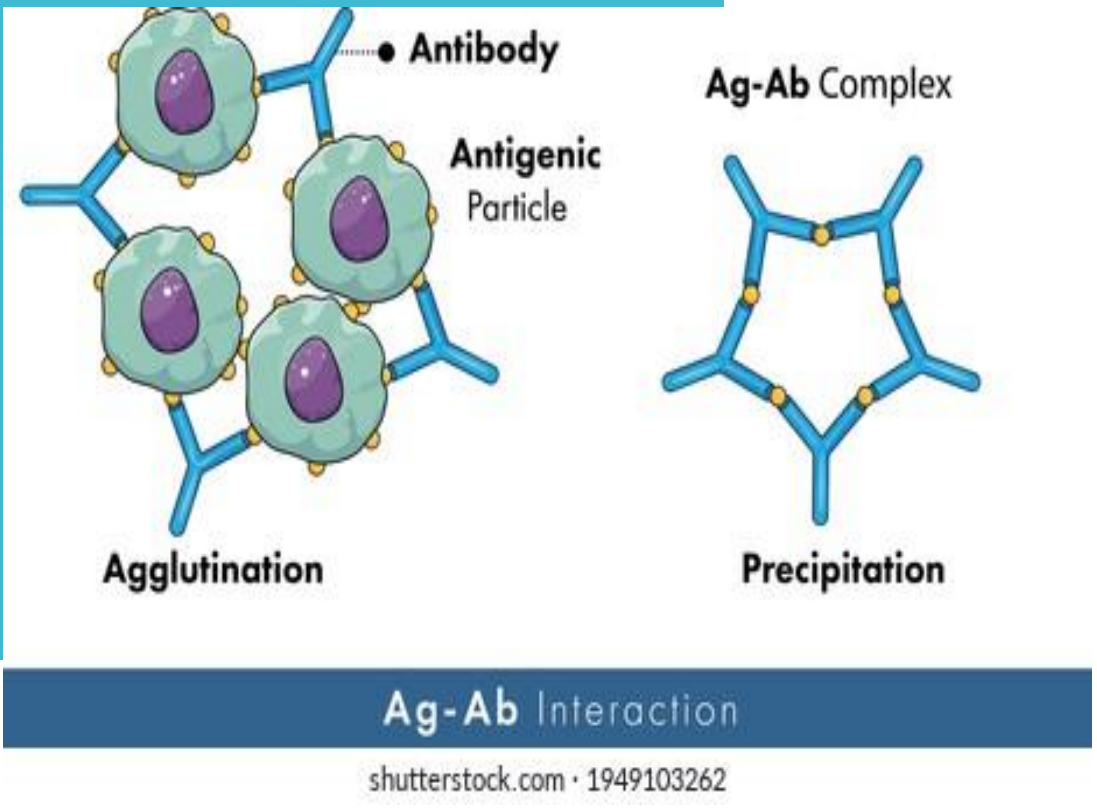
Precipitation and Agglutination Reactions

LEARNING OUTCOMES

- Discuss affinity and avidity and their influence on antigen–antibody reactions.
- Define precipitation and agglutination and differentiate between the two types of reactions.
- Describe the relative concentrations of antigen and antibody in the three zones of the precipitin curve and discuss why optimal precipitation occurs in the zone of equivalence.
- Summarise the principle of radial immunodiffusion (RID).
- Recognise how immunoglobulin M (IgM) and immunoglobulin G (IgG) differ in their ability to participate in agglutination reactions.
- Define and give an example of each of the following: (a) direct agglutination (b) passive agglutination

Precipitation and Agglutination Reactions



- The combination of an antigen with a specific antibody plays an important role in the laboratory in diagnosing many different diseases.
- Immunoassays have been developed to detect either **antigens** or **antibodies** and vary from easily performed **manual** tests to highly complex **automated assays**.
- The first such assays were based on the principles of **precipitation** or **agglutination**.
- **Precipitation** involves combining soluble antigens with soluble antibodies to produce insoluble complexes that are visible.
- **Agglutination** is the process by which particulate antigens, such as cells, aggregate to form larger complexes when a specific antibody is present.
- **Precipitation and agglutination** are considered unlabeled assays because a marker label is not needed to detect the reaction.
- **Labelled assays**, which were developed much later, will be considered in another lab.

