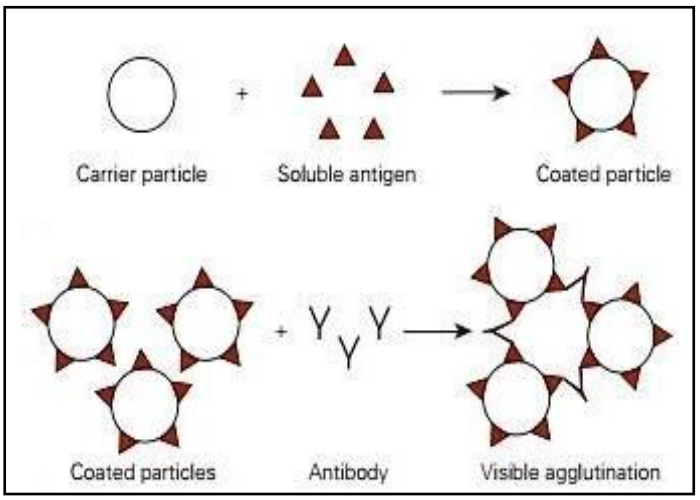
1- Direct (active) agglutination test

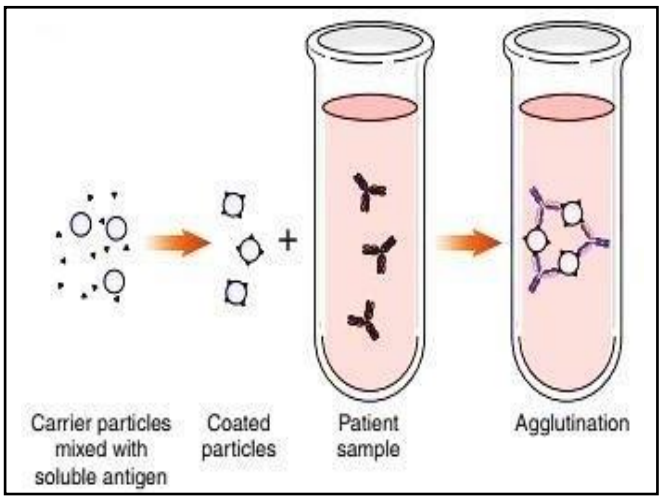
- The reaction between antibody in the patient's serum and antigen found naturally on particles. **For example:** (Widal test, Rose Bengal test, Blood group typing, etc).



2- Indirect (passive) agglutination test

- The antigens or antibodies are coated on the surface of carrier particles such as carbon particles, latex particles, or RBCs. **For example:** Rheumatoid factor (RF) test, C-reactive protein (CRP) test, and Antistreptolysin-O (ASO) test.





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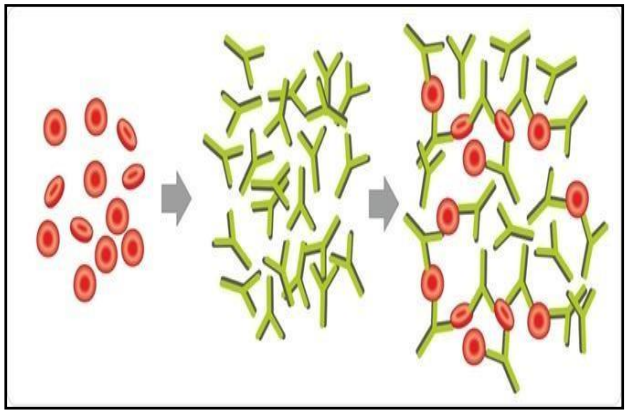
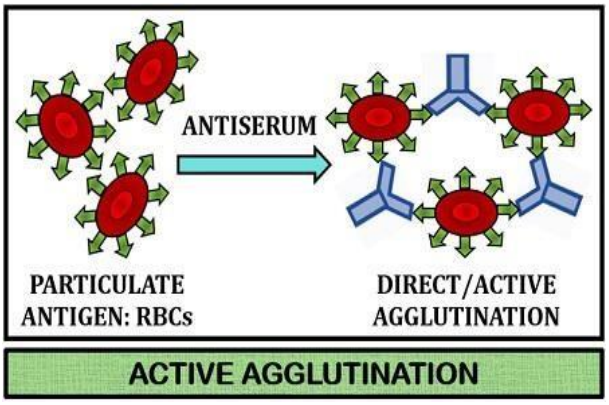
1- Direct (active) heamagglutination test:

