Antigen–Antibody Reactions

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This Lecture was loaded in blackboard and you can find the material in: Jawetz, Meinik & Adelberg's MEDICAL MICROBIOLOGY, 27 th Edition & Essential of Clinical Immunology, 6th Edition

Learning objectives:

- ✓ Determine the importance of serology in medicine.
- ✓ Describe the principle of each serological test.
- ✓ List the uses for each serological tests.
- ✓ Observe the reading of titer.

Antigen–Antibody Reactions

LO.1

- Antigen – antibody reactions are performed to determine the presence of either the antigen or antibody (serological tests).
- In laboratory, they help in diagnosis of infectious diseases , auto immune diseases , hypersensitivity, in epidemiological surveys and in the follow up of patients.
- Detection of Hormone, CD markers & different tumor markers.

Two important parameters in serological tests LO.1

Sensitivity* & *Specificity

Sensitivity: The ability of the test to detect even very minute quantities of antigen or antibody.

- When the test is highly sensitive, false negative results may be absent or minimal.

Specificity: The ability of the test to detect reactions between homologous Ags & Abs only, and with no other.

- In highly specific test, false positive reactions are absent or minimal.

Types of Antigen–Antibody Reactions

LO.2

1. Precipitation reaction
2. Agglutination reaction
3. Complement fixation test
4. Immunofluorescence
5. Radioimmuno assay
6. Enzyme immunoassay

I. Precipitation tests

LO.2

- This test applied to **soluble Ag**, which when react with specific Abs, precipitate will be formed in the zone of equivalence.

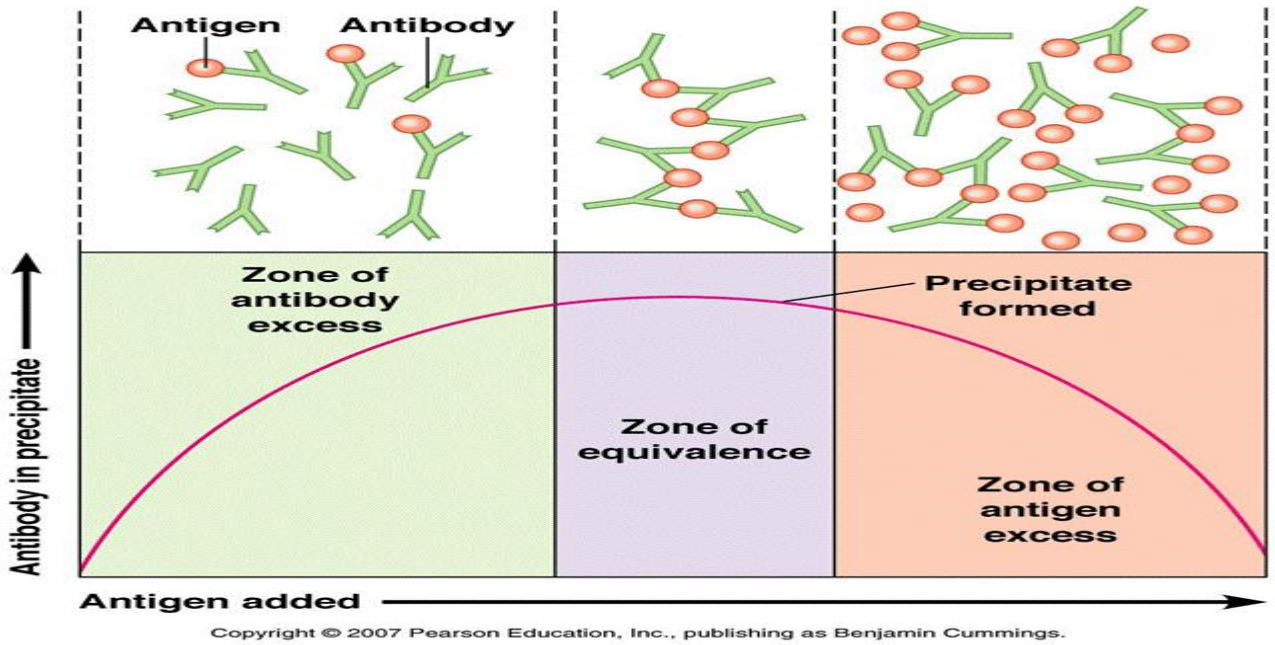
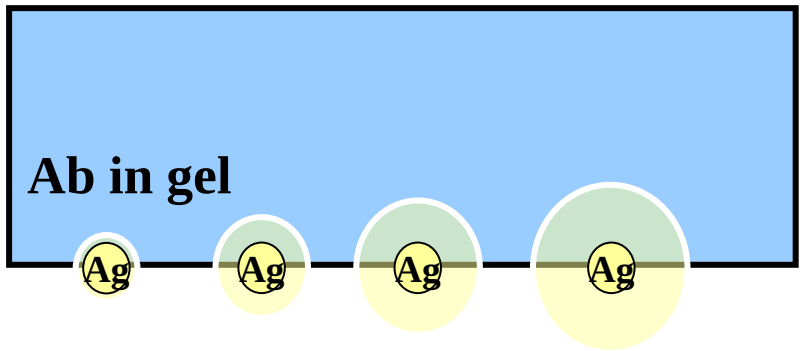


