Precipitation & Chromatographic Immunoassays

Department of Microbiology – College of Medicine

3rd Year Medical Students

Academic Year 2025-2026

Antigen-Antibody Binding

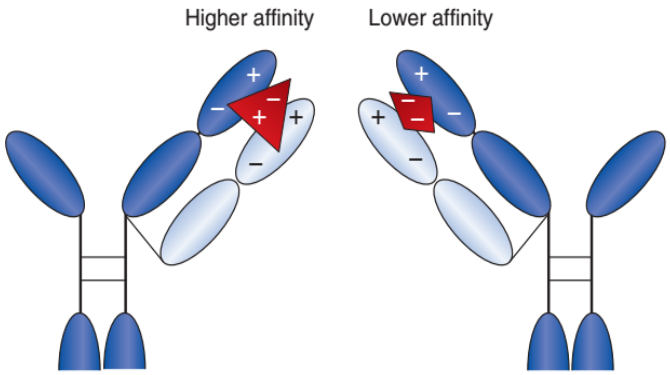


FIGURE 10–1 Affinity is determined by the three-dimensional fit and molecular attractions between one antigenic determinant and one antibody-binding site. The antigenic determinant on the left has a better fit and charge distribution than the epitope on the right and hence will have a higher affinity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

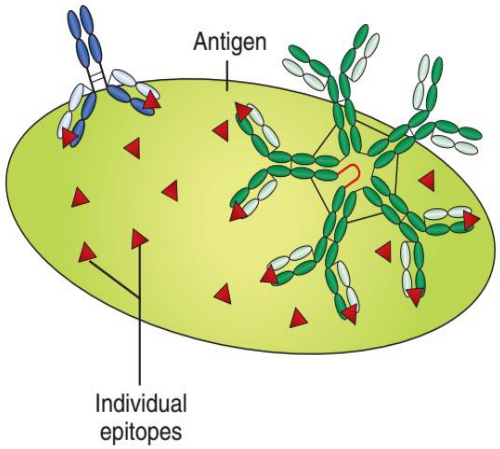


FIGURE 10–2 Avidity is the sum of the forces binding multivalent antigens to multivalent antibodies. In a comparison between immunoglobulin G (IgG) and IgM, IgM has the most potential binding sites for antigen and thus a higher avidity. Note that the monomer subunits in IgM can swing up or down in order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

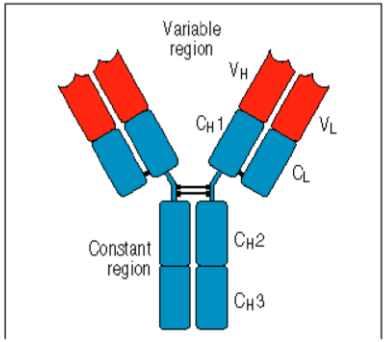
- More specific tests!
- One antibody molecule may initially attract numerous different antigens. Still, what determines whether the bonding will be stable:
  - epitope's shape
  - the way it fits together with the binding sites on an antibody molecule.
- Antibodies are also capable of reacting with 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??

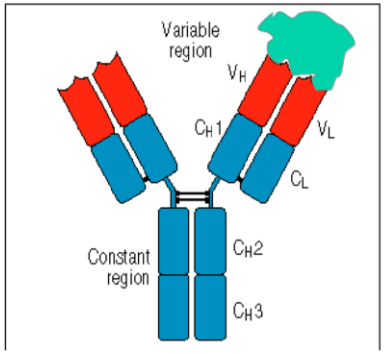
In which Ab elicited by one Ag can cross-react with an unrelated Ag. This is because the 2 different Ags share an identical epitope for e.g.

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.

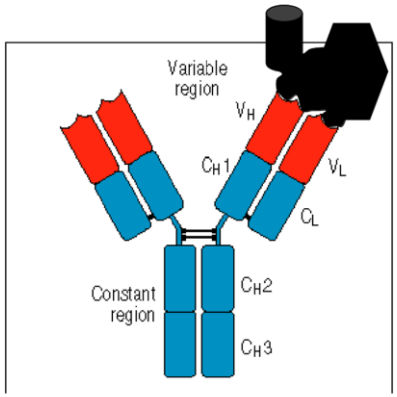
Cross-reactive Antigens



Cross-reactive Antigens



Cross-reactive Antigens



Introduction to Immunoassays

- **Immunoassays** are analytical techniques based on the specific binding between an antigen and its corresponding antibody.
- They are widely used in clinical laboratories to detect, identify, or quantify molecules such as infectious agents, hormones, tumor markers, immune proteins, and drugs.
- Their high specificity derives from the unique molecular recognition between the epitope and the antibody-binding site.

