ADVANCED DIAGNOSTIC TESTS

CONTENT:

- Enzyme-linked immunosorbent assay
- Immunoflosence
- Agglutination Testing
- Complement Fixation Test
- PCR Test
- DNA Probe Test

ELISA : Enzyme-linked immunosorbent assay

- ELISA an immunoassay method is one of immunoassay method using antibodies to capture an antigen and an enzyme labeled antibody to estimate the amount of antigen

- **Principle**: ELISA combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme, can provide a useful measurement of antigen or antibody concentration.
  -There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen

- The presence of a particular substance simply as “+” or “-” :This expression is called **qualitative detection**, means that the substance is present more than detection limit or less

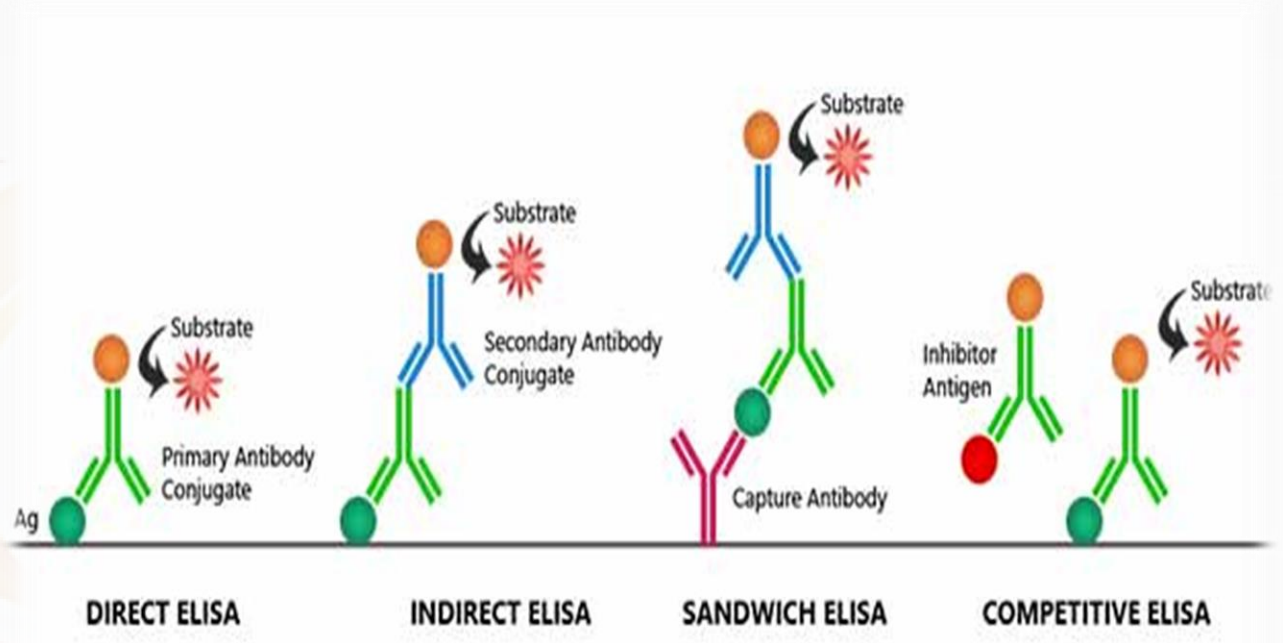
- **Quantitative measurement**, gives information about “how much” quantity

- Important components in immunoassay - Antibody (antiserum) , Antigen & Hapten

- **Procedure**
  -Antibody coating,
  -protein capturing,
  -detection antibody,
  -Streptavidin-enzyme conjugate,
  -Addition of substrate &
  -Analysis

- **ELISA Types**

  The four main types of ELISAs are indirect, direct, sandwich, and competitive.