Figure 18.3 - Overview

Radial Immunodiffusion (Mancini)

LO.2

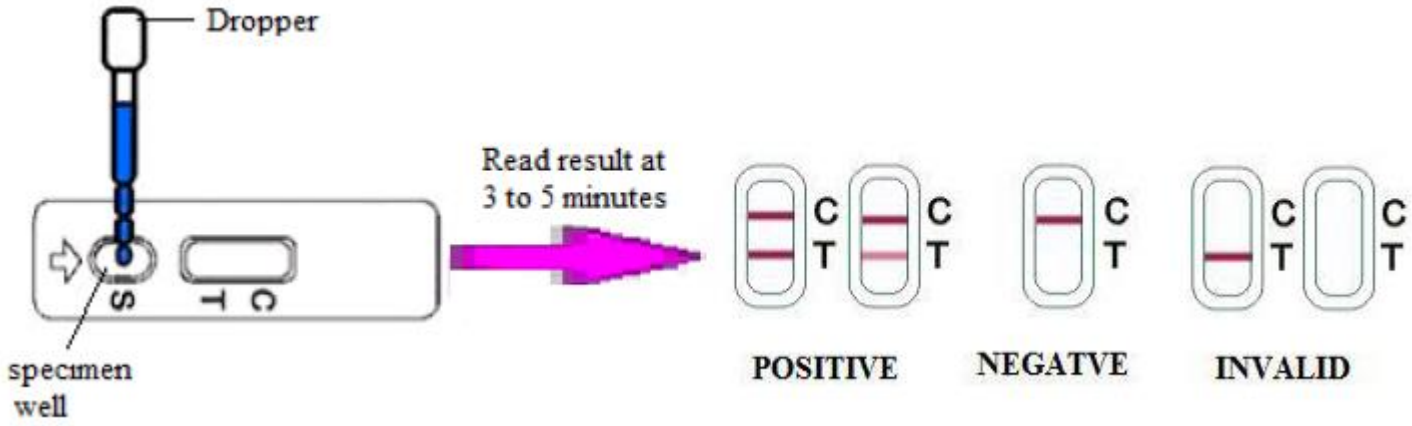
- Method
  - Ab in gel
  - Ag in a well
- Interpretation
  - Diameter of ring is proportional to the concentration
- Quantitative
  - Ig levels
  - Complement level



Cassette (Pregnancy test)

LO.2

- **Pregnancy test:**
- (Nitrocellulose paper impregnated with anti – HCG to detect HCG in urine).



*C= Control, T= Test

Agglutination reaction

LO.2

- Definition:- tests that have as their endpoint the agglutination (clumping) of a particulate antigen (as bacteria , RBC, or latex - beads).
- Ags will be clumped or agglutinate by specific Ab
- Antibodies cause the cross-linking of particulate antigen, usually found on a cell
- When the antigen is an erythrocyte the term hemagglutination is used.