Types of Immunoassays

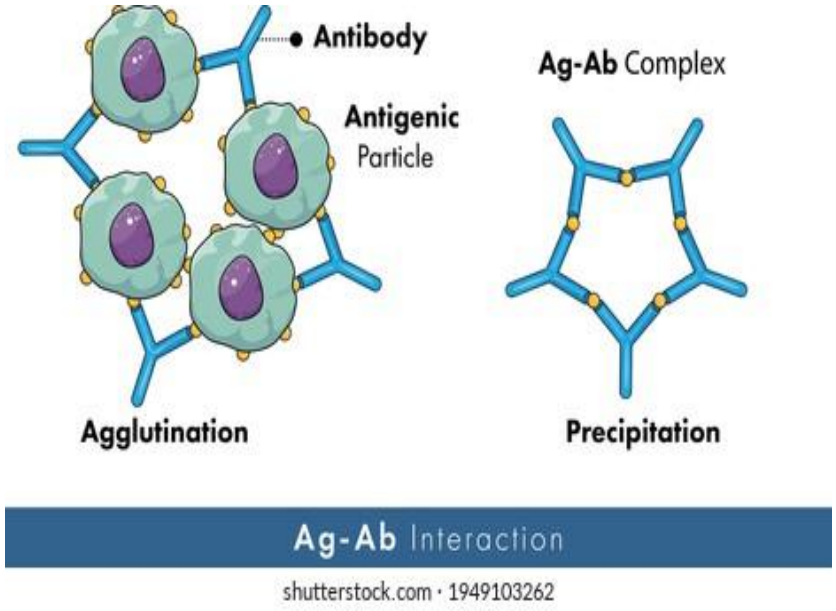
Immunoassays are categorized into two major groups:

- **1. Unlabeled Immunoassays**
  These assays rely on naturally visible antigen–antibody interactions. Detection occurs without the need for an attached marker.
  Examples include precipitation, agglutination, and chromatographic (lateral-flow) assays.
- **2. Labeled Immunoassays**
  These depend on an attached label such as an enzyme, fluorophore, chemiluminescent molecule, or radioisotope.
  Examples include ELISA, CLIA, FIA, and RIA (2nd Semester).

Unlabeled Immunoassays

principal unlabeled immunoassays:

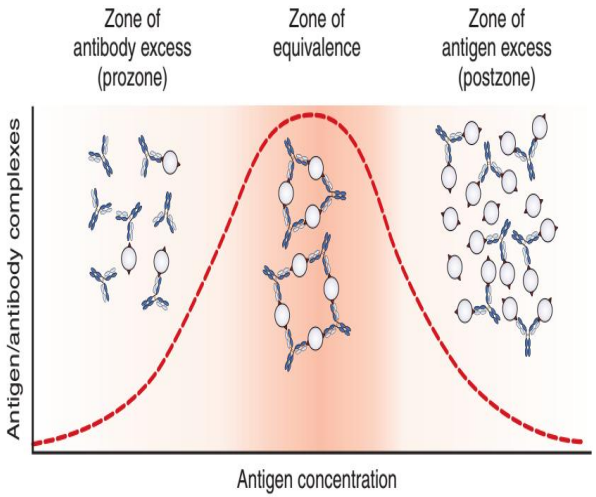
- **Precipitation reactions**, in which soluble antigen and soluble antibody form visible immune complexes.
- **Chromatographic immunoassays**, which use capillary migration and labeled antibodies to produce rapid visual results (e.g., hCG pregnancy test).
- **Agglutination reaction.** Agglutination is an unlabeled immunoassay in which particulate antigens (such as red blood cells, bacteria, or latex beads) react with their specific antibodies to form visible clumps.