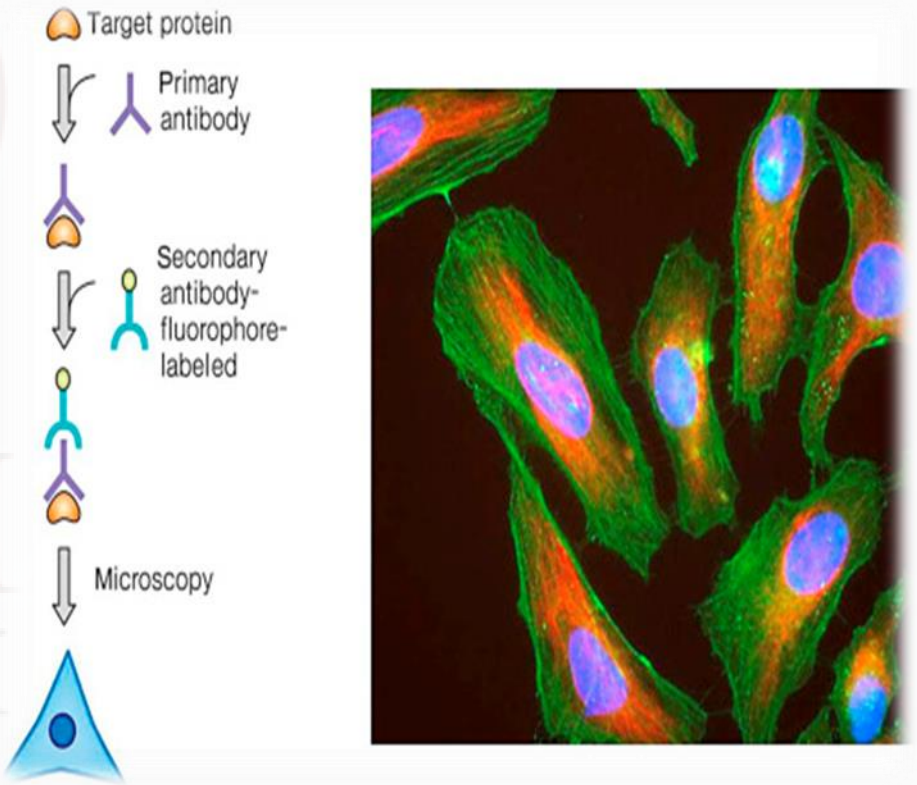


https://socratic.org/questions/in-the-elisa-test-what-do-primary-antibodies-secondary-antibodies-do

- **An ELISA test may be used to diagnose:**
  HIV, which causes AIDS, Lyme disease, Pernicious anemia, Rotavirus
  Syphilis, Toxoplasmosis, Varicella-zoster virus, which causes chickenpox and shingles
  Zika virus

IMMUNOFLUORESCENCE

- Immunofluorescence (IF) is a common morphological approach used to determine the distribution of subcellular components.
- Antibodies that conjugated with fluorescent dyes are required in IF assay. The antibody specifically recognizes the antigen by binding to the epitope of target, and the fluorophore will be detected under a fluorescent microscope. Hence, subcellular components can be visualized in a dark background .



https://www.sciencedirect.com/topics/medicine-and-dentistry/immunofluorescence-test

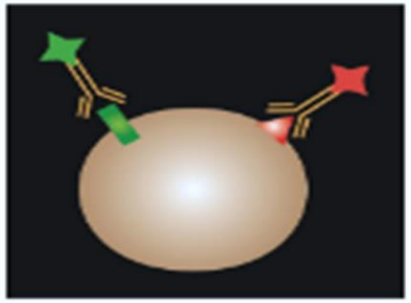
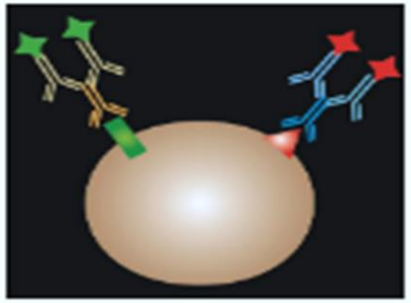
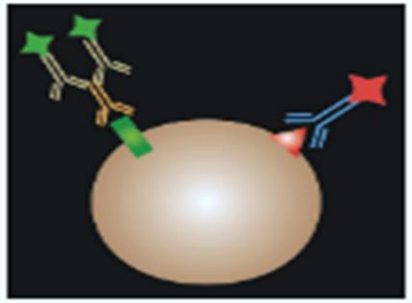
There are three types of IF: direct IF, indirect IF and combined IF