Agglutination reaction

LO.2

- Haemagglutination test.
- Latex agglutination test.
- Agglutination test in suspension (Tube agglutination test)

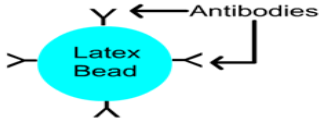
Latex agglutination test

LO.2

- *Inert latex beads provide a convenient carrier for Ag or Ab, examples*:

Latex beads are coated with antigens

- Antigens on a carrier molecule, such as latex, combine with patient's sample for antibody detection



- Antibody is bound to the carrier molecule, which is then mixed with patient's sample to detect antigen

Latex agglutination test

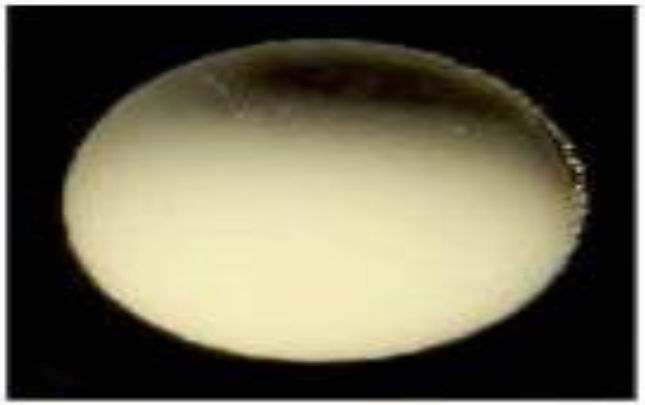
LO.2

- **Pregnancy test:** the conventional method (particles coated with anti-HCG agglutinated when mixed with urine sample containing HCG).

- **CRP test:** is a blood test that measures the amount of a protein called C-reactive protein in your blood. C-reactive protein measures general levels of inflammation in your body. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP within 2 minutes.

- **Rheumatoid factor test** (particles coated with IgG to detect IgM (rheumatoid factor) in patient serum).

