كلية المستقبل الجامعة / قسم تقنيات المختبرات الطبية

(التحليلات المرضية)

المرحلة الرابعة / المناعة العملي

المحاظرة السابعة

The enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is a common laboratory technique which is used to measure the concentration of an analytic (usually antibodies or antigens) in solution.

The basic ELISA, or enzyme immunoassay (EIA), is distinguished from other antibody-based assays because separation of specific and non-specific interactions occurs via serial binding to a solid surface, usually a polystyrene multiwell plate, and because quantitative results can be achieved.

The steps of the ELISA result in a **colored end** product which correlates to the amount of analytic present in the original sample.

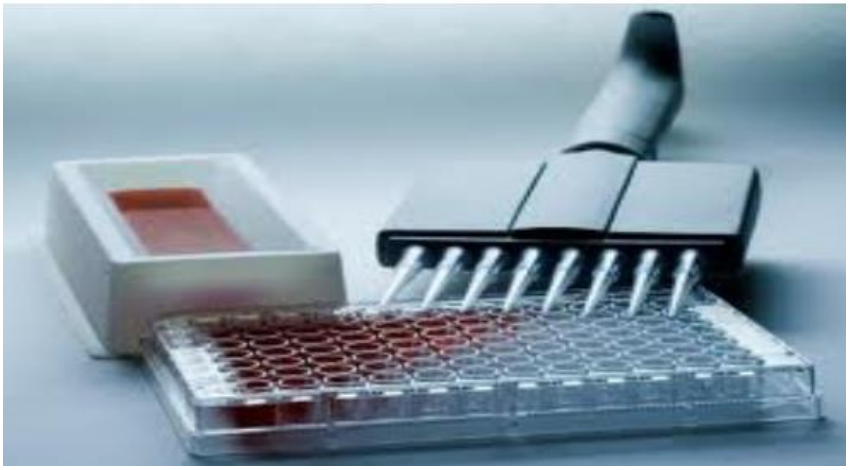
ELISAs are quick and simple to carry out, and since they are designed to rapidly handle a large numbers of samples in parallel, they are a very popular choice for the evaluation of various research and diagnostic targets. Figure 1 shows a typical ELISA result.

ELISAs were first developed in the early 1970s as a replacement for radioimmunoassays. An ELISA test may be used to diagnose:

- **-HIV, which causes AIDS**
- **-Lyme disease**
- **-Rotavirus.**
- **-Squamous cell carcinoma**
- **-Syphilis**
- **-Toxoplasmosis**
- **-Varicella-zoster virus, which causes chicken pox and shingles**

Basic ELISA Procedure:-

ELISAs begin with a coating step, where the first layer - either an antigen or an antibody - is adsorbed to a polystyrene 96 well plate. Since the assay uses surface binding for separation, several washes are repeated between each ELISA step to remove unbound materials. During this process it is essential that excess liquid is removed in order to prevent the dilution of the solutions added in the next stage. For greatest consistency specialized plate washers are used.



Four Typical ELISA Formats:-

The ELISA provides a wealth of information in its simplest formats, but it can also be used in more complex versions to provide enhanced signal, more precise results, or if certain reagents are not available. The four typical ELISA formats are described briefly below.

