AL-Mustaqbal University Collage.
Department of Pathological Analysis Technique.
Subject:- Advanced laboratory techniques.
Lecture-No. 12.
ELISA

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.

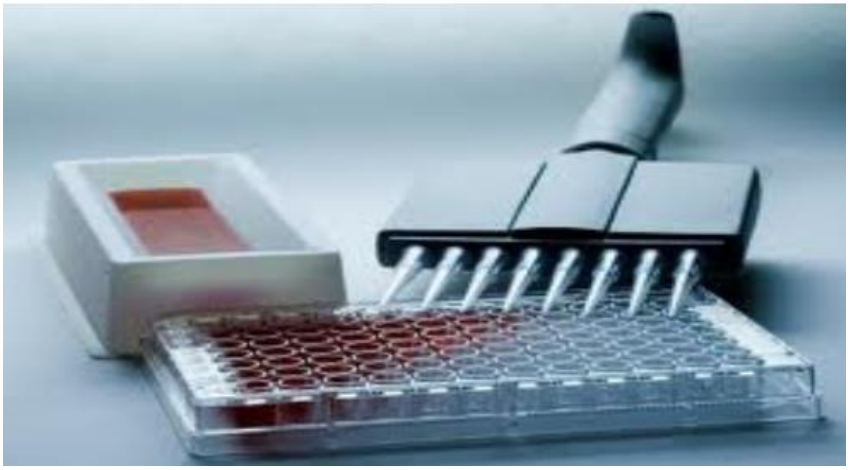
ELISAs are quick and simple to carry out, and since they are designed to rapidly handle a large numbers of samples in parallel, they are a very popular choice for the evaluation of various research and diagnostic targets. Figure 1 shows a typical ELISA result.

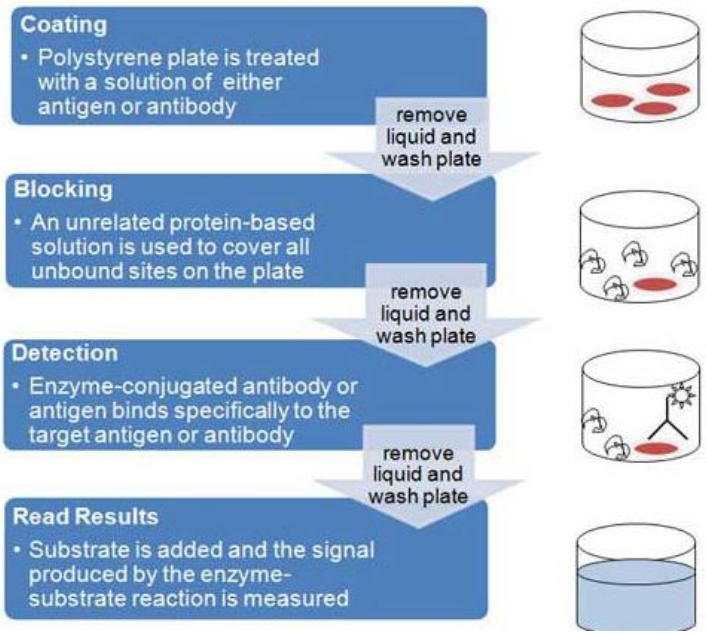
ELISAs were first developed in the early 1970s as a replacement for radioimmunoassays. They remain in wide use in their original format and in expanded formats with modifications that allow for multiple analyzes per well, highly sensitive readouts, and direct cell-based output. An ELISA test may be used to diagnose:

- **-HIV, which causes AIDS**
- **-Lyme disease**
- **-pernicious anemia**
- **-Rocky Mountain spotted fever (RMSF)**
- **-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.
ELISAs can be quite complex, including various intervening steps and the ability to measure protein concentrations in heterogeneous samples such as blood. The most complex and varying step in the overall process is detection, where multiple layers of antibodies can be used to amplify signal.





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.

