ELISA

Types

ELISA Types

- ELISA (enzyme-linked immunosorbent assay) is the most widely used technology in enzyme immunoassay technology. Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries, such as ELISA application in food industry.

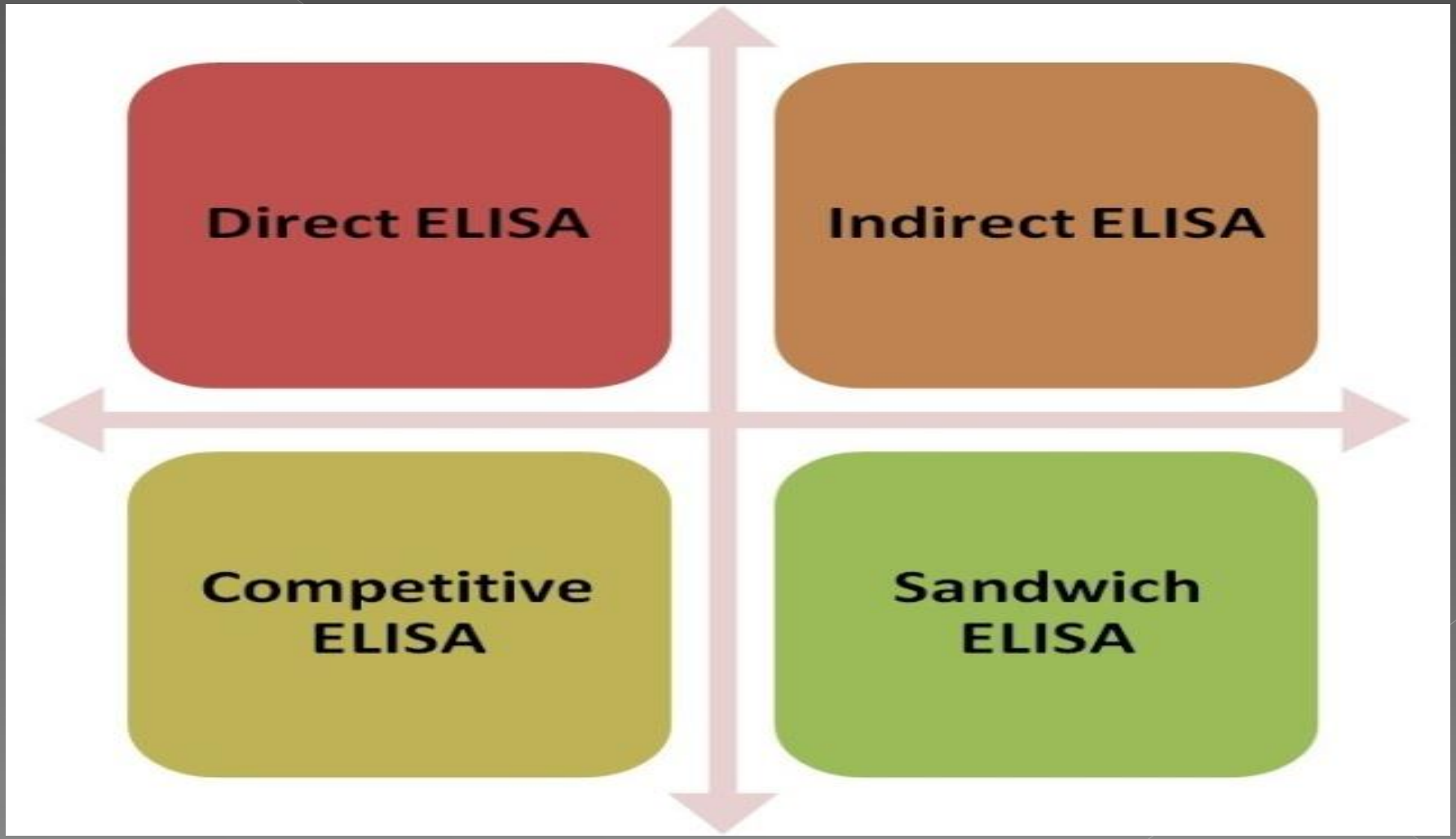
ELISA Principle

- Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs 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.