LO.2



Negative

1+

2+

3+

4+

Latex agglutination tests

LO.2

Rheumatoid factor test

C-reactive protein

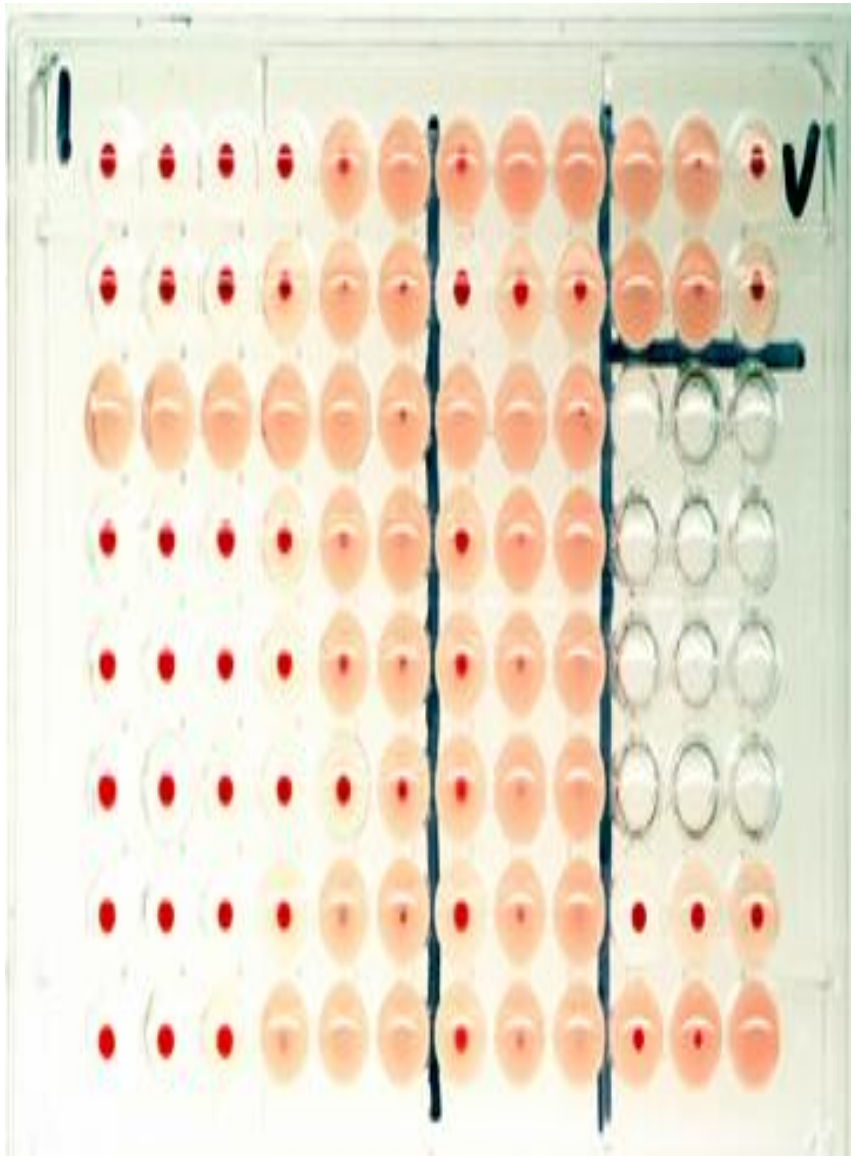
Complement Fixation

Day 1

- Serum with antibodies
- Serum without antibodies
- Antigen binds to antibodies
- Unbound antigen
- Complement binds to Ag/Ab complex
- Unbound complement

Day 2

- Hemolysin sensitized red blood cells serve as an indicator
- Hemolysin sensitized red blood cells serve as an indicator
- No lysis Positive
- Lysis Negative



LO.2

Assays Based on labeling (conjugate) system

LO.2

- Immunofluorescent assay test (IFAT)
- Radioimmunoassay (RIA)
- Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assay (ELISA)

LO.2

- Enzyme-linked immunosorbent assay.
- Is a plate-based immunoassay technique designed for **detecting and quantifying** substances such as peptides, proteins, antibodies and hormones.

- In qualitative ELISA: (+ OR -)
- In quantitative ELISA: The optical density or florescent units of the sample is interpolated into a standard curve.

Advantages of ELISA

LO.2

- Less costly and safest.
- Easy visualization of results with high level of accuracy.
- Specific and highly sensitive assay that can detect protein at the pico-molar to nano-molar range.
- Easily automated for performance of large numbers of tests.
- Require minimal reagents.
- Qualitative detection or Quantitative measurement of either antigen or antibody.
- Wells can be coated with antigens or antibodies.
- Can be done by personnel with only minimal training.

Principle:

LO.2

- To detect a specific antibody- antigen reaction by assessing the ***conjugated enzyme activity.***
- The enzyme convert a colourless substrate to a measurable colored product, indicating the presence of the antibody - antigen [Ab-Ag] binding.
- The detection enzyme can be linked **directly to the primary antibody** or introduced through a **secondary antibody** that recognizes the primary antibody.
- The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

Enzyme linked immunosorbent assay (ELISA)

LO.2

Method of the test:

Patient serum

+

Ag/Ab labeled Horseradish peroxidase enzyme

+

Substrate

=

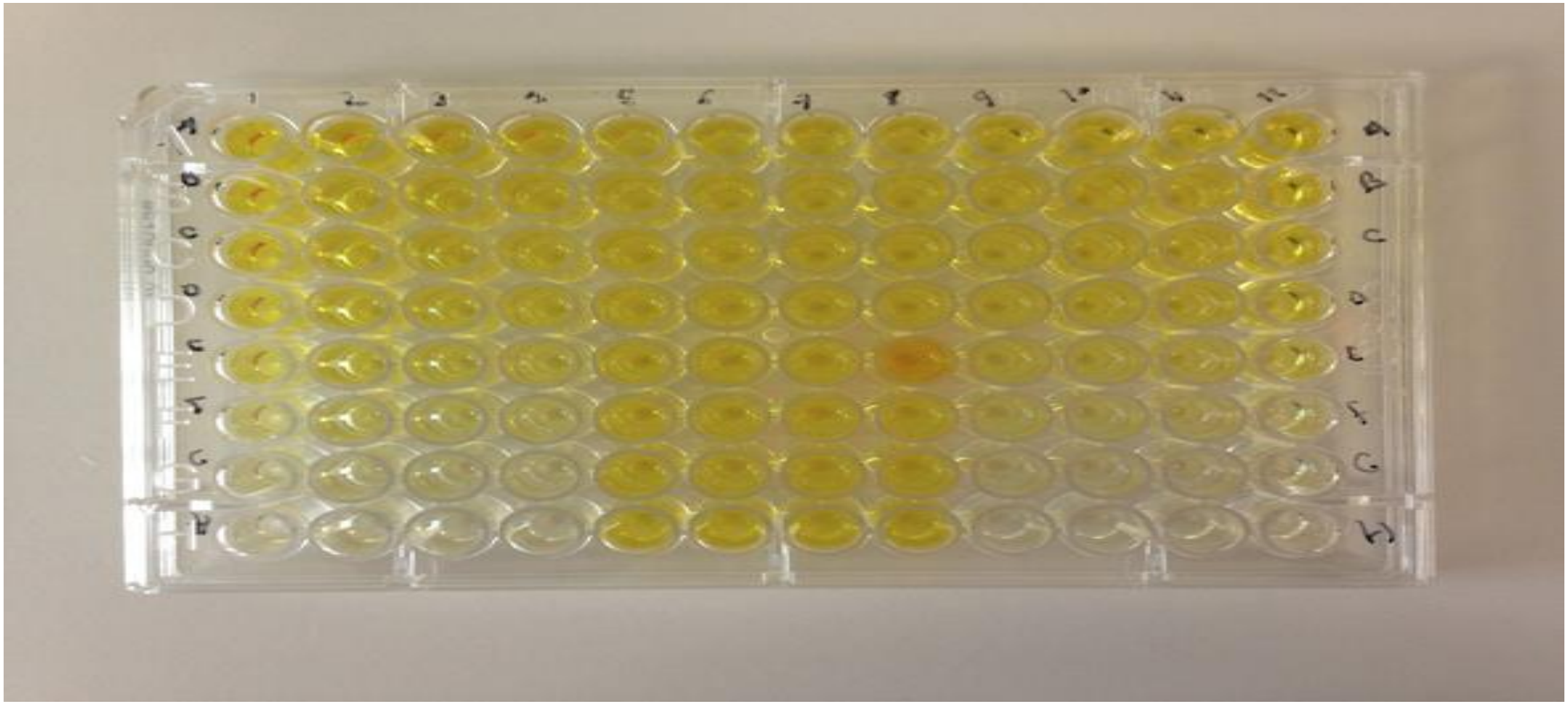
Enzymatic activity (color reaction)

