Methods of detection of virus

Three Approaches:

1. **Direct detection of virus**

• Electron Microscopy (Transmission Electron Microscopy).
• Antigen detection tests.
• Molecular Methods: PCR & Nucleic Acid Probes.

2. **Virus Isolation (Indirect detection)**

• Animal inoculation.
• Inoculation of eggs.
• *In vitro* Cell Culture.

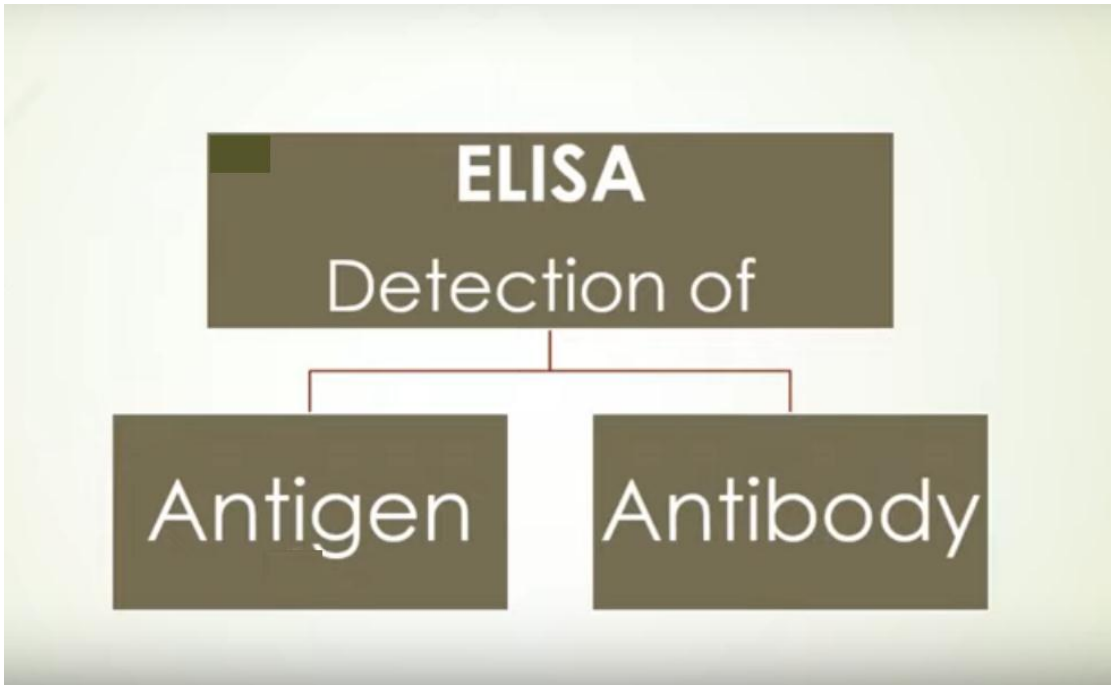
3. **Serology (Detection of Antibodies)**