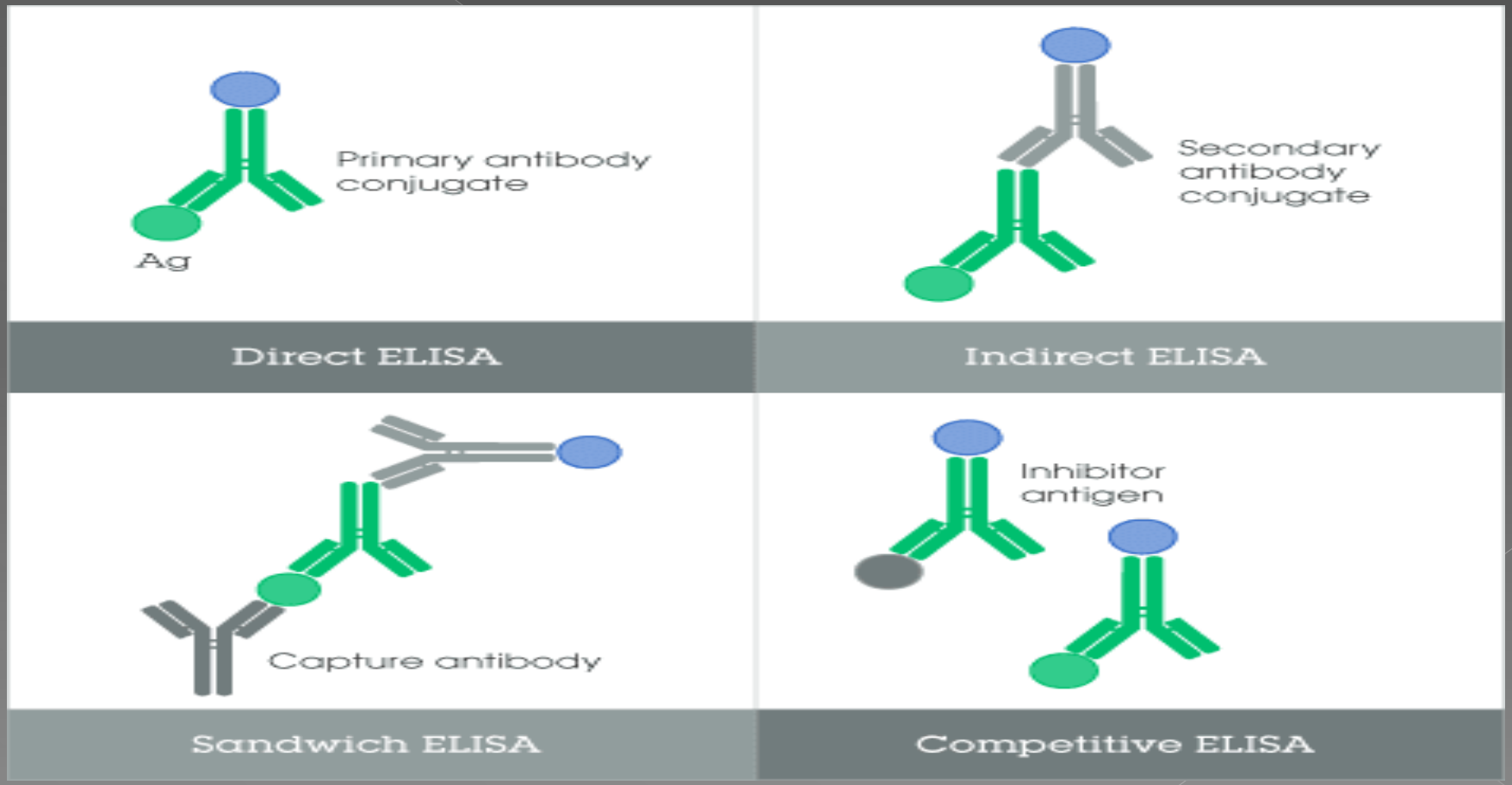
A general ELISA is a five-step procedure:

1) coat the microtiter plate wells with antigen;
2) block all unbound sites to prevent false positive results;
3) add primary antibody (e.g. rabbit monoclonal antibody) to the wells;
4) add secondary antibody conjugated to an enzyme (e.g. anti-mouse IgG);
5) reaction of a substrate with the enzyme to produce a colored product, thus indicating a positive reaction.

ELISA Types


Direct ELISA
Indirect ELISA
Competitive ELISA
Sandwich ELISA

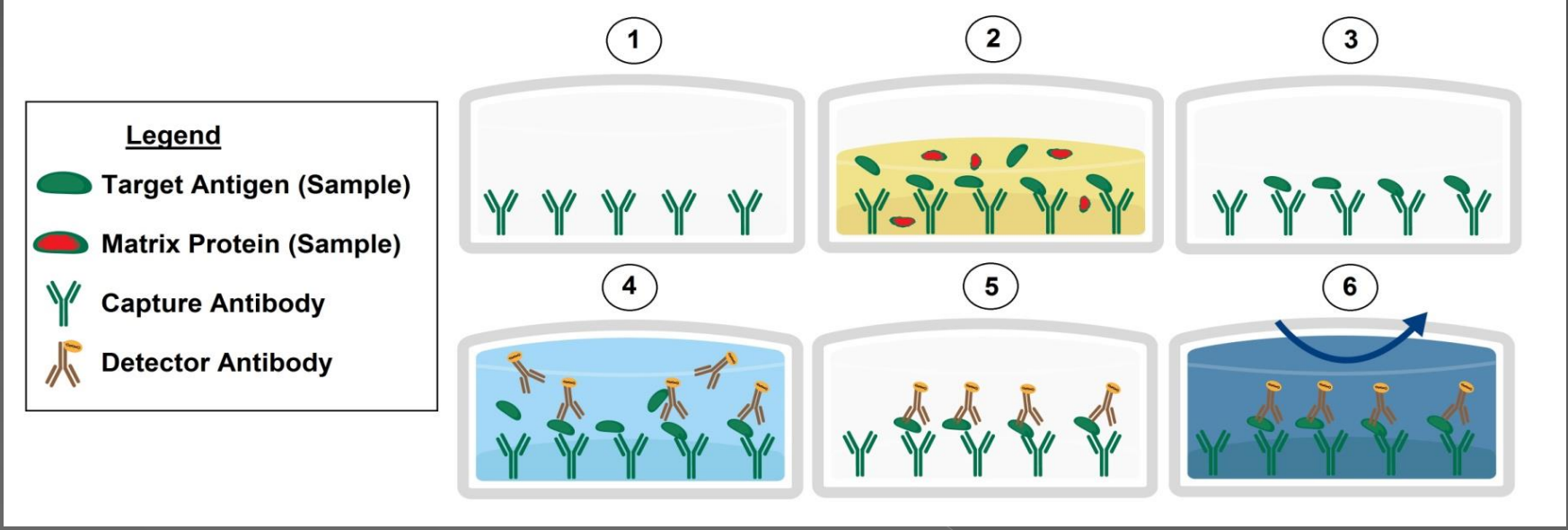
The different types of ELISA (direct, indirect, sandwich, and competitive)



Sandwich

- An immobilized capture antibody first binds the target protein analyte. Subsequently a detector antibody in liquid phase will bind ("sandwich") the capture antibody immobilized target analyte. Detection of the analyte is achieved with catalysis of a substrate mediated by an enzyme conjugated to the detector antibody or a secondary detector complex. Detection output is proportional to the quantity of analyte in the test sample.
- Sandwich ELISA (or sandwich immunoassay) is the most commonly used format. This format requires two antibodies specific for different epitopes of the antigen. These two antibodies are normally referred to as matched antibody pairs. One of the antibodies is coated on the surface of the multi-well plate and used as a capture antibody to facilitate the immobilization of the antigen. The other antibody is conjugated and facilitates the detection of the antigen.