Antigen-Antibody Binding

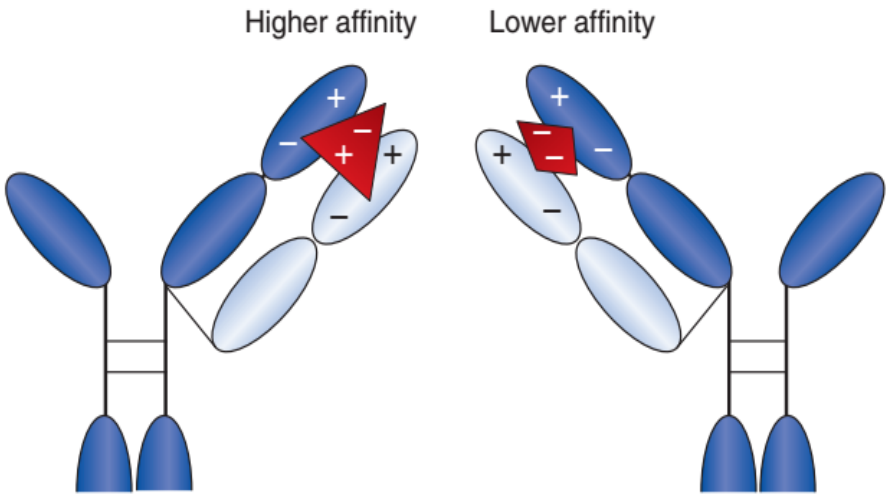


FIGURE 10–1 Affinity is determined by the three-dimensional fit and molecular attractions between one antigenic determinant and one antibody-binding site. The antigenic determinant on the left has a better fit and charge distribution than the epitope on the right and hence will have a higher affinity for the antibody.

- The primary union of binding sites on an antibody with specific epitopes on an antigen depends on two characteristics of the antibody:
- **Affinity** and **Avidity:** These characteristics are important because they relate to the sensitivity and specificity of testing in the clinical laboratory.
- **Affinity** is the initial force of attraction that exists between a single Fab site on an antibody molecule and a single epitope or determinant site on the corresponding antigen.

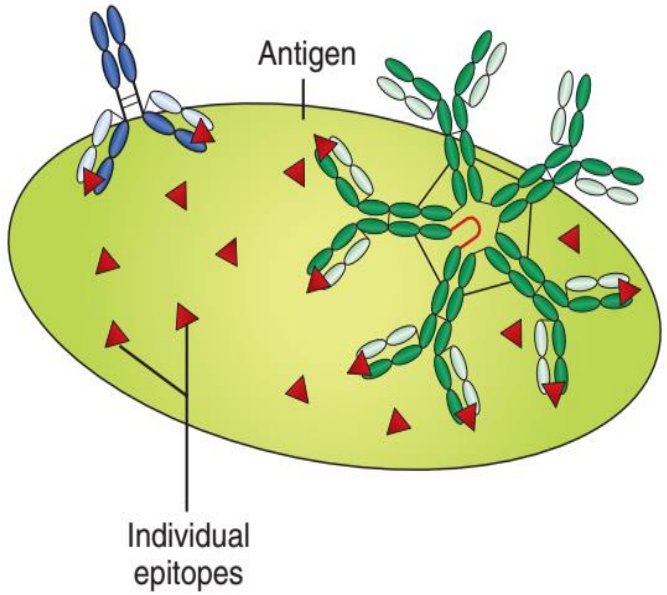


FIGURE 10–2 Avidity is the sum of the forces binding multivalent antigens to multivalent antibodies. In a comparison between immunoglobulin G (IgG) and IgM, IgM has the most potential binding sites for antigen and thus a higher avidity. Note that the monomer subunits in IgM can swing up or down in order to bind antigen more effectively.

- **Avidity:** represents the overall strength of antigen–antibody binding and is the sum of the affinities of all the individual antibody–antigen combining sites.
- Avidity refers to the strength with which a multivalent antibody binds a multivalent antigen
- It measures the overall stability of an antigen–antibody complex that keeps the molecules together after binding.
- A high avidity can compensate for a low affinity.
- Different classes of antibodies differ in their avidities. The more bonds that form between antigen and antibody, the higher the avidity is.
- Immunoglobulin M (IgM), for instance, has a higher avidity than IgG because IgM has the potential to bind 10 different epitopes.
- Both affinity and avidity contribute to the stability of the antigen–antibody complexes, which is essential to detect the presence of an unknown, whether it is antigen or antibody.
- The ideal conditions in the clinical laboratory would be an antibody with a high affinity and a high avidity or strength of binding.
- The higher the values are for both affinity and avidity, the more antigen–antibody complexes are formed and the more sensitive the test.

Cross-reactivity

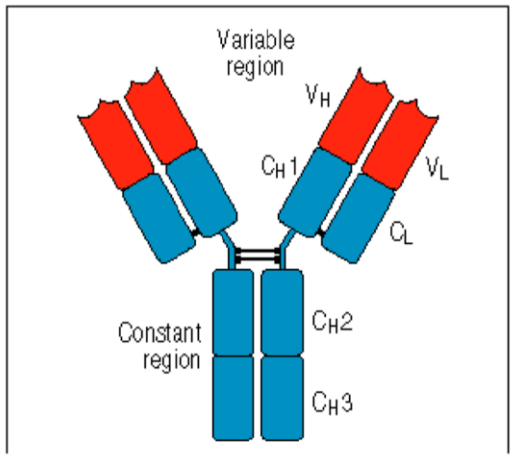
- More specific tests!
- One antibody molecule may initially attract numerous different antigens. Still, what determines whether the bonding will be stable:
  - epitope's shape
  - the way it fits together with the binding sites on an antibody molecule.
- Antibodies are also capable of reacting with antigens resembling the original antigen that induced antibody production, a phenomenon known as **cross-reactivity**.
- The more the cross-reacting antigen resembles the original antigen, the stronger the bond will be between the antigen and the binding site.