- Direct IF - is using a single primary antibody that is conjugated with fluorescent dye

- Indirect IF -is using two antibodies for the staining: primary antibody that specifically binds to epitope and a matched secondary antibody conjugated with fluorescence dye

- Combined IF - is a combination of direct and indirect IF staining

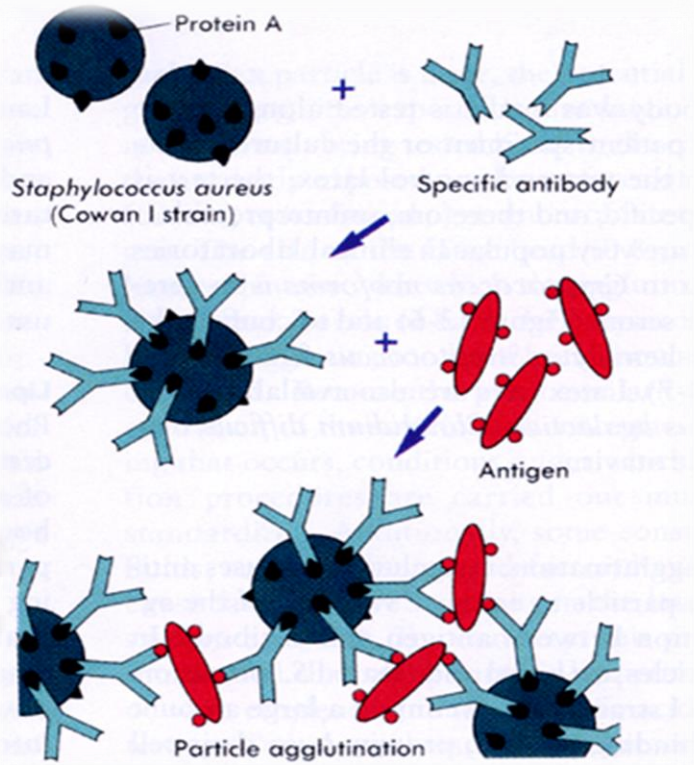
Comparison of direct, indirect and combined IF

IF type	Direct	Indirect	Combined
Schematic diagram			
Advantages	• Rapid staining • Available for antibodies from the same host • Less non-specific background signal	• Secondary signaling amplification • A single secondary antibody can detect multiple primary antibodies from the same host • A primary antibody can be matched to diverse secondary antibodies with different fluorescence dyes	• Secondary amplification for weak signaling • Available for antibodies from the same host
Disadvantages	• Less sensitive because of lack of secondary signaling amplification	• Time-consuming • Require antibodies from different hosts • Possibility of antibody cross-reactivity	• Multiple-step staining

AGGLUTINATION TESTING

- When the specific antibodies (agglutinins) bind to surface antigens of bacteria/virus or any antigens immobilized in particulate matter (such as latex particle) and cause the formation of a visible clumps, such test is called agglutination test.
- It is the development of antigen–antibody complexes in the form of particle clumps due to the interaction between the insoluble form of antigens.