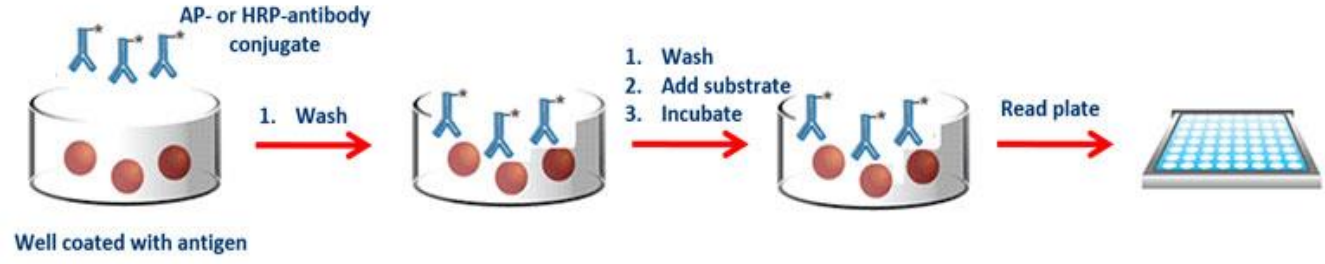
1- Direct ELISA:-

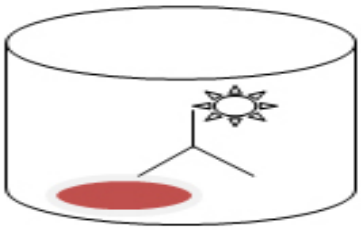
In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA

This type of ELISA has two main advantages:

1-It is faster, since fewer steps are required

2-It is less prone to error, since there are fewer steps and read so are not easily reusable.





2-Indirect ELISA:-

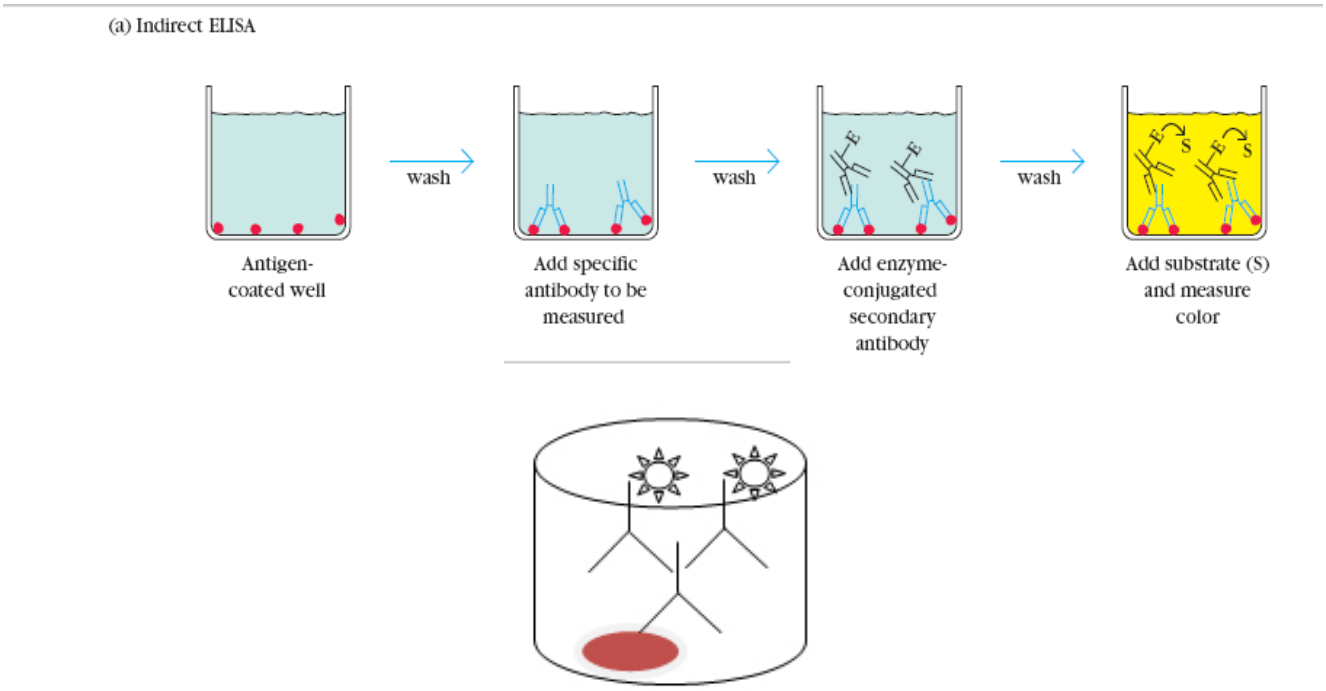
Antigen coated to a polystyrene multiwell plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species antibody and is often polyclonal.