What is Cross-reactivity??

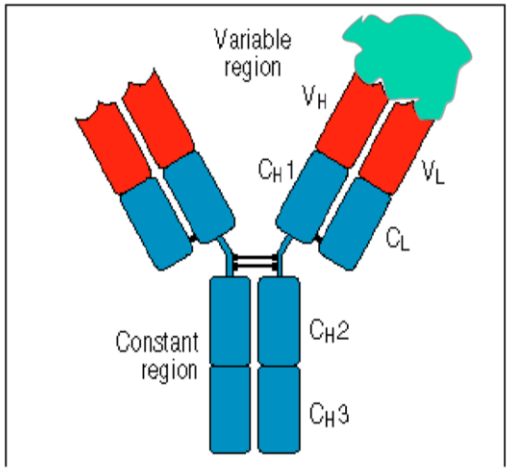
In which Ab elicited by one Ag can cross-react with an unrelated Ag. This is because the 2 different Ags share an identical epitope for e.g.

1. ABO blood-group Ag cross-reacts with microbial Ags present in the common intestinal bacteria, which induces the formation of Ab in individuals lacking the similar blood-group Ag on their red blood cells.
2. Streptococcal pyogenes cell protein(M protein), of which Ab against it will cross-react with several myocardial & skeletal muscle proteins, resulting in the development of Rheumatic fever and glomerulonephritis.