Precipitation Reactions: Definition

- Precipitation occurs when a soluble antigen reacts with a soluble antibody to form **insoluble antigen–antibody complexes**.
- These complexes become visible only when antigen and antibody are present in optimal proportions, allowing the formation of a large, stable lattice structure.

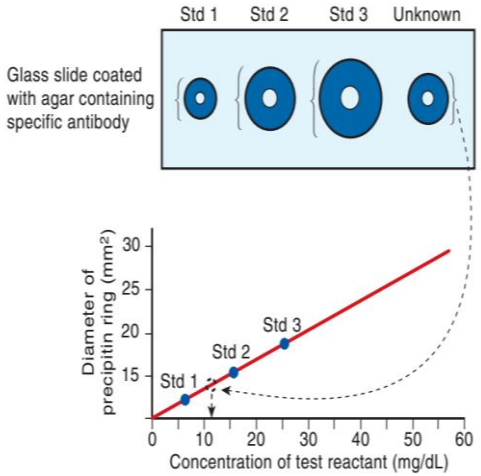
The Precipitin Curve



- **Precipitation reactions** depend on the relative concentrations of antigen and antibody in the reaction mixture. When the two components combine, they form antigen–antibody complexes of varying sizes.
- The extent of visible precipitation is determined by the balance between the antigen and the antibody in the system.
- **Prozone:** excess antibody → no precipitation.
- **Equivalence:** optimal ratio → visible precipitation.
- **Postzone:** excess antigen → weak or absent precipitation.

These zones explain possible **false-negative** results in clinical serology.

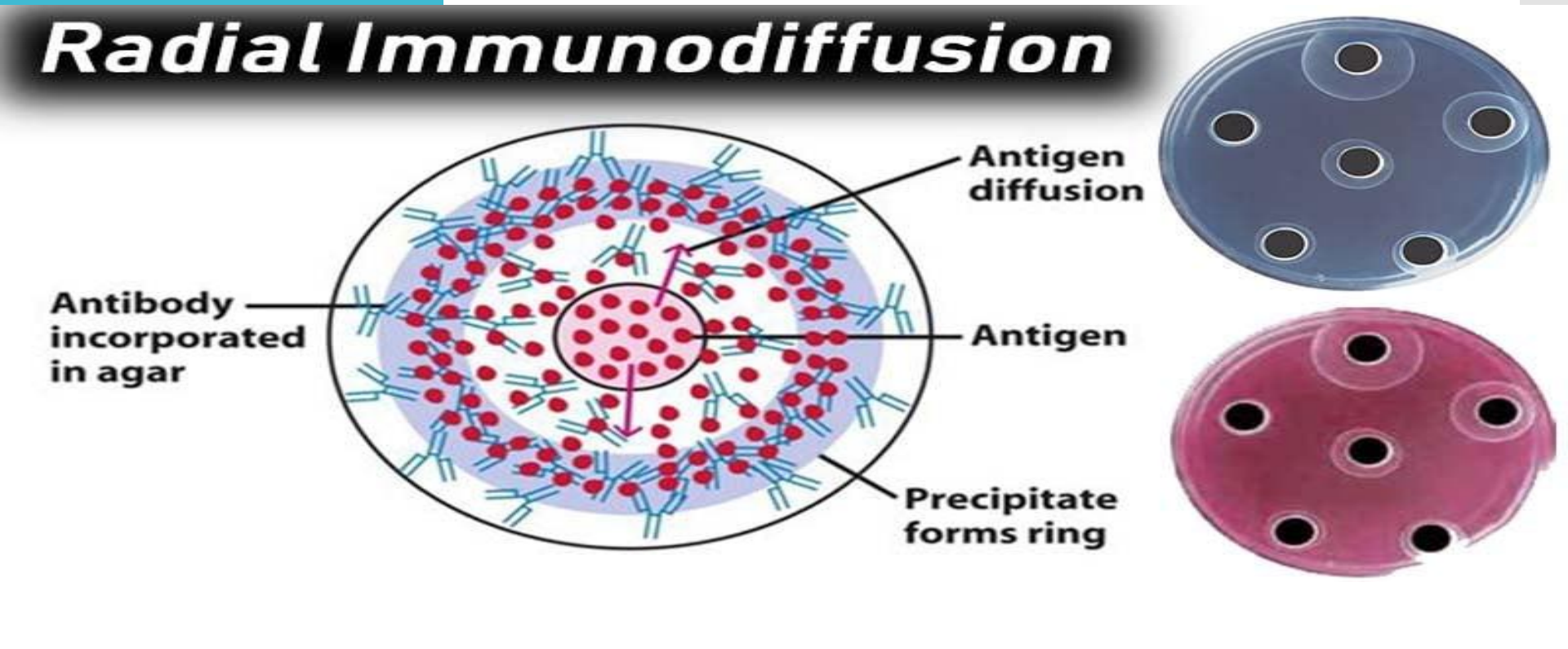
Radial Immunodiffusion (RID)