Coagglutination Test



https://microbeonline.com/serologic-methodsagglutination/

➢- **Can be performed in:**

Surface of glass slides: Rapid reading/evaporation

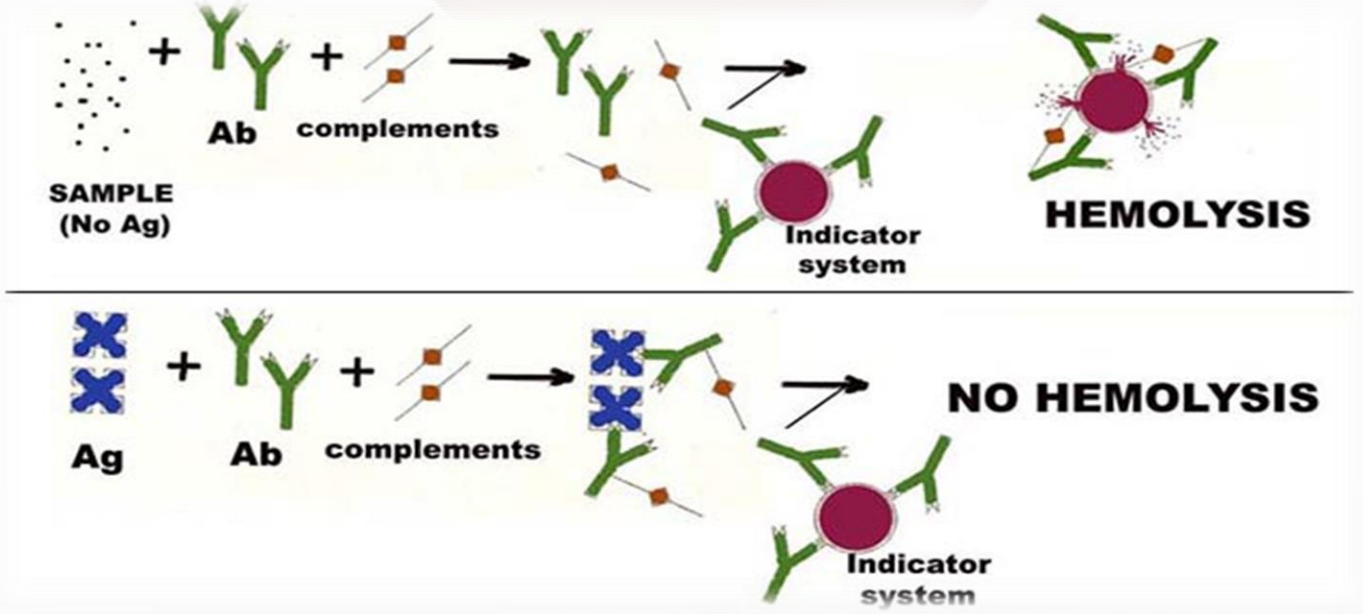
Test tubes: More sensitive because of longer incubation

➢**TYPES:** - Bacterial agglutination test

- Slide Agglutination test
- Particle agglutination test
- Hemagglutination
- Latex agglutination test for Ag detection
- Latex agglutination test for Ab detection
- Coagglutination (COAG)

COMPLEMENT FIXATION TEST

- It is a method for demonstrating the presence of antibody in patient serum. It is based on the principle that when antigen and antibodies of the IgM or the IgG classes are mixed, complement is “fixed” to the antigen-antibody complex. If this occurs on the surface of RBCs, the complement cascade will be activated and hemolysis will occur .