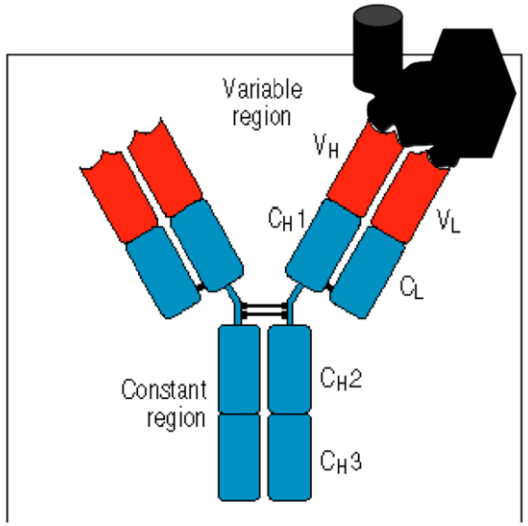
Cross-reactive Antigens



Cross-reactive Antigens



Cross-reactive Antigens

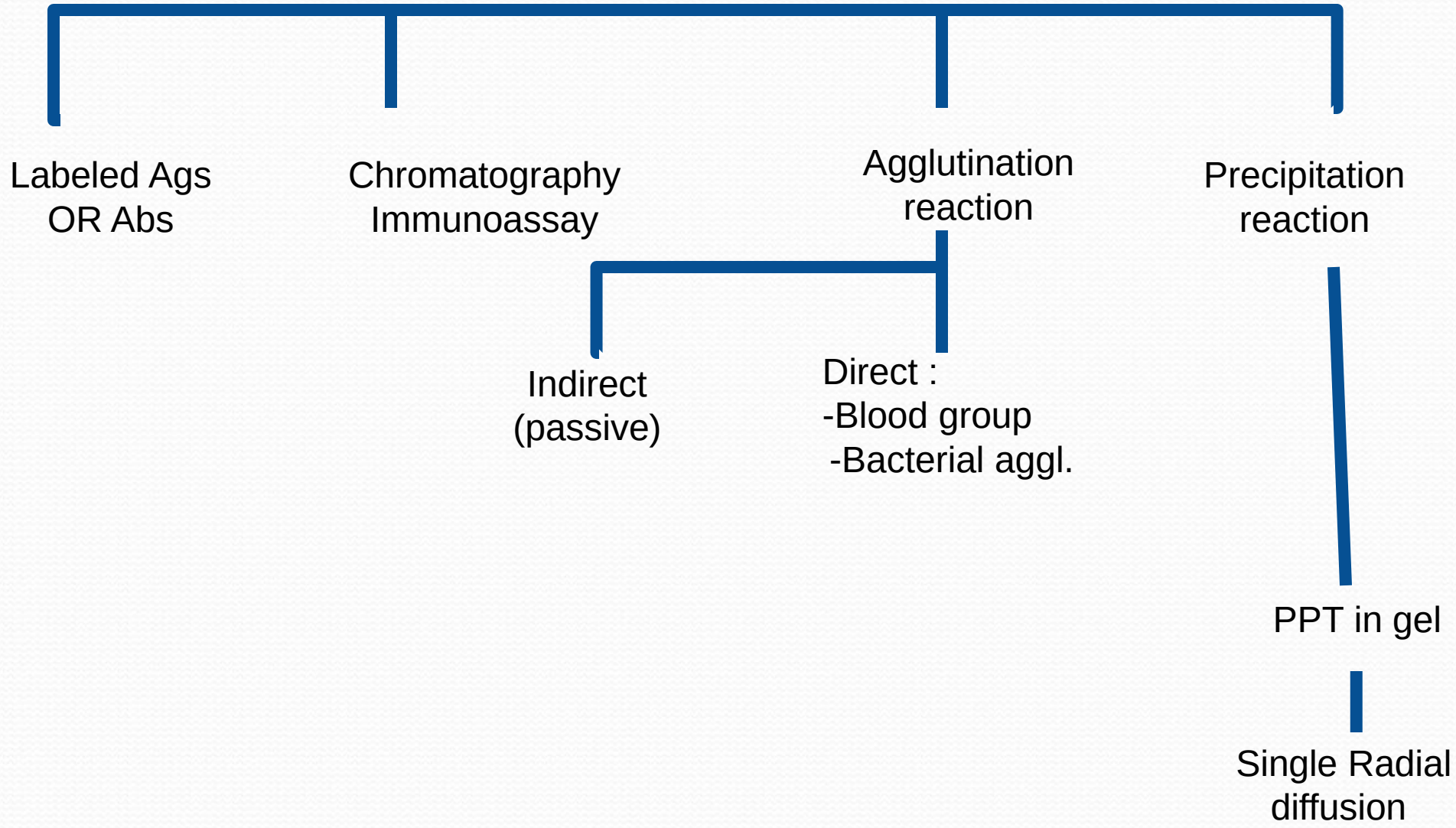


ANTIGEN-ANTIBODY INTERACTIONS

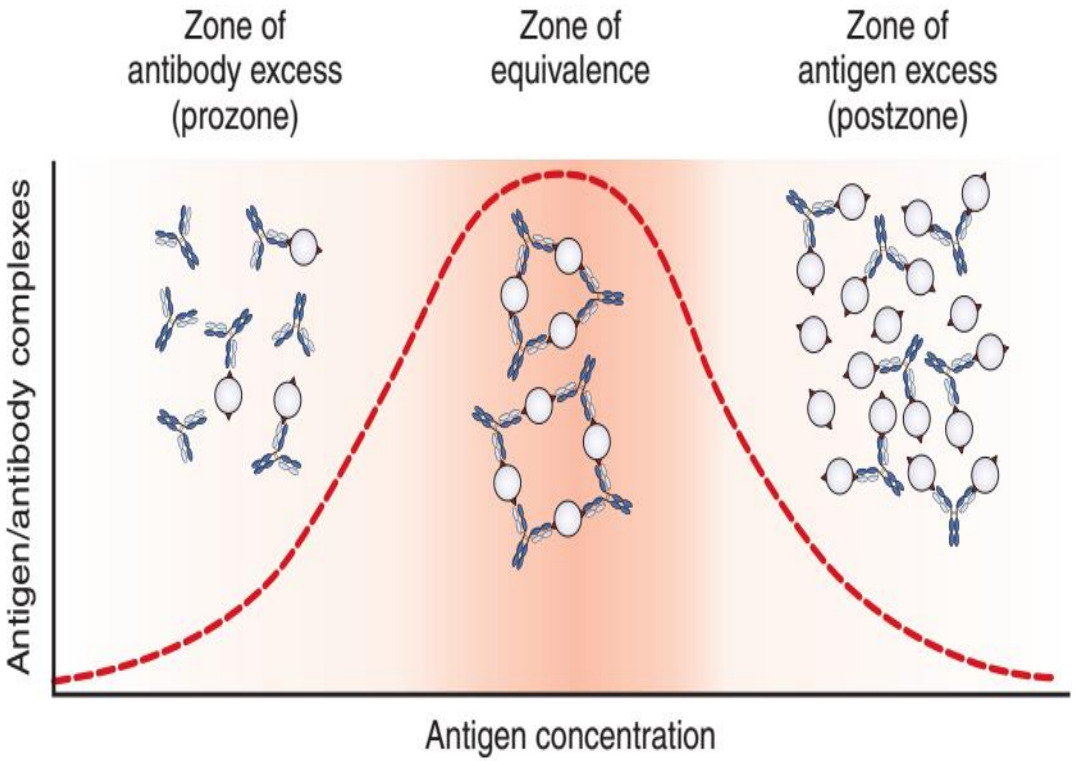
Many diagnoses would not be possible without laboratory procedures that identify antibodies or antigens in the patient

- Interaction of antigen and antibody occurs *in vivo*, and in clinical settings it provides the basis for all serologically based tests.
- The formation of immune complexes produces a **visible reaction** that is the basis of **precipitation and agglutination tests.**