- Radial immunodiffusion is a **single-diffusion precipitation technique**.
- The antibody is distributed uniformly within an agar matrix.
- Antigen diffuses radially from a well into the gel.
- A precipitin ring forms at the point of antigen–antibody equivalence.
- The ring diameter is directly proportional to the antigen concentration.

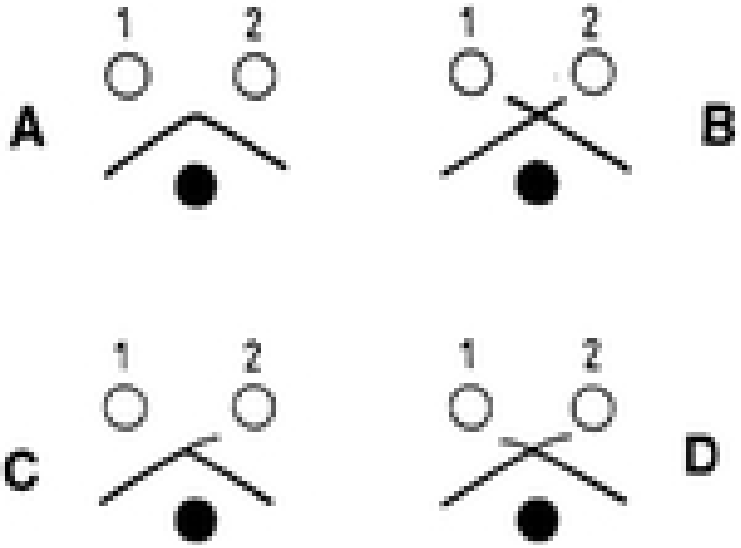
Clinical Applications of RID

- RID is used for quantitative measurement of:
- Immunoglobulin classes (IgG, IgA, IgM)
- Complement components (C_3, C_4)
- Specific serum proteins
- It is particularly useful in detecting **abnormal elevations** of immunoglobulins in immunoproliferative disorders such as multiple myeloma.



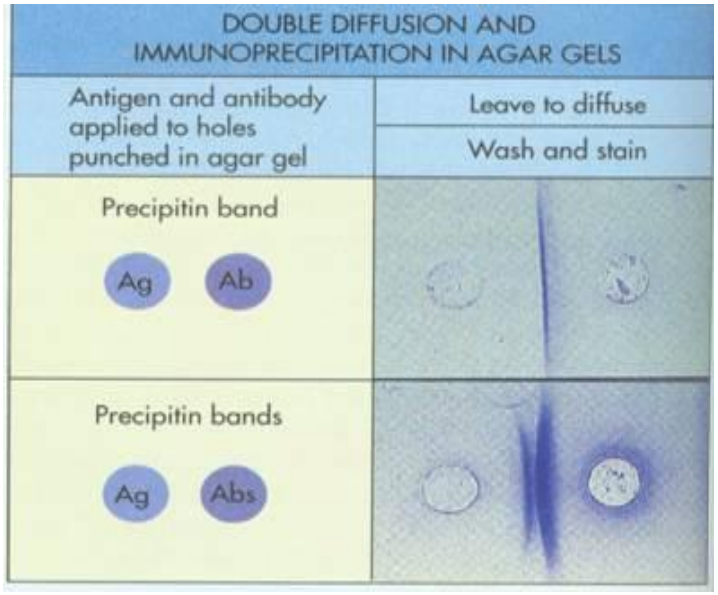
Ouchterlony Double Diffusion

- This assay is particularly useful **Ouchterlony double diffusion** is a qualitative precipitation technique in which both antigen and antibody diffuse through agar and form visible precipitin lines at the zone of equivalence.
  The method is used to determine:
  - **Antigenic identity** (shared epitopes)
  - **Partial identity** (overlapping but non-identical epitopes)
  - **Non-identity** (unrelated antigens)