↓

Read by spectrophotometer

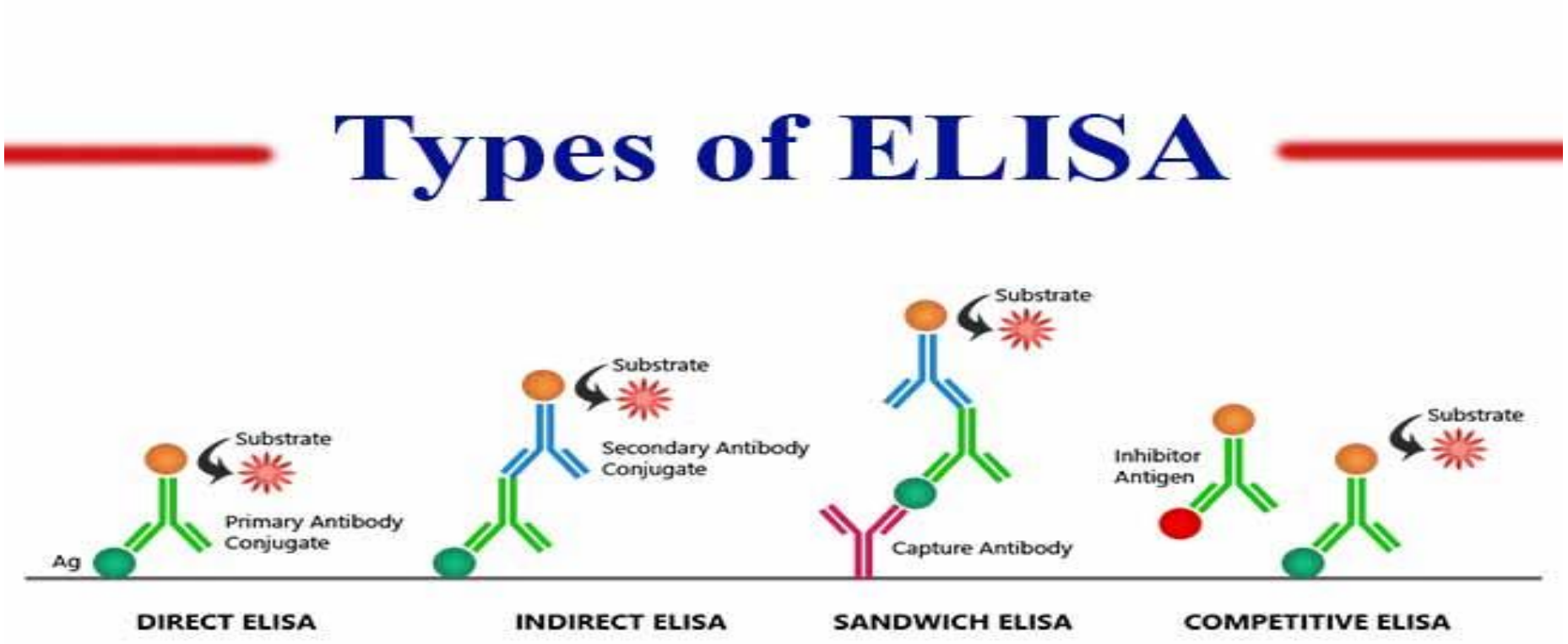
- **Dense color (high enzymatic activity) = High Ag or Ab in Pts specimen**

LO.2



Types of (ELISA)