Antigen-Antibody Interaction

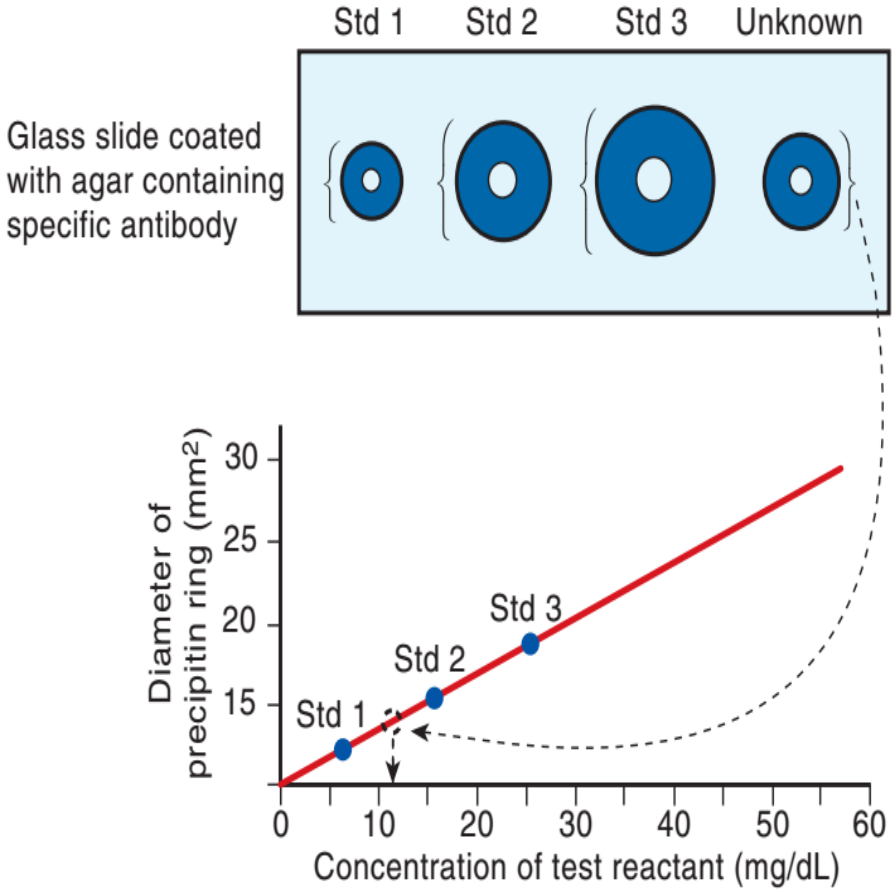


Precipitation Curve



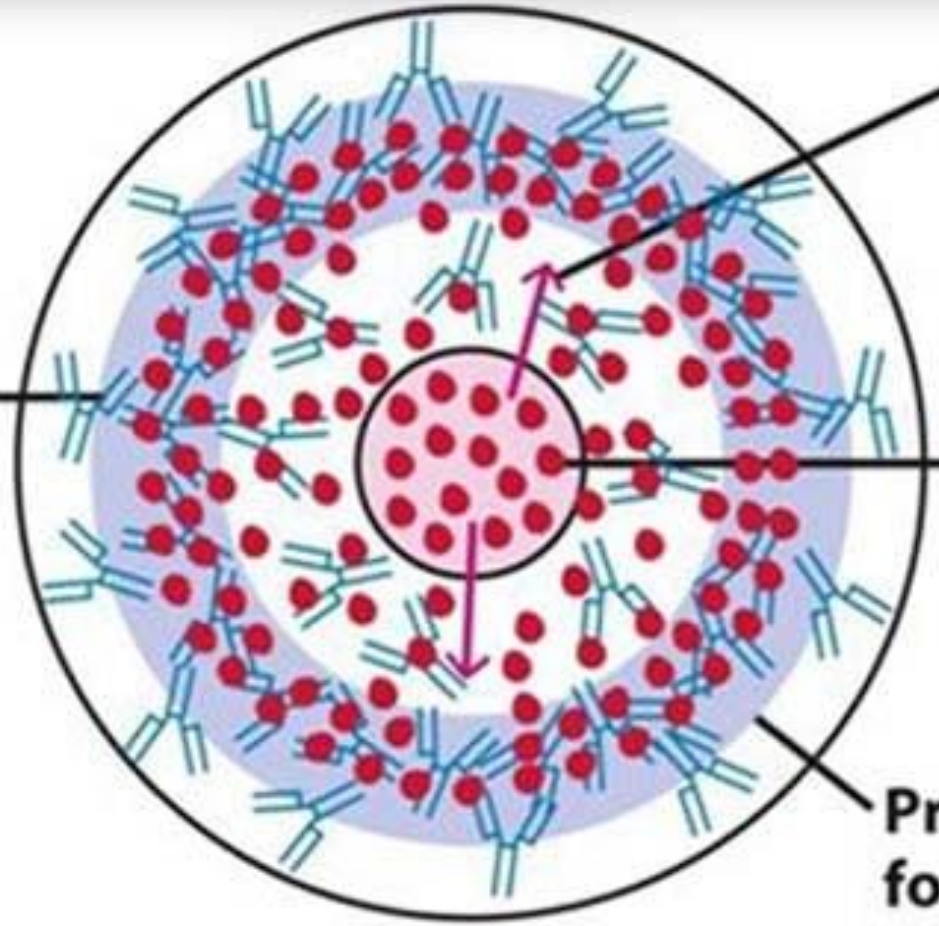
- In addition to the affinity and avidity of the antibody involved, precipitation depends on the relative proportions of antigen and antibody present.
- Optimal precipitation occurs when the relative amounts of antigen- and antibody-binding sites are equivalent, and neither is in excess compared to the other.
- Three Zones of the Precipitation Reaction: The precipitin curve shows how the amount of precipitation varies with increasing antigen concentrations when the amount of antibody is kept constant.
- Optimal precipitation occurs in the zone of **equivalence**.
- A large excess of antibody results in a **prozone**, whereas a large excess of antigen creates a **postzone.**