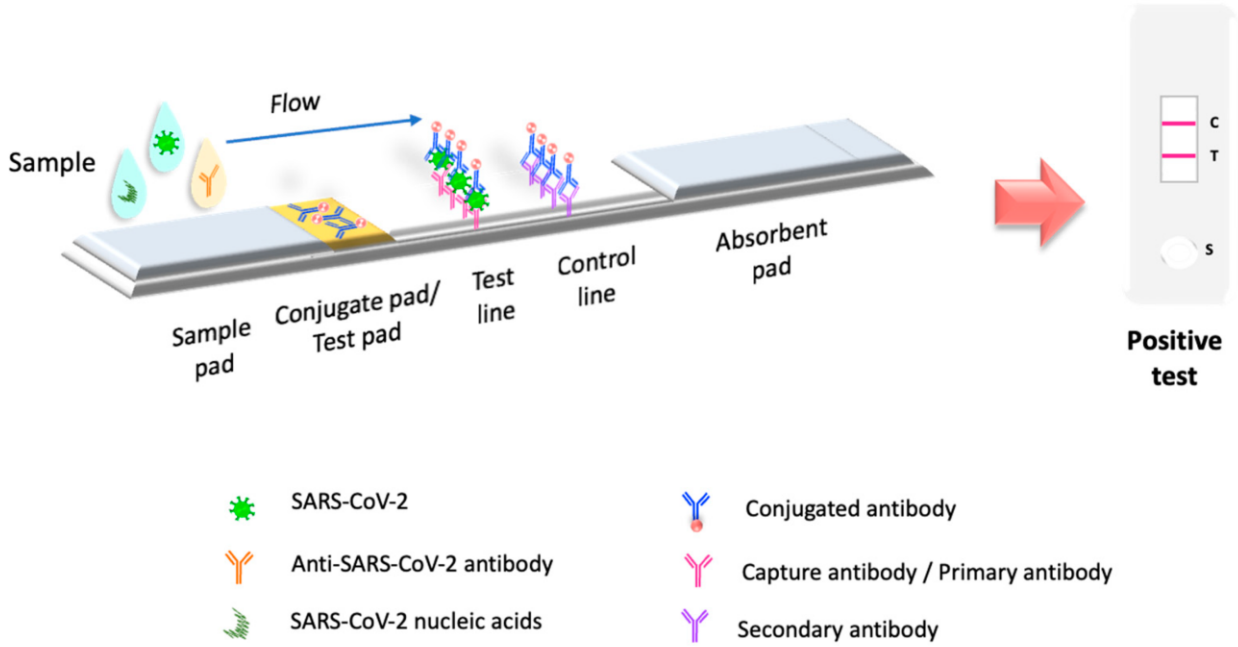
Applications of the Ouchterlony

- Verification of **antigenic integrity** and **purity** of immunological preparations.
- Detection of **cross-reactivity** between related antigens.
- **Vaccine and antiserum quality control**: assessing batch consistency, confirming antigen specificity, and identifying contamination or degradation.
- Comparison of antigenic relationships in microbial or protein samples.



Chromatographic Immunoassay

- Chromatographic immunoassays (lateral-flow assays) are rapid diagnostic tests based on **capillary migration of a liquid sample** through a membrane containing labeled antibodies.
  The antigen–antibody reaction produces a visible line, allowing simple and rapid test interpretation.