https://microbenotes.com/types-of-complement-fixation-test/

TYPES

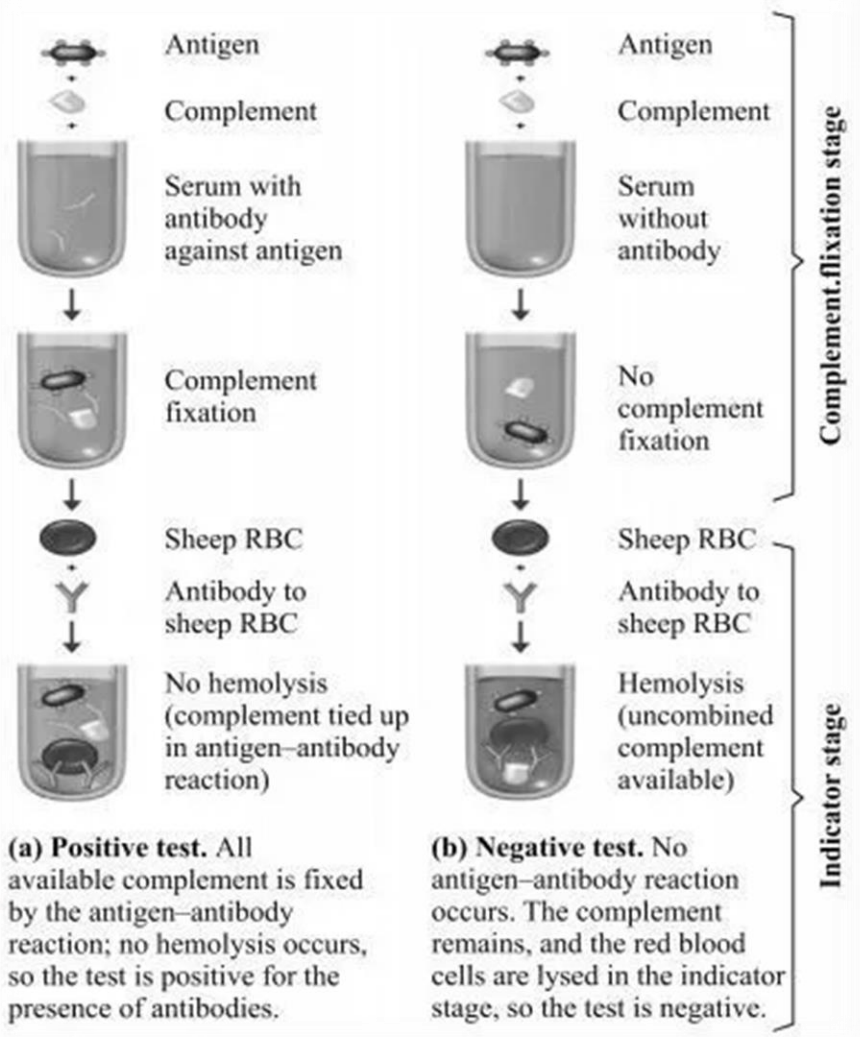
- Indirect complement fixation test
- Congulatinating complement absorption test
- Immune adherence
- Cytolytic tests

➢PROCEDURE

Complement Fixation Test (CFT) consists of two stage:

-First step (Complement fixation stage) a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement.

-Second step (Indicator Stage): The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

https://microbeonline.com/complement-fixation-test-principle-procedure-results/

➢ADVANTAGES

Ability to screen against a large number of viral and bacterial infections at the same time.
Economical

➢DISADVANTAGES

Not sensitive – cannot be used for immunity screening
Time consuming and labor intensive
Often non-specific e.g. cross-reactivity between HSV and VZV

PCR TEST

- Its principle is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. This method can generate tens of billions of copies of a particular DNA fragment (the sequence of interest, DNA of interest, or target DNA) from a DNA extract (DNA template).
- **STEPS**