Newer Techniques of Serology

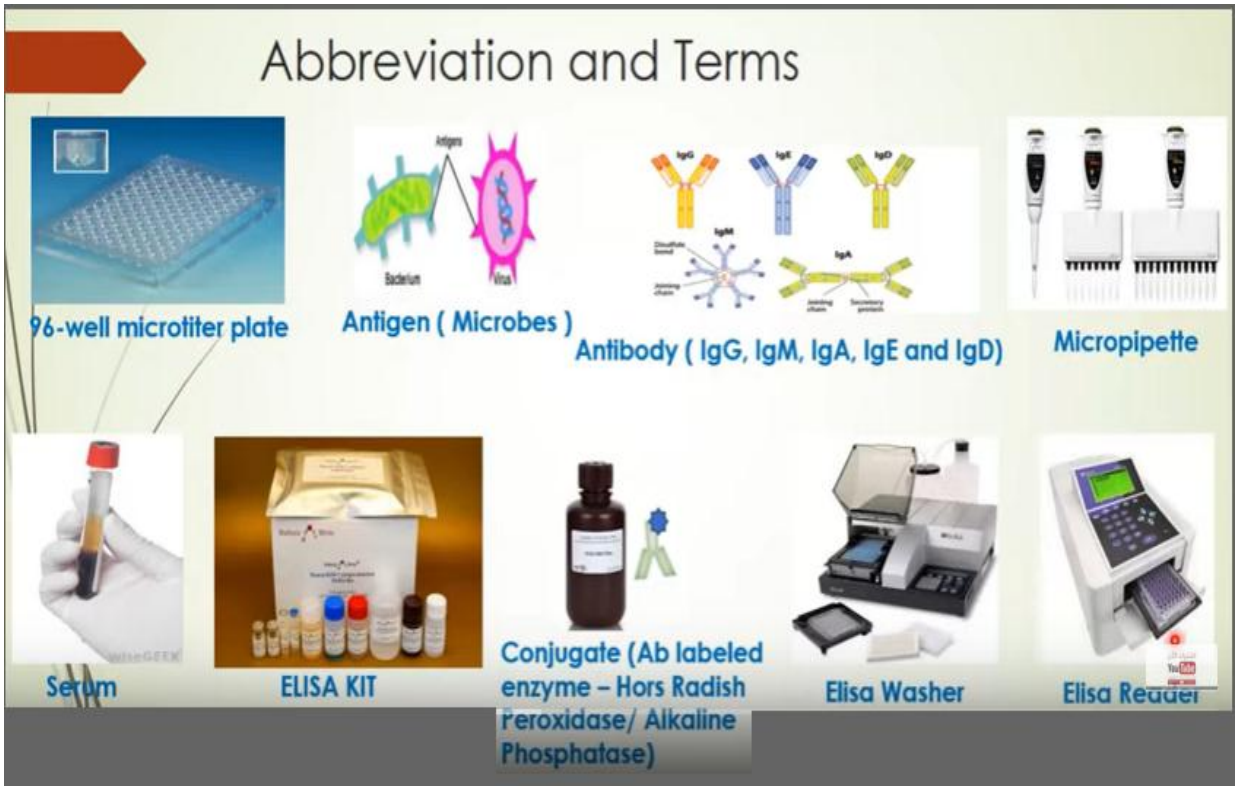
- Enzyme linked immunosorbent assay (ELISA).
- Particle agglutination**.**
- Radioimmunoassay (RIA).

ELISA

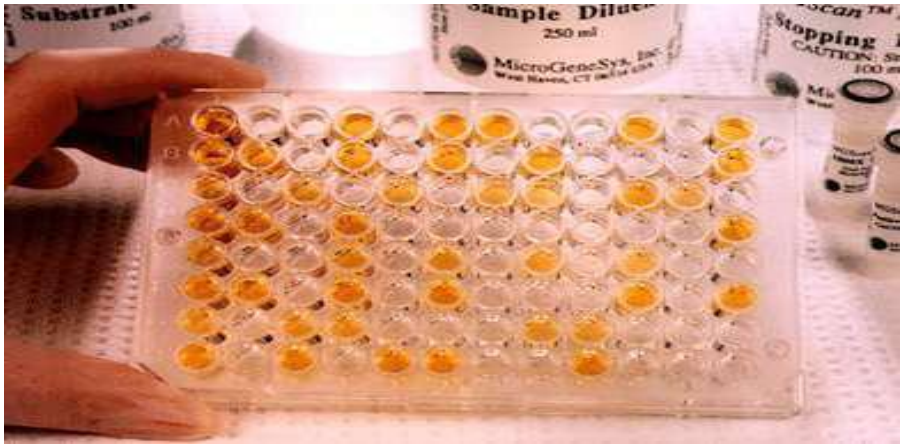
ELISA (enzyme-linked immunosorbent assay) is a method used to quantitatively detect an antigen within a sample. An antigen is a toxin or other foreign substance, for example a flu virus or environmental contaminant, which causes the vertebrate immune system to mount a defensive response. The range of potential antigens is vast, so ELISAs are used in many areas of research and testing to detect and quantify antigens in a wide variety of sample types. Cell lysates, blood samples, food items, and more can be analyzed for specific substances of interest using ELISAs.
There are four major types of ELISAs: direct, indirect, competitive and sandwich. Each type is described below with a diagram illustrating how the analytics and antibodies are bonded and used.