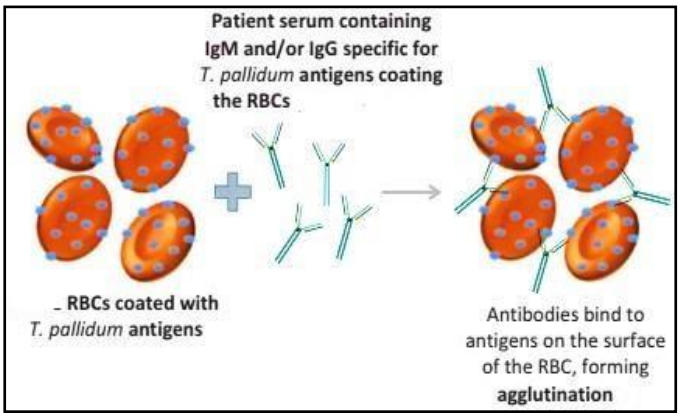
- The best example of this occurs in ABO blood group typing of RBCs.
- Patient RBCs mixed with antiserum to determine the presence or absence of the A and B antigens. Group A RBCs will agglutinate with anti-A antibody and Group B RBCs will agglutinate with anti-B antibody.

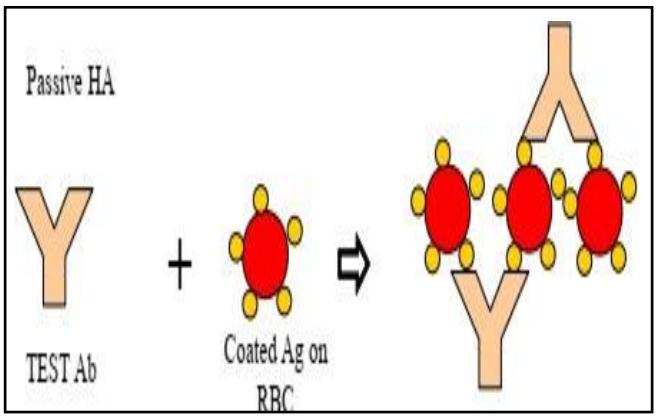




2- Indirect (passive) heamagglutination test:

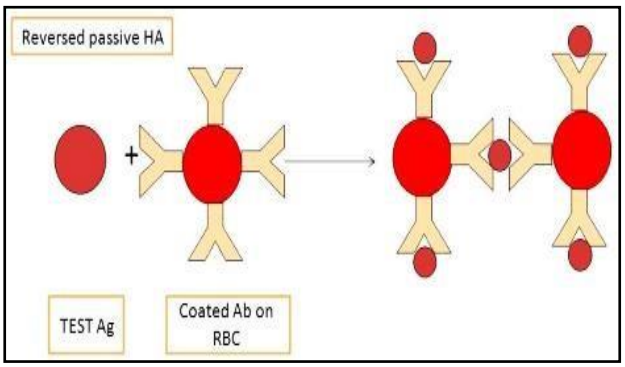
- Agglutination of RBCs coated with some antigen to detect specific antibody in patient serum **For example:** Treponema Pallidum Haemagglutination Test.

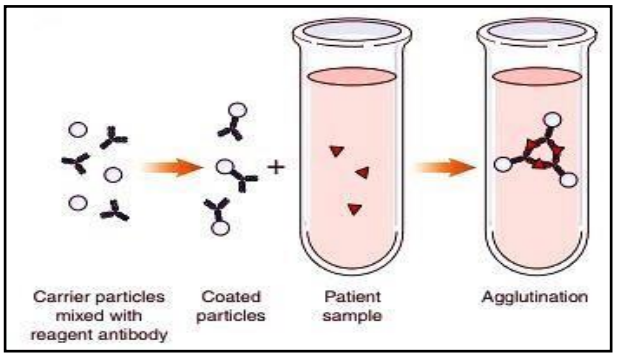




3- Reverse passive heamagglutination test

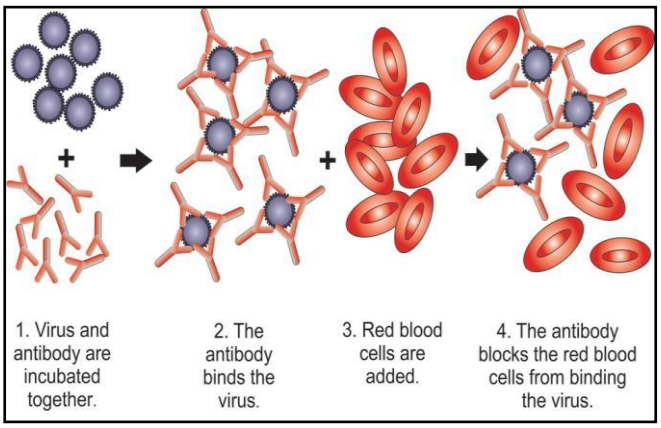
- Agglutination of RBCs coated with antibodies to detect the specific antigen in the sample. The reverse passive heamagglutination is used to detect viral antigens in the serum.