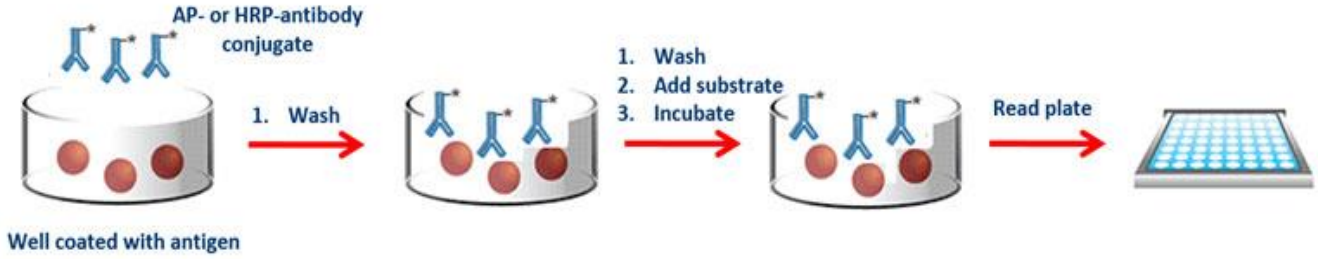
1- Direct ELISA:-

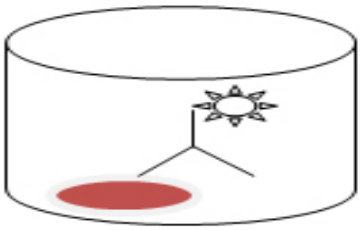
An antigen coated to a multiwell plate is detected by an antibody that has been directly conjugated to an enzyme. This can also be reversed, with an antibody coated to the plate and a labeled antigen used for detection, but the second option is less common.

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.



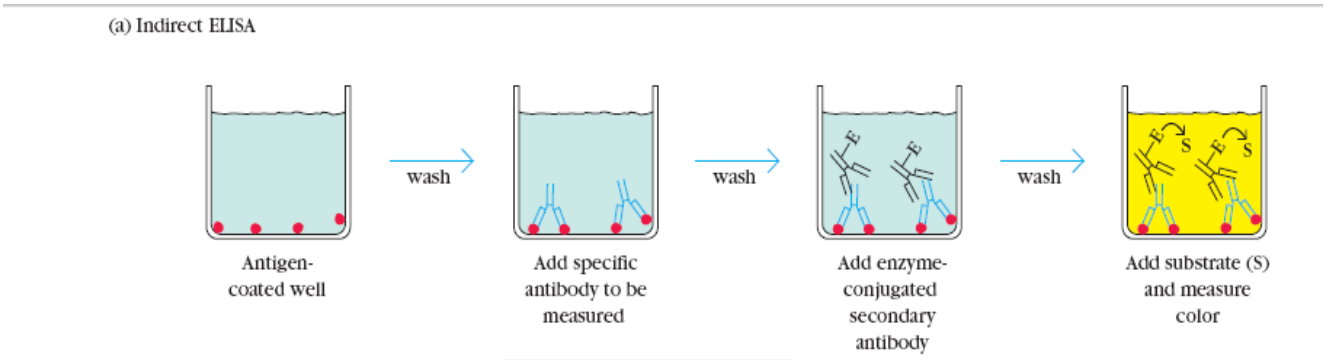


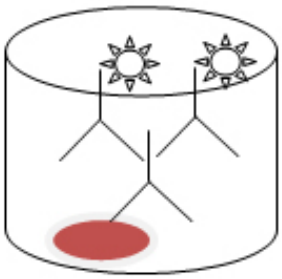
2-Indirect ELISA:-

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 typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule.
The first antibody, termed the capture antibody, is coated to the polystyrene plate. Next, the analyte or sample solution is added to the well.
A second antibody layer, the detection antibody, follows this step in order to measure the concentration of the analyte.
Polyclonals can also be used for capture and/or detection in a sandwich ELISA provided that variability is present in the polyclonal to allow for both capture and detection of the analyte through different epitopes.
If the detection antibody is conjugated to an enzyme, then the assay is called a direct sandwich ELISA. If the detection antibody is