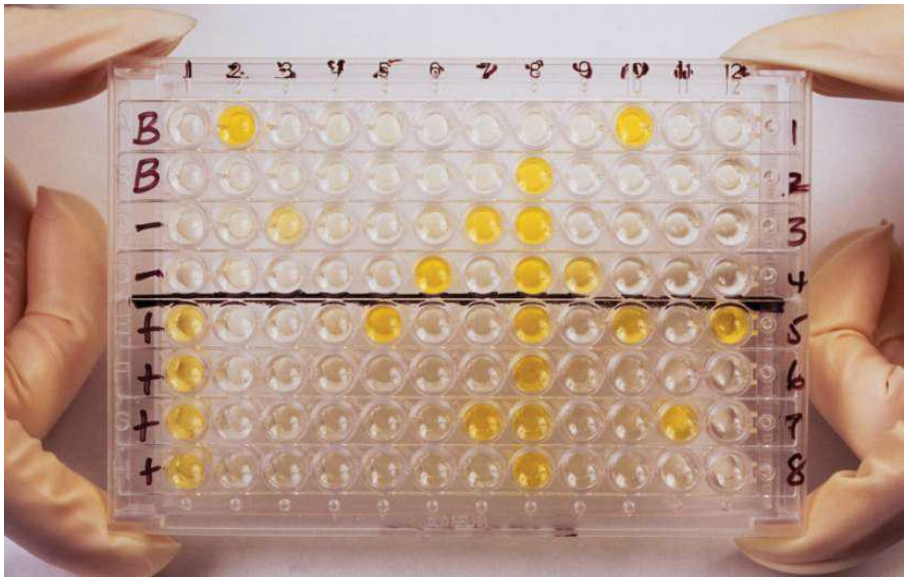
Materials needed in ELISA



ELISA for HIV antibody



Micro plate ELISA for HIV antibody: colored wells indicate reactivity



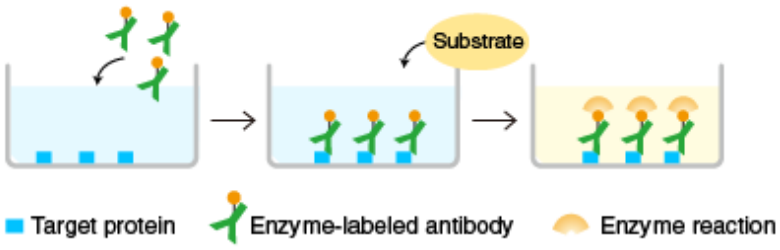
Types of ELISA

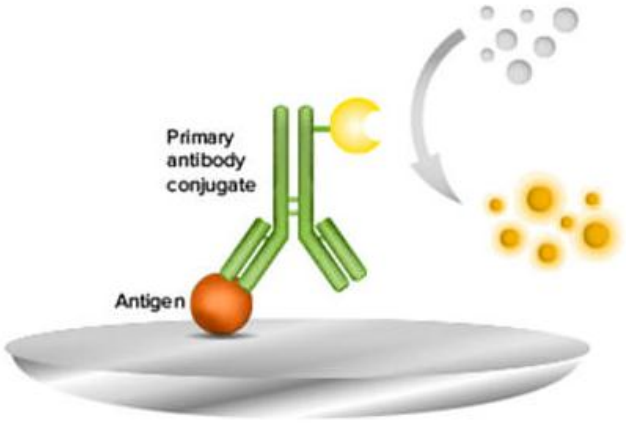
Frequently there are 4 types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Direct ELISA
2. Indirect ELISA
3. Sandwich ELISA
4. Competitive ELISA

Direct ELISA

In a direct ELISA, the antigen is bound to the bottom of the microplate well, and then it is bound by an antibody that is specific to the antigen and also conjugated to an enzyme or other molecule that enables detection.