Radial Immunodiffusion



- **Radial immunodiffusion (RID)** is a single-diffusion technique involving the migration of antigens only.
- In this method, the reagent antibody is uniformly mixed into the support gel.
- The antigen contained in commercial standards or the patient sample is applied to wells cut into the gel.
- During incubation, the antigen diffuses out from each well and combines with specific antibodies in the agarose to form rings of precipitation around the wells.
- The rings expand in size until the zone of equivalence is reached and a stable lattice network is formed.
- The area of the ring obtained is a measure of the antigen concentration in a particular well.
- The antigen concentration within a patient sample can be derived from a standard curve obtained by using antigens of known concentrations.
- One technique for the measurement of RID was developed by Mancini and is known as the endpoint method.
- In this technique, an antigen is allowed to diffuse to completion; when equivalence is reached, there is no further change in the ring diameter.
- Equivalence occurs between 24 and 72 hours. The square of the ring diameter is directly proportional to the concentration of the antigen.
- The concentration of the test reactant in the unknown sample can be determined from a standard curve generated from standards with known concentrations of the reactant.

Radial Immunodiffusion



Clinical Correlations

Immunoproliferative Diseases

Normally, the immune system produces polyclonal immunoglobulins, generated by many clones of B cells as they respond to different antigens in our environment. Patients with cancers involving B cells or plasma cells produce high levels of a monoclonal antibody that originate from the single malignant clone. These conditions are referred to as *immunoproliferative diseases* and include multiple myeloma, which is most commonly characterized by the production of monoclonal IgG, IgA, or free light chains, and Waldenström macroglobulinemia, in which patients produce monoclonal IgM. See Chapter 18 for details.

- RID has been used to measure immunoglobulin classes and subclasses as well as complement components and other serum proteins.
- Immunodiffusion is simple to perform and requires no instrumentation but requires reagents, has a long turnaround time to results, and is subject to technical artefacts.
- For these reasons, it has been largely replaced by automated techniques in the clinical laboratory.

Table 10–1 Comparison of Precipitation Techniques

TECHNIQUE	APPLICATIONS	SENSITIVITY (µg Ab/mL)	PRINCIPLE
Nephelometry	Immunoglobulins,	1–10	Light that is scattered at an angle is measured,
	other serum proteins		body present.
Radial immunodiffusion (RID)	Immunoglobulins, complement	10–50	Antigen diffuses out into a gel that is infused with antibody. Measurement of the precipitin ring diameter indicates the concentration of the antigen.

Principles of Agglutination Reactions

- **Agglutination,** similar to precipitation, is a two-step process that results in the formation of a stable lattice network.
- The first step, **sensitization**, involves antigen–antibody combination through single antigenic determinants on the particle.
- The second step, **lattice formation**, involves the development of crosslinks that form visible aggregates. Lattice formation represents the stabilization of antigen-antibody complexes with the binding together of multiple antigenic determinants

