Agglutination

Chapter Nine

Agglutination

- Whereas precipitation reactions involve soluble antigens, **agglutination** is the visible aggregation of particles caused by combination with specific antibody.
- Agglutination is actually a two-step process, involving sensitization or initial binding followed by lattice formation, or formation of large aggregates.

Agglutination

- Antibodies that produce such reactions are often called agglutinins.
- Types of particles participating in such reactions include erythrocytes, bacterial cells, and inert carriers such as latex particles.
- Each particle must have multiple antigenic or determinant sites, which are cross-linked to sites on other particles through the formation of antibody bridges or lattices.
Agglutination

- Agglutination, like precipitation, is a two-step process that results in the formation of a stable lattice network.
- The first reaction involves antigen–antibody combination through single antigenic determinants on the particle surface and is often called the sensitization step.

Agglutination

- The affinity and avidity (discussed in Chapter 8) of an individual antibody determine how much antibody remains attached.
- IgM with a potential valence of 10 is over 700 times more efficient in agglutination than is IgG with a valence of 2.

Agglutination

- The second stage, representing the sum of interactions between antibody and multiple antigenic determinants on a particle, is dependent on environmental conditions and the relative concentrations of antigen and antibody.
Agglutination

- Antibody must be able to bridge the gap between cells in such a way that one molecule can bind to a site on each of two different cells.
- Figure 9-1 depicts the two-stage process.

Agglutination

- The surface charge must be controlled for lattice formation, or a visible agglutination reaction, to take place.
- One means of accomplishing this is by decreasing the buffer’s ionic strength through the use of low-ionic-strength saline (LISS).

Agglutination

- The addition of albumin in concentrations of 5 to 30 percent also helps to neutralize the surface charge and allows red cells to approach each other more closely.
- Other techniques that enhance agglutination include increasing the viscosity, using enzymes, agitating or centrifuging, and altering the temperature or the pH.
Agglutination

- Agglutination reactions can be classified into several distinct categories: direct, passive, reverse passive, agglutination inhibition, and coagglutination.

Agglutination

- Direct agglutination occurs when antigens are found naturally on a particle.
- One of the best examples of direct agglutination testing involves known bacterial antigens used to test for the presence of unknown antibodies in the patient.

Agglutination

- One such example is the Widal test, a rapid screening test to help determine the possibility of typhoid fever.
- The antigens used in this procedure include Salmonella O (somatic) and H (flagellar) antigens.
Agglutination

- If an agglutination reaction involves red blood cells, then it is called hemagglutination.
- The best example of this occurs in ABO blood group typing of human red blood cells.
- Positive reactions can be graded to indicate the strength of the reaction (see Fig. 9-2).

Agglutination

This type of hemagglutination reaction is simple to perform, is relatively sensitive, and is easy to read.

A titer that yields semiquantitative results can be performed in test tubes or microtiter plates by making serial dilutions of the antibody.
Agglutination

- The reciprocal of the last dilution still exhibiting a visible reaction is the **titer**, indicating the antibody’s strength.
- **Passive, or indirect, agglutination** employs particles that are coated with antigens not normally found on their surfaces.

A variety of particles, including erythrocytes, latex, gelatin, and silicates, are used for this purpose.
- The use of synthetic beads or particles provides the advantage of consistency, uniformity, and stability.
- Reactions are easy to read visually and give quick results.

Latex particles are inexpensive, are relatively stable, and are not subject to cross-reactivity with other antibodies.
- A large number of antibody molecules can be bound to the surface of latex particles, so the number of antigen binding sites is large.
- In addition, the large particle size facilitates reading of the test.
Agglutination

- In reverse passive agglutination, antibody rather than antigen is attached to a carrier particle.
- This type of testing is often used to detect microbial antigens.
- Figure 9-3 shows the differences between passive and reverse passive agglutination.

Use of monoclonal antibodies has greatly cut down on cross-reactivity, but there is still the possibility of interference or nonspecific agglutination.

Such tests are most often used for organisms that are difficult to grow in the laboratory or for instances when rapid identification will allow treatment to be initiated more promptly.
Agglutination

- In all of these reactions, rheumatoid factor will cause a false positive as it reacts with any IgG antibody, so this must be taken into account.