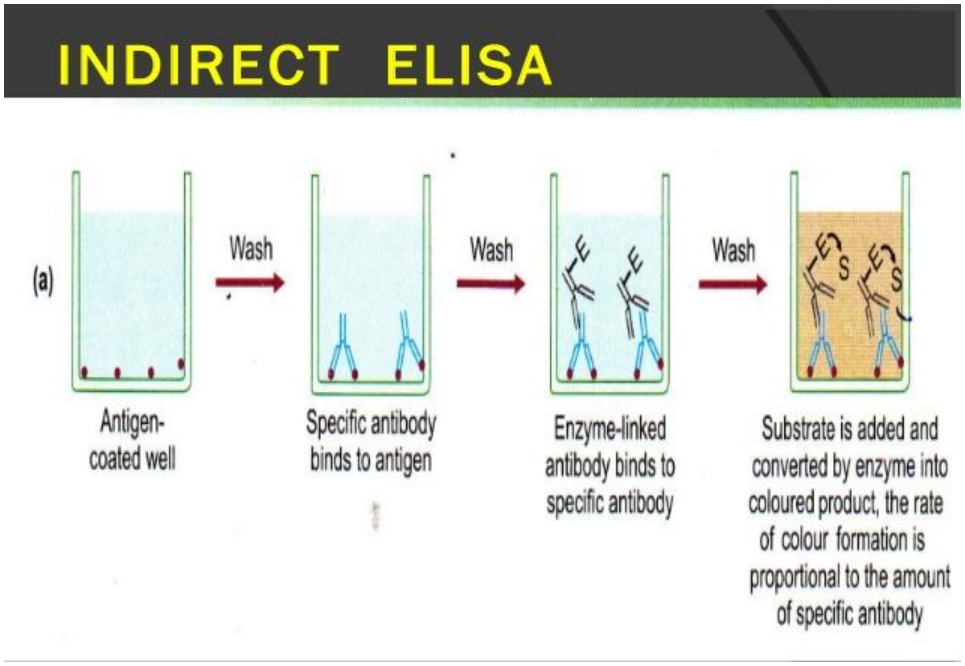


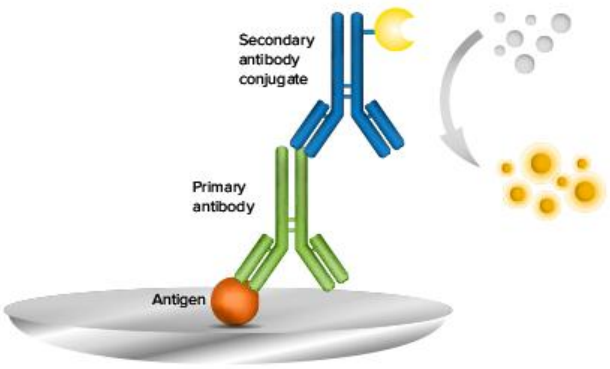


Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react

with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form colored products. The amount of colored end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.





Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.
3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.

4. Wash the plate, so that unbound antibody is removed.
5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6. Wash the plate, so that unbound enzyme-linked antibodies are removed.
7. Add substrate which is converted by the enzyme to produce a colored product.
8. Reaction of a substrate with the enzyme to produce a colored product.