Sandwich



1. Micro-plate wells are pre-coated with capture antibody and blocked.
2. Sample is incubated in the wells, where the target sample analyte is bound by the immobilized capture antibody.
3. Wells are washed to remove unbound non-specific sample matrix.
4. A detector antibody, typically conjugated to an enzymatic reporter molecule (ex. HRP) or an affinity molecule (ex. Biotin, where a secondary detector complex is used) is incubated in the wells where it binds to the captured analyte.
5. Wells are again washed to remove unbound detector antibody.
6. Colorimetric, fluorescent or luminescent detection is then performed. The enzymatic reporter (conjugated to the detector antibody or to a secondary detector complex added in an additional step) catalyzes a detection substrate producing a signal which is proportional to the quantity of analyte.

Direct ELISA

- The antigen is immobilized to the surface of the multi-well plate and detected with an antibody specific for the antigen and directly conjugated to HRP or other detection molecules.

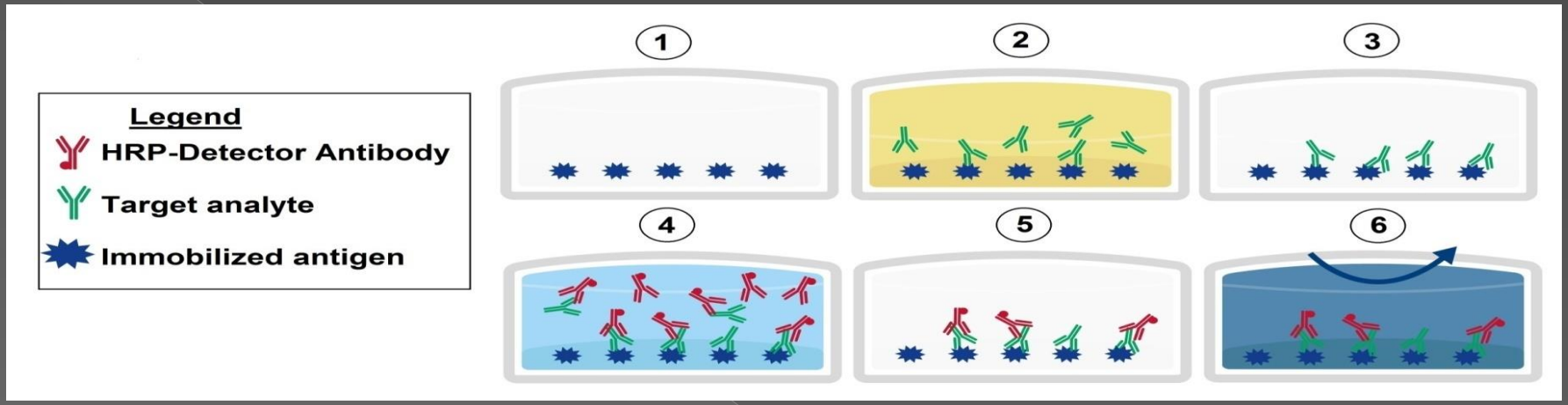
Sandwich

Advantages	Drawbacks
• Highly specific, requires binding of two antibodies	
• Capable of accurate quantitative measurements	• Complex and longer duration protocol (Shortened protocols exist, but only with highly developed assays due to additional non-specific interactions)
• Broad dynamic range of detection	
• Very sensitive - secondary detection reagents provide detection signal amplification	• More expensive due to additional reagents and complex assay development
• Specificity - High sensitivity facilitates larger sample dilution, mitigating interference factors	• Assay technique can greatly influence, poor technique yields less adequate data, exacerbated by numerous assay processing steps
• Good reproducibility and assay recovery (Provided good technique is employed)	• May not work for small molecules or antigens without sufficient binding epitopes for two antibodies
• Common for, but not limited to, typical protein antigen measurements	