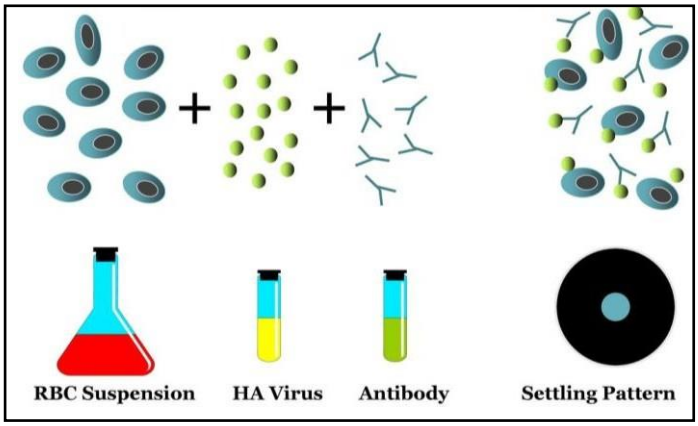




Heamagglutination inhibition test :

- Is used to detect antibodies in the serum against virus infection. Some viruses such as Influenza virus, Measles virus, and some bacteria such as Vibrio cholera can be bound with the RBCs and agglutination occurs.
- In this method, patient serum is mixed with viral culture and then RBCs are added.
- If heamagglutination occurs, it indicates the absence of antibodies in serum.
- If heamagglutination does not occur, it indicates the presence of antibodies in serum, it will attach to the viral surface and inhibition the agglutination reaction.





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Antiglobulin (Coombs) test:

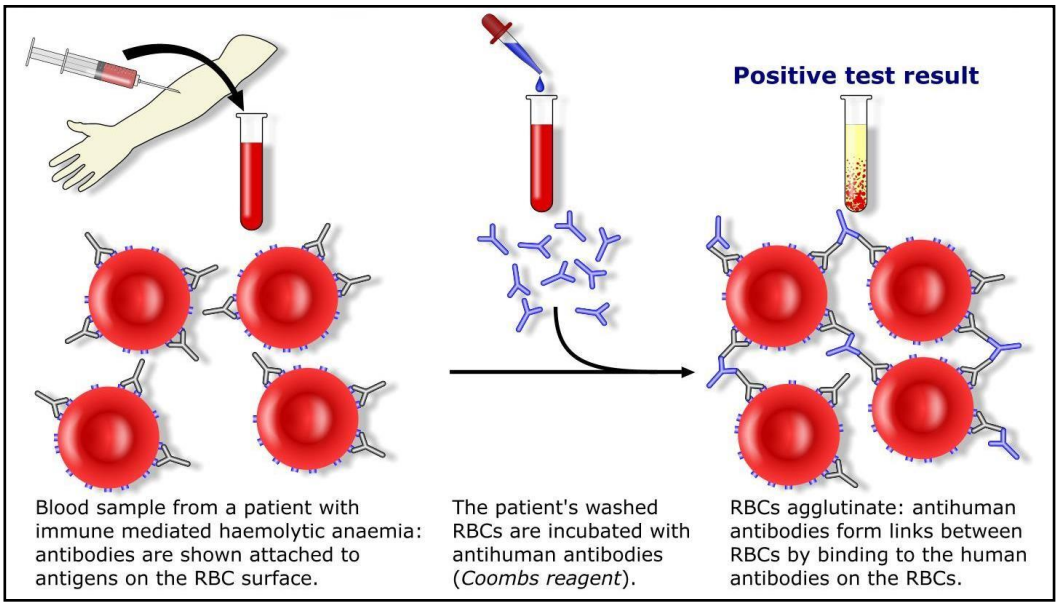
1- Direct Coombs test:

- The test detects antibodies attached to the surface of RBCs and may be causing the destruction of those cells.
- In this method, RBCs are washed and then the addition of Coombs reagent (Anti-human IgG) (AHG).
- If agglutination occurs that means the test is positive, antibodies are bound to the RBCs.
- **Direct Coombs test is used for diagnosis of:**

✓ Hemolytic transfusion reaction.

✓ Hemolytic disease of the fetus and newborn.

✓ Autoimmune hemolytic anemia.



2- Indirect Coombs test:

- The test detects antibodies in the patients serum that could act against RBCs.
- In this method, the patient serum is incubated with RBCs of a known type followed by the addition of Coombs reagent (AHG).
- If agglutination occurs, that means the test is positive.
- **Indirect Coombs test is used for diagnosis of:**
- Cross-matching before blood transfusion.
- In prenatal testing of pregnant women.