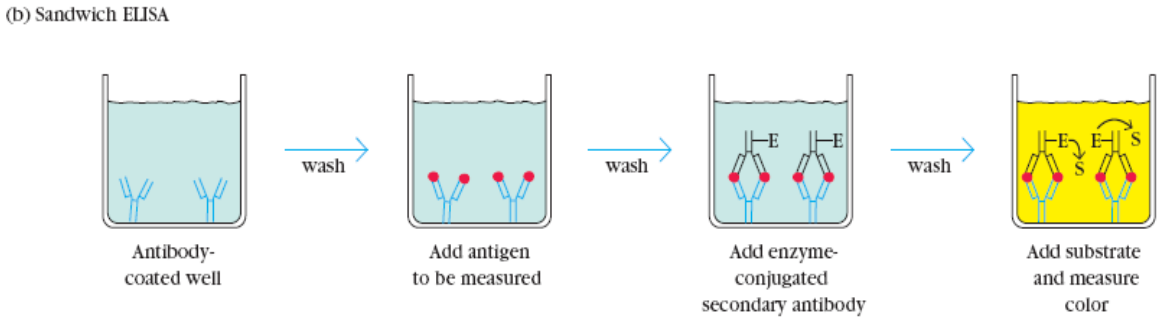
unlabeled, then a second detection antibody will be needed resulting in an indirect sandwich ELISA.

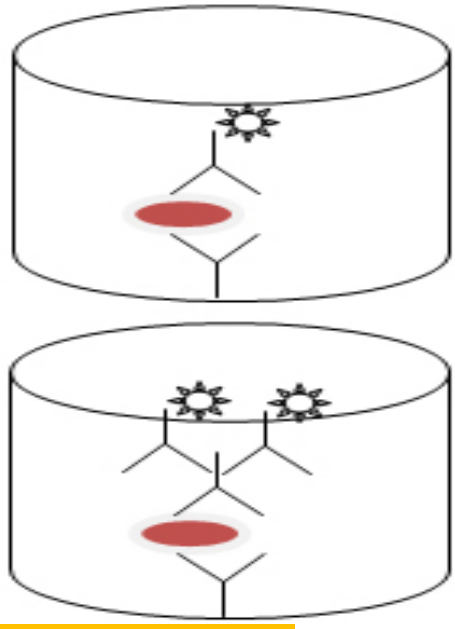
This type of assay has several advantages:

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.



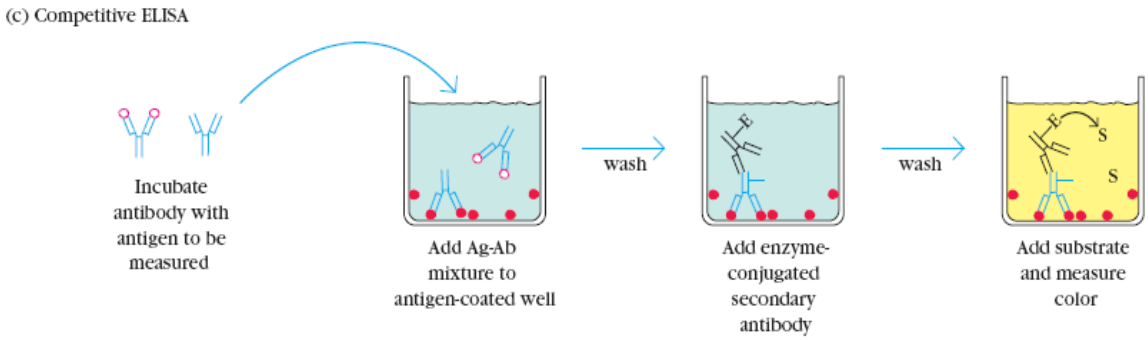


4-Competition or Inhibition ELISA:-

This is the most complex ELISA, and is used to measure the concentration of an antigen (or antibody) in a sample by observing interference in an expected signal output.

It is most often used when only one antibody is available to the antigen of interest or when the analyte is small, i.e. a hapten, and cannot be bound by two different antibodies.

Therefore, with a competition ELISA, one is actually measuring antigen concentration by noting the extent of the signal reduction. If the detection antibody is labeled, then this would be a direct competition ELISA and if unlabeled, then this would be an indirect competition ELISA.



ELISA applications

-Its sensitivity allows the assay of nanogram amounts without great difficulty

-Enzymoimmunoassays for antimicrobial antibodies, antigen detection, and for hormone and drug quantitation have been successfully developed and commercialized.

-Rapid diagnosis EIA have been developed and successfully introduced

-pregnancy diagnosis capture the antigen (hCG).

-diagnosis of infectious diseases, including streptococcal sore throat, respiratory syncytial virus infections, viral influenza, HIV infection (not approved for use in the U.S.), etc.

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) in order to precisely calculate the concentrations of antigen in various samples.

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.

3-Semi-quantitative:

ELISAs can be used to compare the relative levels of antigen in assay samples, since the intensity of signal will vary directly with antigen concentration.

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.