Indirect (or Reverse-Phase)

- Specific target antigen is coated in wells. The immobilized antigen acts as "bait" where the assay target analyte then binds and is subsequently detected. Enzymatic detection output is proportional to the quantity of analyte in the test sample.
- Similar to direct ELISA assays, the antigen is immobilized to the surface of the multi-well plate. However, a two-step process is required for detection whereby a primary antibody specific for the antigen binds to the target, and a labeled secondary antibody against the host species of the primary antibody binds to the primary antibody for detection. The method can also be used to detect specific antibodies in a serum sample by substituting the serum for the primary antibody.

Indirect (or Reverse-Phase)



1. Micro-plate wells are pre-coated with target specific antigen ("bait") and blocked.
2. Samples are incubated in the wells, where target analyte in the sample binds the immobilized target antigen.
3. Wells are washed to remove unbound non-specific sample matrix.
4. An analyte specific detector antibody conjugated to a reporter (ex. HRP) or affinity molecule (ex. Biotin, where a secondary detector is used) is incubated and binds the target analyte.
5. Wells are washed to remove unbound detector antibody.
6. Colorimetric, fluorescent or luminescent detection is then performed. The enzymatic reporter (conjugated to the detector antibody or to a secondary detector complex added in an additional step) catalyzes a detection substrate producing a signal which is proportional to the quantity of analyte.

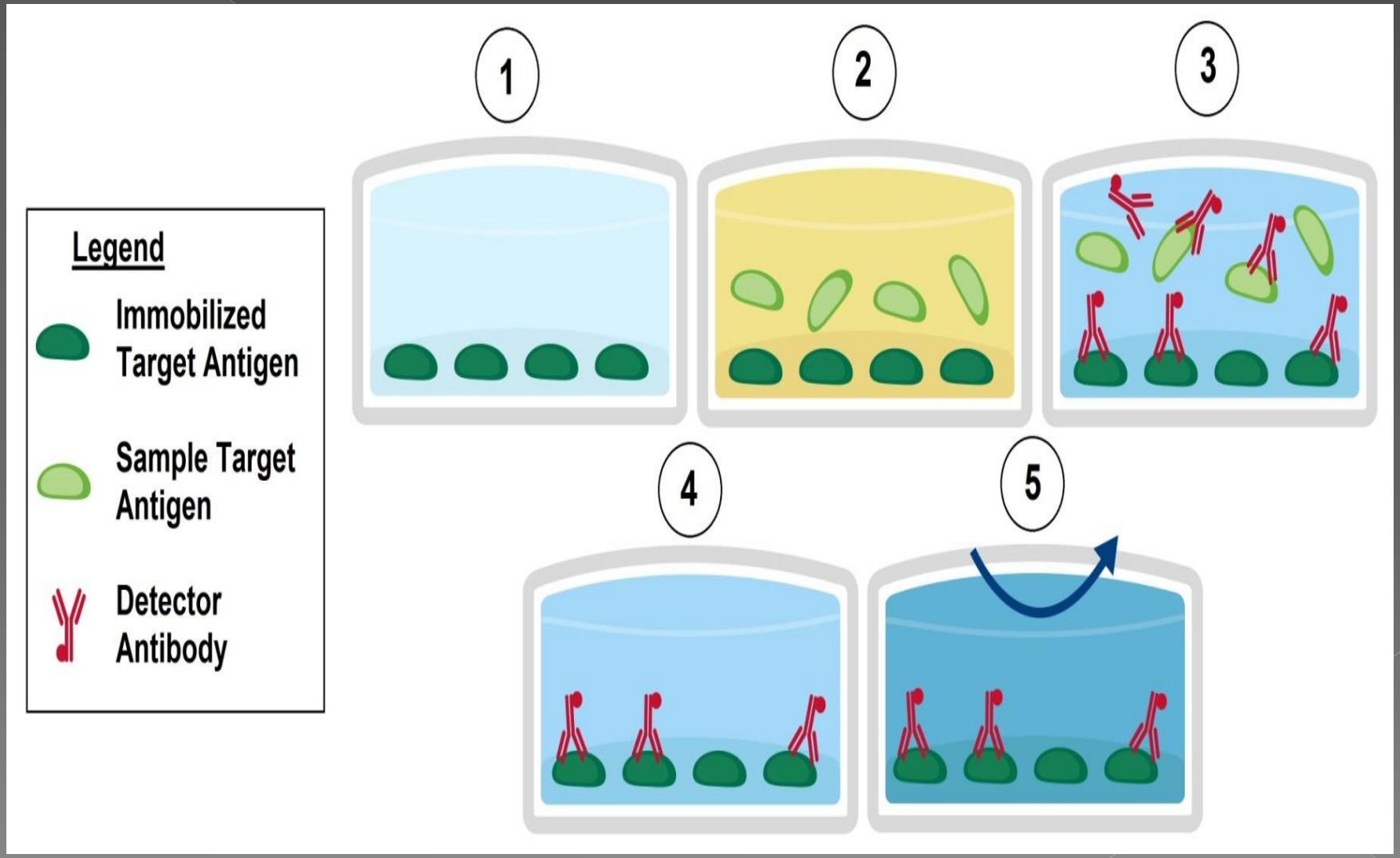
Indirect (or Reverse-Phase)