Agglutination

- Agglutination inhibition reactions are based on competition between particulate and soluble antigens for limited antibody-combining sites, and a lack of agglutination is an indicator of a positive reaction.

Agglutination

- Typically, this type of reaction involves haptens that are complexed to proteins; the hapten–protein conjugate is then attached to a carrier particle.
- The patient sample is first reacted with a limited amount of reagent antibody that is specific for the hapten being tested.
Agglutination

- Indicator particles that contain the same hapten one wishes to measure in the patient are then added.
- If the patient sample has no free hapten, the reagent antibody is able to combine with the carrier particles and produce a visible agglutination.

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Agglutination

- In this case, however, agglutination is a negative reaction, indicating that the patient did not have sufficient hapten to inhibit the secondary reaction (see Fig. 9-4).
- Hemagglutination inhibition reactions use the same principle, except red blood cells are the indicator particles.

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Agglutination

Figure 9-4

![Diagram of agglutination process]
Agglutination

- This type of testing has been used to detect antibodies to certain viruses, such as rubella, mumps, measles, influenza, parainfluenza, HBV, herpes virus, respiratory syncytial virus, and adenovirus.

Agglutination

- **Coagglutination** is the name given to systems using bacteria as the inert particles to which antibody is attached.
- *Staphylococcus aureus* is most frequently used, because it has a protein on its outer surface, called protein A, which naturally adsorbs the FC portion of antibody molecules.

Agglutination

- The active sites face outward and are capable of reacting with specific antigen (see Fig. 9-5).
- These particles exhibit greater stability than latex particles and are more refractory to changes in ionic strength.
- However, because bacteria are not colored, reactions are often difficult to read.
Agglutination

Figure 9-5

The antihuman globulin test, also known as the Coombs’ test, is a technique that detects nonagglutinating antibody by means of coupling with a second antibody. It remains one of the most widely used procedures in blood banking.

Agglutination

- The antihuman globulin test is antibody to human globulin that is made in animals or by means of hybridoma techniques.
- Such antibody will react with the FC portion of the human antibody attached to red blood cells.
- Agglutination takes place because the antihuman globulin is able to bridge the distance between cells that IgG alone cannot.
Agglutination

- The **direct antiglobulin test** is used to demonstrate in vivo attachment of antibody or complement to an individual's red blood cells.
- This test serves as an indicator of autoimmune hemolytic anemia, hemolytic disease of the newborn, sensitization of red blood cells caused by the presence of drugs, or a transfusion reaction.

Agglutination

- The **indirect antiglobulin test**, or indirect **Coombs' test**, is used to determine the presence of a particular antibody in a patient, or it can be used to type patient red blood cells for specific blood group antigens.
- Washed red blood cells and antibody are allowed to combine at 37°C, and the cells are then carefully washed again to remove any unbound antibody.

Agglutination

- When antihuman globulin is added, a visible reaction occurs where antibody has been specifically bound.
- This test is most often used to check for the presence of clinically significant alloantibody in patient serum when performing compatibility testing for a blood transfusion.
- See **Figure 9-6** for an illustration of the test.
Agglutination

Quality Control and Quality Assurance

- Although agglutination reactions are simple to perform, interpretation must be carefully done.
- Techniques must be standardized as to concentration of antigen, incubation time, temperature, diluent, and the method of reading.

- The possibility of cross-reactivity and interfering antibody should always be considered.
- Cross-reactivity is caused by the presence of antigenic determinants that resemble one another so closely that antibody formed against one will react with the other.
Agglutination

- Most cross-reactivity can be avoided through the use of monoclonal antibody directed against an antigenic determinant that is unique to a particular antigen.
- Heterophile antibody and rheumatoid factor are two interfering antibodies that may produce a false-positive result.

Agglutination

- Heterophile antibodies (see Chapter 3) are most often a consideration when red blood cells are used as the carrier particle.
- Other considerations include proper storage of reagents and close attention to expiration dates.
- Refer to Table 9-1 for a list of false-positive and false-negative reactions.