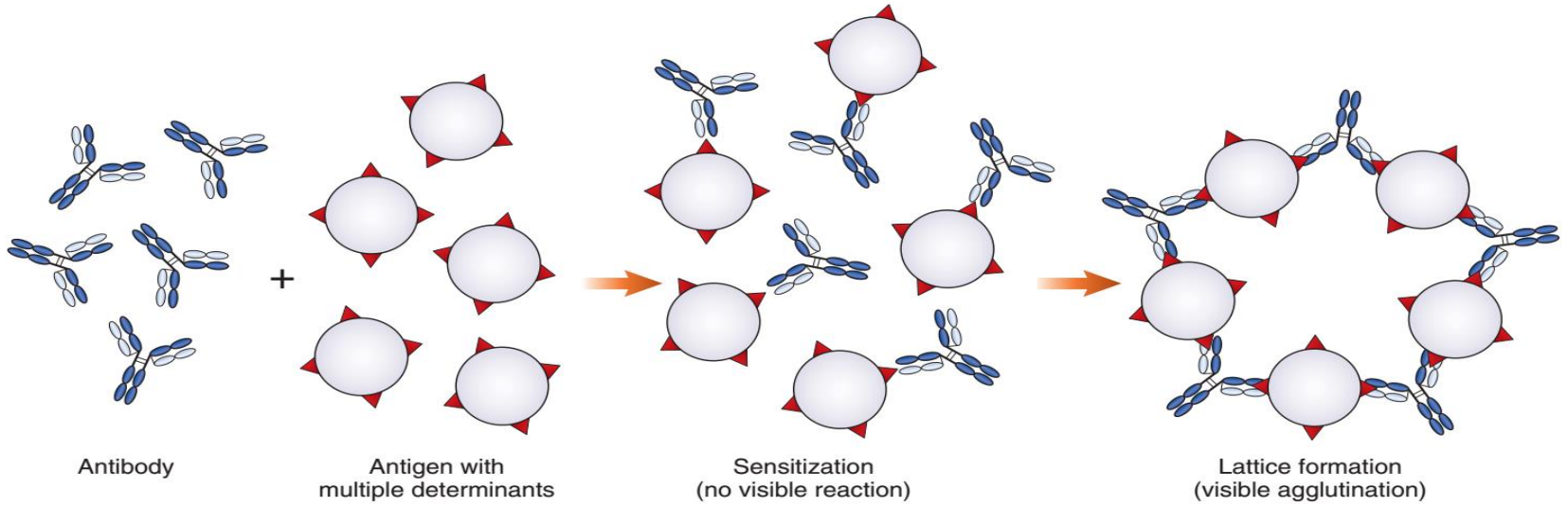
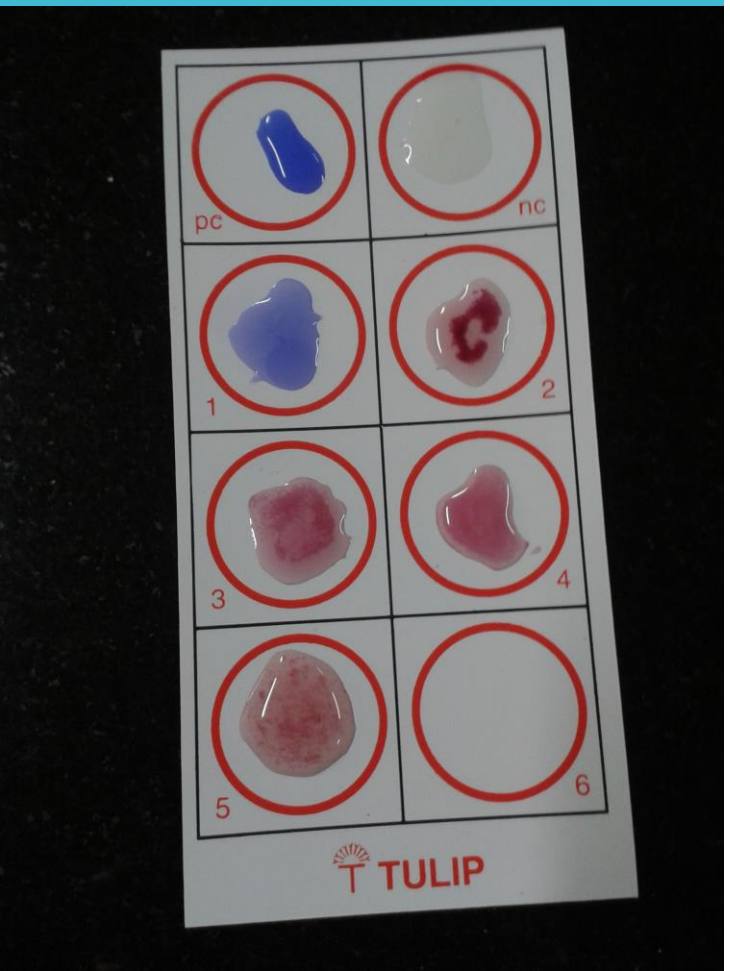


FIGURE 10–8 Phases of agglutination. Sensitization: Single epitopes on the antigen bind to antibody. Lattice formation: Multiple antigen and antibody molecules bind together to form a stable lattice.

Types of Agglutination Reactions

- Agglutination tests are easy to carry out, require no complicated equipment, and can be performed as needed in the laboratory without having to batch specimens.
- Agglutination reactions can be used to identify either antigen or antibody.
- Typically, most agglutination tests are qualitative, simply indicating the absence or presence of antigen or antibody, but dilutions can be made to obtain semiquantitative results.
- Many variations exist that can be categorized according to the type of particle used in the reaction and whether an antigen or antibody is attached.

Direct Agglutination

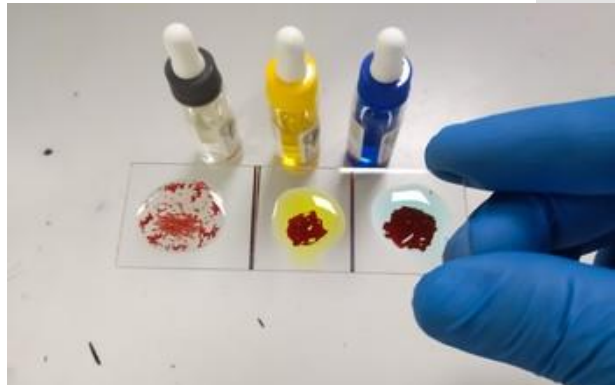


- Occurs when antigens are found naturally on a particle.
- use of known bacterial antigens to test for the presence of bacterial antibodies in a patient.
- Typically, patient serum is diluted into a series of tubes or wells on a slide and reacted with bacterial antigens specific to the suspected disease.
- Detection of antibodies is primarily used in the diagnosis of diseases for which the bacterial agents are extremely difficult to culture.
- One such example is the **Widal test,** a rapid screening test for antibodies to Salmonella typhi antigens, which has been used to help detect **typhoid fever**.
- A significant finding is a fourfold increase in antibody titer through time when dilutions of serum samples are tested with any of these antigens.