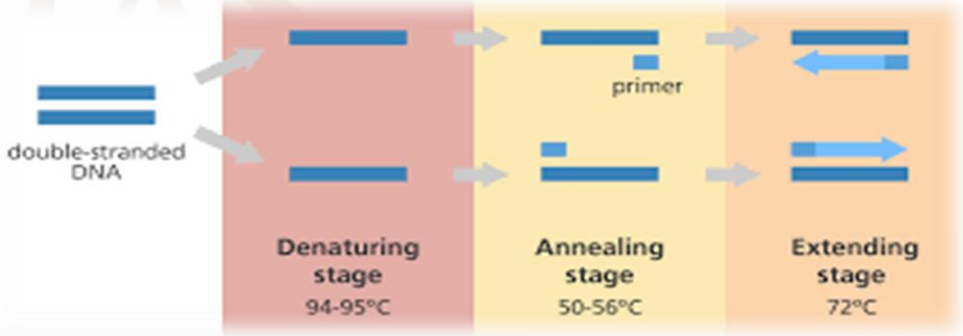
  The initial step is the denaturation or separation of the two strands of the DNA molecule. This is accomplished by heating the starting material to temperatures of about 95 °C (203 °F). Each strand is a template on which a new strand is built.

  Step 1: Denaturation by Heat

  Step 2: Annealing Primer to Target Sequence

  Step 3: Extension

  Step 4: End of the First PGR Cycle

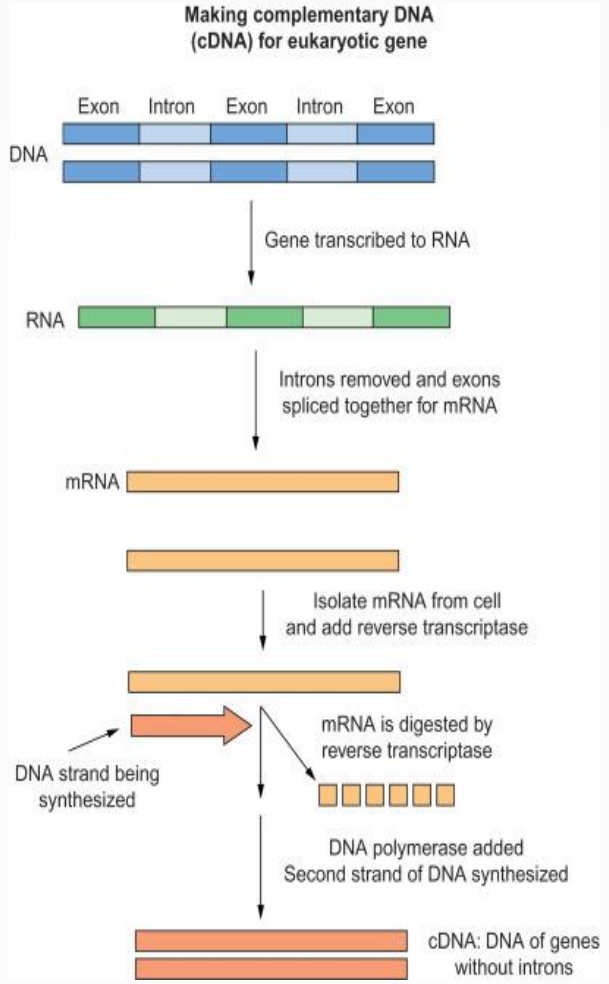


➢ USES

Polymerase chain reaction tests are used to detect HIV's genetic material, called RNA. These tests can be used to screen the donated blood supply and to detect very early infections before antibodies have been developed. This test may be performed just days or weeks after exposure to HIV.

DNA Probe Test

- A DNA probe test is based upon the principle of a nucleic acid hybridiza- tion reaction. This hybridization re- action can be defined as the formation of stable double-strand nucleic acid molecules from complementary single-strand molecules
- **<u>DETECTION METHODS</u>** • Radioactive labels• Non-radioactive label



https://www.sciencedirect.com/topics/immunology-and-microbiology/dna-probe

- USES :This methods uses nucleic acid hybridization with specifically labeled sequences to rapidly detect complementary sequences in the test sample.

- DIAGNOSTIC APPLICATIONS - Infectious Diseases,
  -Genetic Diseases: Sickle cell anemia, Huntington's disease, Alzheimer's Disease

- -Paternity and Forensic Testing

- DNA probes are used to detect mutation, chromosomal translocation, and detection of leukemia.

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THANK YOU