Advantages	Drawbacks
• Shorter assay protocol, simple method provides a robust assay, less dependent on technique	
• Less reagent cost	• Specificity dependent on immobilized "bait" antigen
• Good for quantitative measurements of samples containing relatively abundant analyte	• Shorter dynamic range, more suited to qualitative measurements
• Good reproducibility from small number of assay processing steps	• Average sensitivity, although novel amplification methods are available with extra cost and development
• Flexibility from common detection reagent set	
• Common for, but not limited to, immune response or other infectious disease measurements	

Competitive

- Typically a variation of indirect (or reverse-phase) type assays wherein target analyte in samples competes for detector antibody binding with immobilized target analyte. In some cases a target analyte competes for binding in solution with a labeled standard for immobilized capture antibody binding sites. In either orientation, the method produces an inhibition of the detector and the output signal is inversely proportional to the quantity of analyte in the test sample.
- Also known as inhibition ELISA or competitive immunoassay, this assay measures the concentration of an antigen by detection of signal interference. Each of the previous formats can be adapted to the competitive format. The sample antigen competes with a reference antigen for binding to a specific amount of labeled antibody. The reference antigen is pre-coated on a multi-well plate. The sample is pre-incubated with labeled antibody and added to the wells. Depending on the amount of antigen in the sample, more or less free antibodies will be available to bind the reference antigen. This means the more antigen there is in the sample, the less reference antigen will be detected and the weaker the signal. Some competitive ELISA kits use labeled antigen instead of a labeled antibody. The labeled antigen and the sample antigen (unlabeled) compete for binding to the primary antibody. The lower the amount of antigen in the sample, the stronger the signal due to more labeled antigen in the well.

Competitive



Competitive

Advantages	Drawbacks
• Typically shortest and easiest assay protocol, simple method provides a very robust assay	• Development can be difficult to optimize competitve assay components
• Very low reagent costs	• Shortest dynamic range and provides primarily qualitatitive measurements
• Good reproducibility from small number of assay processing steps	• Lowest level of sensitivity, although novel amplification methods are available with extra cost and development
• Common for, but not limited to, small molecule and bio-chemical measurements	

References

- https://www.abcam.com
- https://www.sinobiological.com
- https://www.bio-rad
- https://www.thermofisher.com