This method has several advantages:

1-Increased sensitivity, since more than one labeled antibody is bound per primary antibody

2-Flexibility, since different primary detection antibodies can be used with a single labeled secondary antibody

3-Cost savings, since fewer labeled antibodies are required.



3-Sandwich ELISA:-

Sandwich ELISAs are the most common type of ELISA. Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.

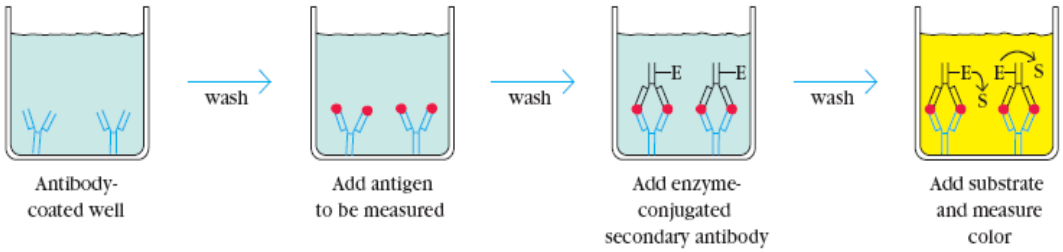
This type of assay has several advantages:

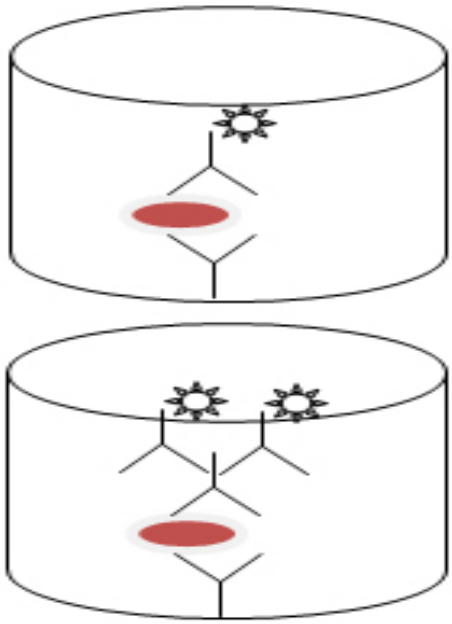
1-High specificity, since two antibodies are used the antigen/analyte is specifically captured and detected

2-Suitable for complex samples, since the antigen does not require purification prior to measurement

3-Flexibility and sensitivity, since both direct and indirect detection methods can be used.

(b) Sandwich ELISA





4-Competition or Inhibition ELISA:-

- Principle of the test is that two specific antibodies, one conjugated with enzyme and the other present in test serum (if serum is positive for antibodies), are used.
- Competition occurs between the two antibodies for the same antigen.
- Appearance of color indicates a negative test (absence of antibodies), while the absence of color indicates a positive test (presence of antibodies).
- The central event of competitive ELISA is a competitive binding process executed by original antigen (sample antigen) and add-in antigen.
- The procedures of competitive ELISA are different in some respects compared with other forms of ELISA.

ELISA Results:-

The ELISA assay yields three different types of data output:

1-Quantitative:-

ELISA data can be interpreted in comparison to a standard curve (a serial dilution of a known, purified antigen) .

2-Qualitative:-

ELISAs can also be used to achieve a yes or no answer indicating whether a particular antigen is present in a sample, as compared to a blank well containing no antigen or an unrelated control antigen.

Sensitivity

ELISAs are one of the most sensitive immunoassays available. The typical detection range for an ELISA is 0.1 to 1 of ml or 0.01 ng to 0.1 ng, with sensitivity dependent upon the particular characteristics of the antibody –antigen interaction.