Advantages

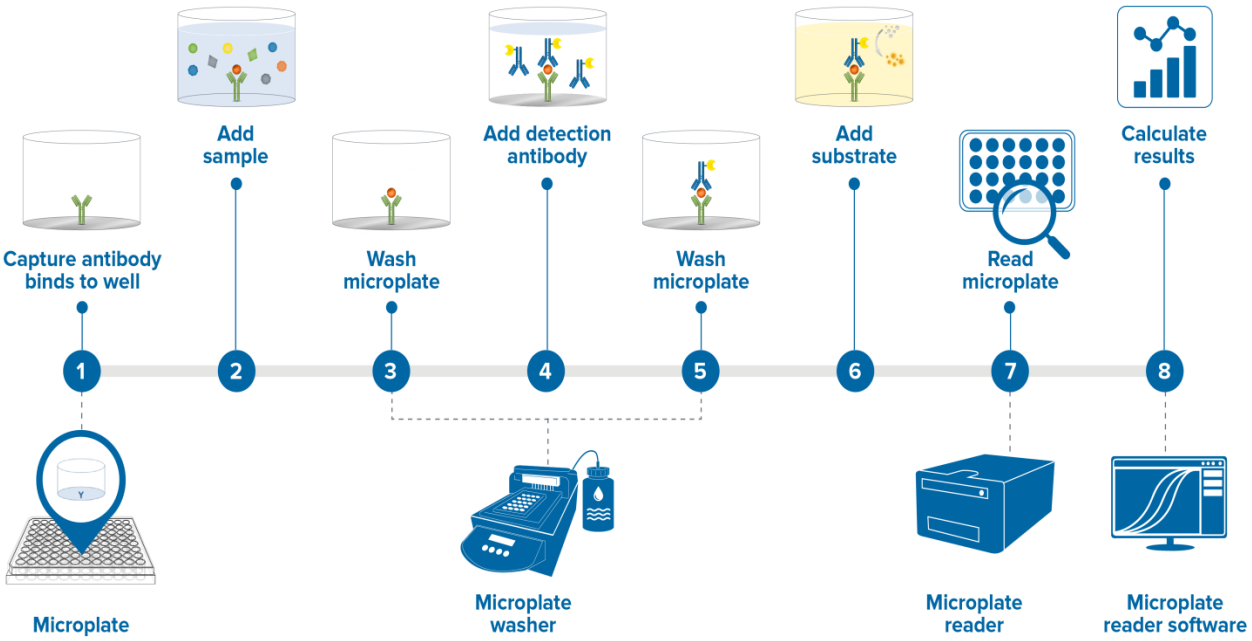
- Increased sensitivity, since more than one labeled antibody is bound per primary antibody.
- A wide variety of labeled secondary antibodies are available commercially.
- Maximum immunoreactivity of the primary antibody is retained because it is not labeled.
- Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection.
- Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody.
- Cost savings, since fewer labeled antibodies are required.
- Different visualization markers can be used with the same primary antibody.

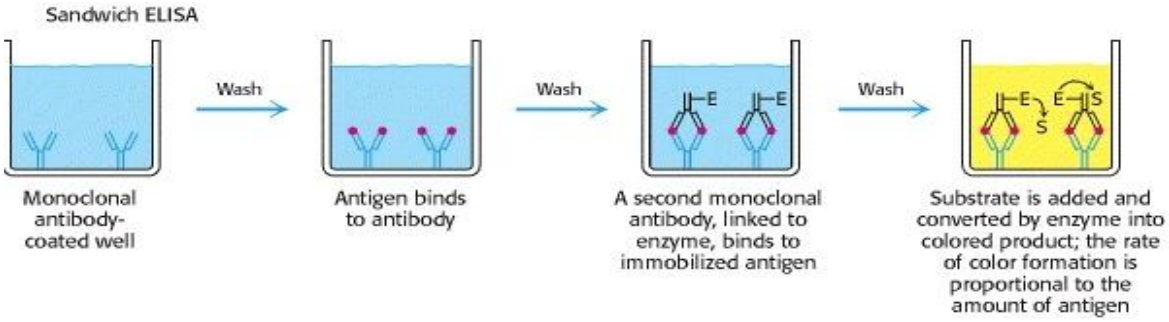
Disadvantages

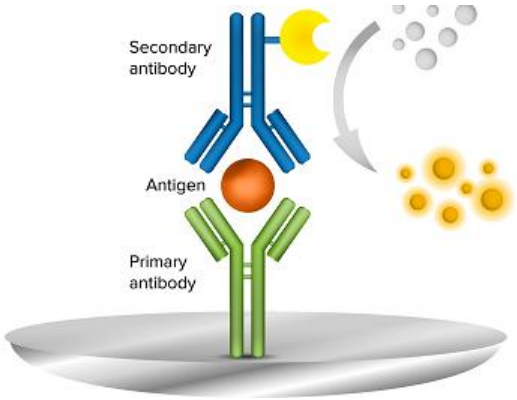
- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form colored products.


Capture antibody binds to well
Add sample
Wash microplate
Add detection antibody
Wash microplate
Add substrate
Read microplate
Calculate results
1
2
3
4
5
6
7
8
Microplate
Microplate washer
Microplate reader
Microplate reader software


Sandwich ELISA
Wash
Wash
Wash
Monoclonal antibody-coated well
Antigen binds to antibody
A second monoclonal antibody, linked to enzyme, binds to immobilized antigen
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen


Secondary antibody
Antigen
Primary antibody

2. Sandwich ELISA

Procedure of sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a colored product.
7. Reaction of a substrate with the enzyme to produce a colored product.