LO.2

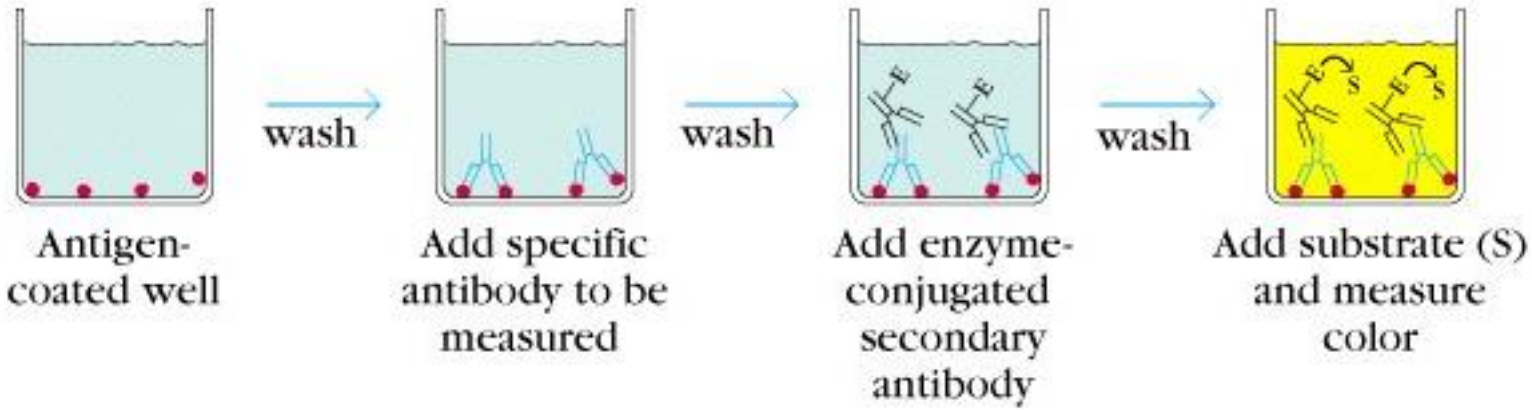


Applications of ELISA

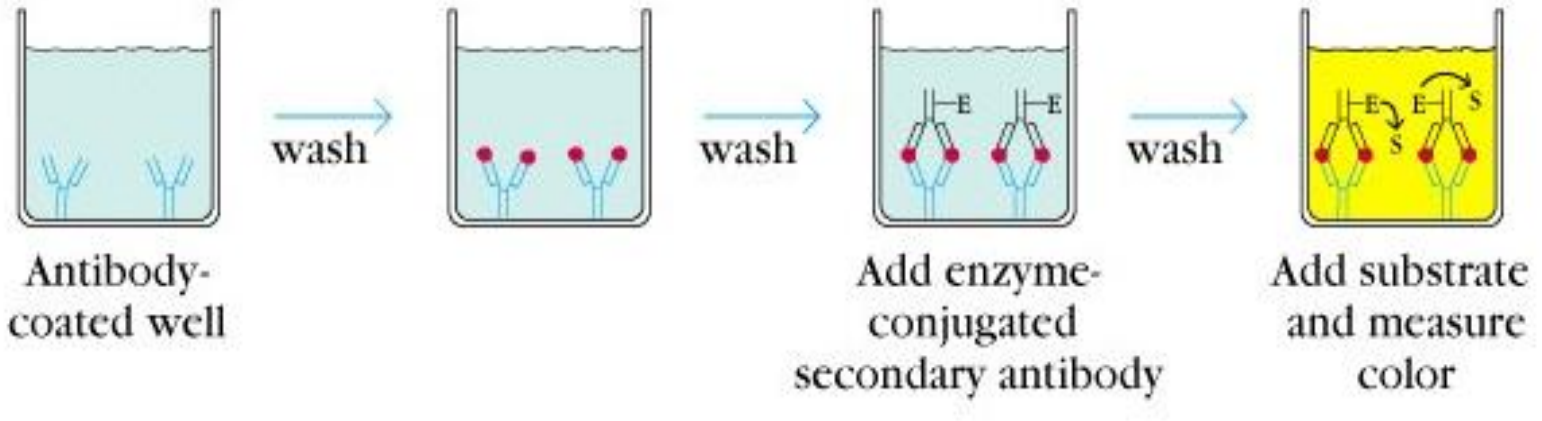
- **Medical Diagnosis:** For the detection of various diseases, including infectious diseases, autoimmune disorders, and cancer
- **Detection of Viral and Bacterial Pathogens:** For the detection of viral and bacterial antigens in clinical samples play a role in the diagnosis and surveillance of infectious diseases. such as hepatitis B surface antigen (HBsAg) and bacterial antigens like Helicobacter pylori.
- **Monitoring Drug Levels:** particularly in therapeutic drug monitoring. like digoxin, phenytoin, and vancomycin in patient serum.
- **Allergy Testing:** to detect specific IgE antibodies against allergens. They help in identifying allergens that trigger allergic reactions in individuals
- **Detection of Hormones:** such as cortisol, thyroid hormones (T3, T4), insulin, and reproductive hormones like estrogen and progesterone.
- **Research and Development**

LO.2

- **Antibody Test**



- **Antigen Test**

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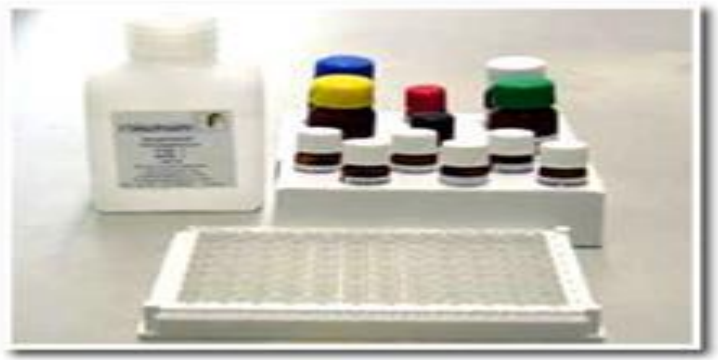


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