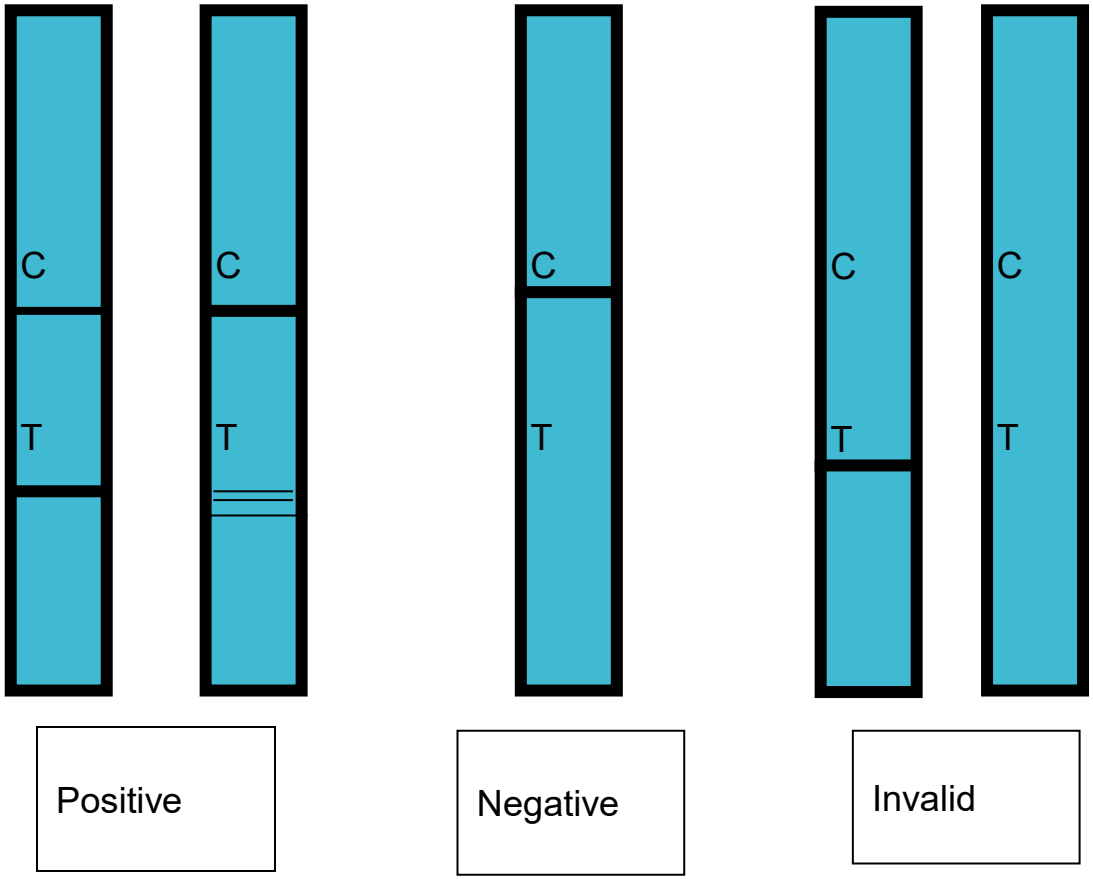


Principle of Chromatographic Test

- A lateral-flow test strip contains the following components:
  - **Sample pad**
  - **Conjugate pad** containing labeled antibodies (e.g., gold nanoparticles)
  - **Test line (T)** with immobilized capture antibodies
  - **Control line (C)** to confirm test validity
  - **Absorbent pad**
- As the sample migrates, the antigen binds to the labeled antibody complex and is captured at the test line. The control line must always appear to ensure proper test function.

- Sample pad → conjugate pad → membrane → absorbent pad.
- Test (T) line indicates antigen detection.
- Control (C) line confirms validity of the test.

Chromatographic immunoassay



hCG Pregnancy Test

- The hCG pregnancy test is a chromatographic immunoassay that detects **human chorionic gonadotropin (hCG)** in urine or serum.
- **Interpretation:**
  - **Positive:** Both the test (T) and control (C) lines are visible.
  - **Negative:** Only the control (C) line is visible.
  - **Invalid:** Absence of the control line, regardless of the test line.
- hCG becomes detectable approximately **7–10 days after conception**.

Summary

- Precipitation forms visible immune complexes.
- RID quantifies immunoglobulins and complement.
- Ouchterlony tests antigenic relationships.
- Chromatography provides rapid diagnostic results (e.g., hCG test).