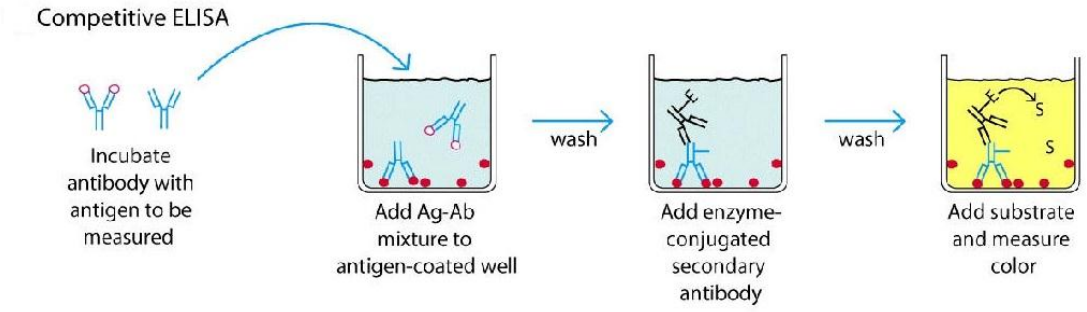
Advantages

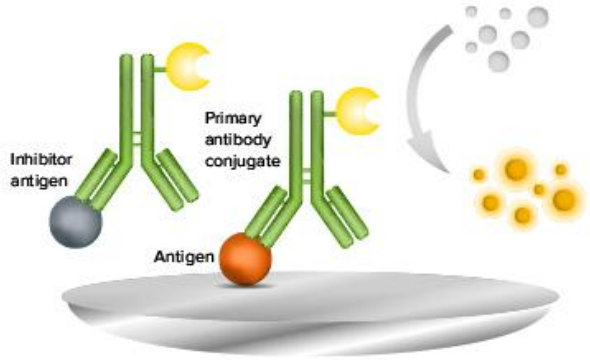
- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.
- Flexibility and sensitivity, since both direct and indirect detection methods can be used.

Competitive ELISA

This test is used to measure the concentration of an antigen in a sample.

In this test, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to the microtitre well which is coated with antigen. The more the antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. After the well is washed, enzyme conjugated secondary antibody specific for isotype of the primary antibody is added to determine the amount of primary antibody bound to the well. The higher the concentration of antigen in the sample, the lower the absorbance.