Hemagglutination (Direct)

FIGURE 10–10 RBC agglutination. The tube on the left is a positive test for RBC agglutination, whereas the tube on the right is a negative test showing that the RBCs have remained in a smooth suspension. *(Courtesy Linda Miller.)*

- If an agglutination reaction involves RBCs, then it is called **hemagglutination.**
- The best example of this occurs in **ABO blood group typing** of human RBCs, one of the world's most frequently used immunoassays.
- Patient RBCs mixed with antisera of the IgM type can be used to determine the presence or absence of the A and B antigens; this reaction is usually performed at room temperature.
- Group A RBCs will agglutinate with anti-A antibody, and Group B RBCs will agglutinate with anti-B antibody.
- This type of agglutination reaction is simple to perform, relatively sensitive, and easy to read



Indirect agglutination
Passive Agglutination

- Employs particles that are coated with antigens not normally found on their surfaces.
- A variety of particles, including latex and gelatin, are used for passive agglutination (indirect).
- Using synthetic beads or particles provides the advantages of consistency and uniformity.
- Reactions are easy to read visually and give rapid results.
- It is used to detect many antibodies, including rheumatoid factor (an anti-IgG found in some autoimmune disorders), antibodies to Group A Streptococcus antigens, and antibodies to viruses such as cytomegalovirus and rubella.
- Commercial tests are usually performed on disposable plastic slide cards or glass slides.
- Kits contain positive and negative controls; if the controls do not give the expected results, the test is not valid.
- Such tests are typically used as screening tools, which are followed by more extensive testing if the results are positive.

Advantages of agglutination reactions

- Rapidity, relative sensitivity, and the fact that if the sample contains a microorganism, the organism does not need to be viable.
- Most tests are simple to perform and require no expensive equipment.
- A wide variety of antigens and antibodies can be tested for in this manner.
- It must be kept in mind, however, that a negative result does not rule out the presence of the disease or the antigen (WHY?) The quantity of antigen or antibody may be below the sensitivity of the test system.
- Although the number of agglutination tests has decreased in recent years, they continue to play an important role in the identification of rare pathogens such as Brucella and more common organisms such as rotavirus and Cryptococcus, for which other testing is complex or unavailable.

Study Guide: Comparison of Agglutination Reactions

TYPE OF REACTION	PRINCIPLE	RESULTS
Direct agglutination	Patient serum is reacted with antigen that is naturally found on a particle.	Agglutination indicates the presence of patient antibody to a natural antigen.
Indirect (passive) agglutination	Patient sample is reacted with particles coated with antigens not normally found on their surfaces.	Agglutination indicates the presence of patient antibody to an artificially attached antigen.

Summary

- Precipitation involves the combination of soluble antigens with soluble antibodies to produce insoluble complexes that are visible.
- The union of antigen and antibody depends on affinity, or the force of attraction that exists between one antibody-binding site and a single epitope on an antigen.
- Avidity is the sum of all attractive forces between multiple binding sites on antigen and antibody.
- Maximum binding of antigen and antibody occurs when the aggregate number of multivalent sites of antigen and antibody are approximately equal.
- The concentrations of antigen and antibody that yield maximum binding represent the zone of equivalence. In this zone, the multivalent sites of antigen and antibody are approximately equal.
- In the prozone, when the antibody is being tested against a standard concentration of antigen, an antibody is in excess as compared with antigen, and precipitation or agglutination cannot be detected.
- In the postpone, the antigen is in excess compared with the concentration of antibody, so manifestations of antigen–antibody combination, such as precipitation and agglutination, are undetectable.
- Agglutination is the process by which particulate antigens, such as latex beads, RBCs, or gel particles, react with specific antibodies to form large aggregates or clumps.
- The process of agglutination can be divided into two steps: (1) sensitization, or initial binding of antigen and antibody, which depends on the nature of the antibody and the antigen-bearing surface, and (2) lattice formation, which is governed by such factors as pH, ionic strength, and temperature.
- Because of its larger size, IgM is usually able to mediate lattice formation with antigen without additional enhancement. In contrast, agglutination reactions involving IgG require enhancement techniques that vary physicochemical conditions and the addition of an anti-human immunoglobulin (Coombs reagent) in order to see a visible reaction
- In direct agglutination tests, patient serum is incubated with antigens that are found naturally on the indicator particles.
- In passive agglutination tests, patient serum is reacted with antigens that are artificially attached to such particles.

Reference

- Miller, Linda E; Stevens, Dorresteyn Christine. (2022) Clinical Immunology & Serology A Laboratory Perspective (p. 175). F.A. Davis Company. Kindle Edition.