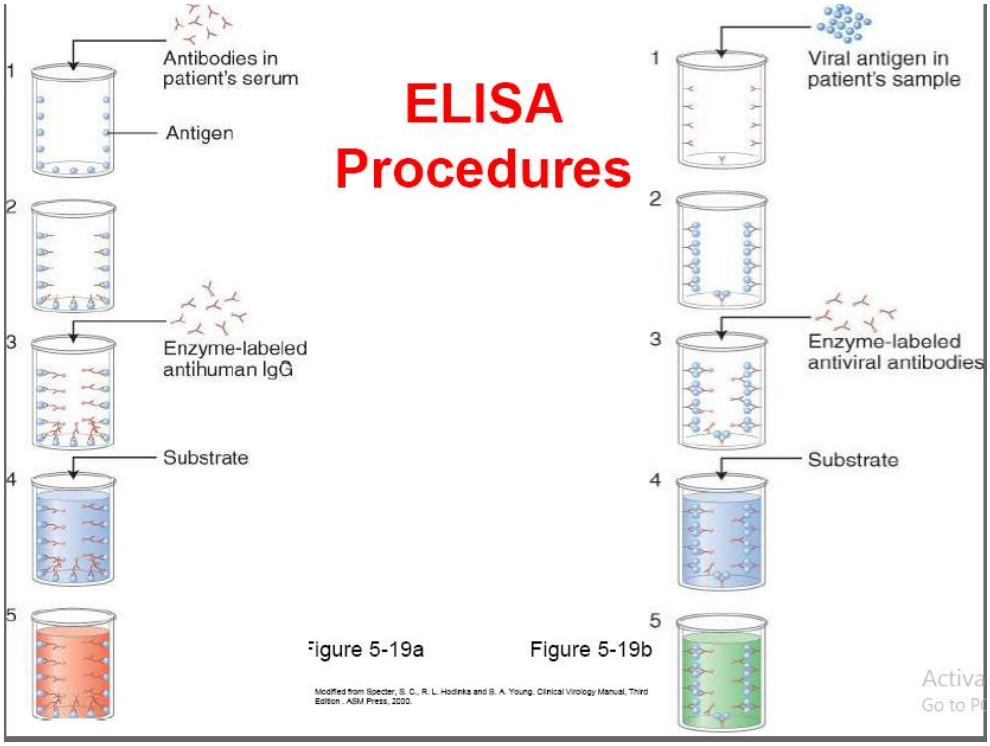
- **3. Competitive ELISA**

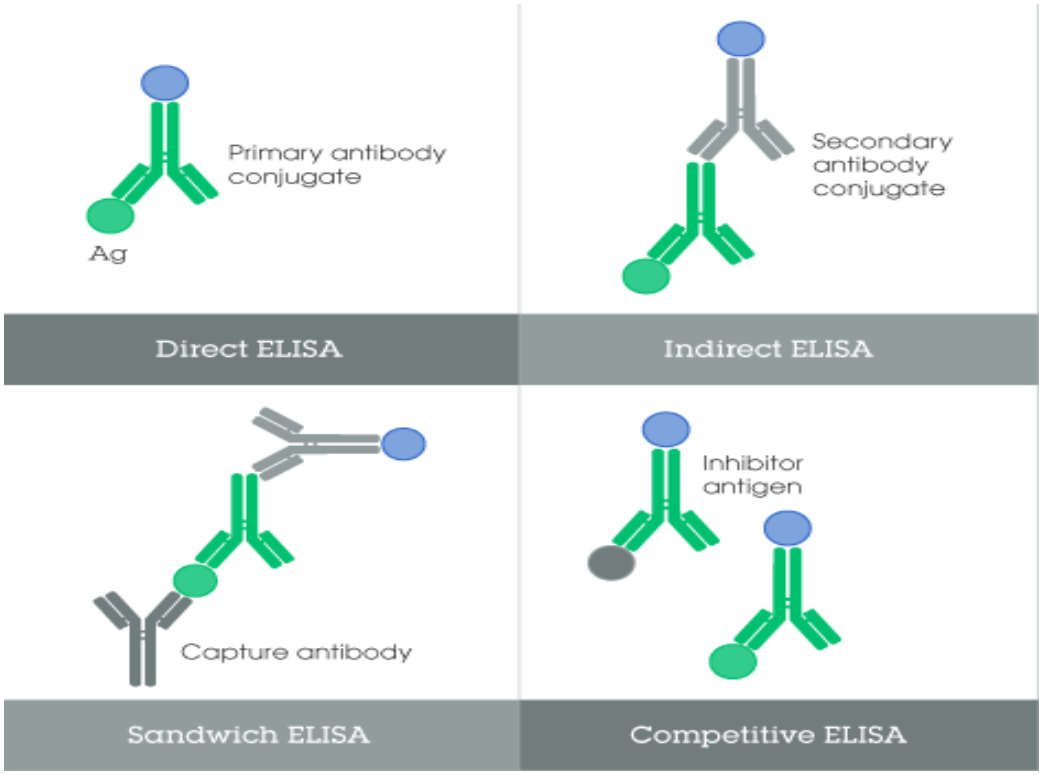
Procedure

1. Antibody is incubated with sample containing antigen.
2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.
3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.


ELISA Procedures
1
Antibodies in patient's serum
Antigen
2
3
Enzyme-labeled antihuman IgG
4
Substrate
5
Figure 5-19a
1
Viral antigen in patient's sample
2
3
Enzyme-labeled antiviral antibodies
4
Substrate
5
Figure 5-19b
Modified from Specter, S. C., R. L. Hodinka and S. A. Young. Clinical Virology Manual, Third Edition . ASM Press, 2000.


Primary antibody conjugate
Ag
Direct ELISA
Secondary antibody conjugate
Indirect ELISA
Capture antibody
Sandwich ELISA
Inhibitor antigen
Competitive ELISA