Online Training Curriculum

Confirmation Methods for Food contaminants

Principles of ELISA

Introduction to the variations of ELISA-Based formats

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ELISA was first described by Engvall and Perlmann in 1971 [1]. This assay was developed to find an alternative to radioimmunoassay (RIA) du to potential safety and health problems coming with the initiation of RIA. In the 1970s, several research teams developed ELISA assays for the clinical diagnostic by targeting microorganisms and, in the early 1980s, assays were developed to allow the detection of peptides, hormones [2].

As the structure and the characteristics of the targeted analytes are not always the same, several ELISA assays have been developed progressively to optimize increase the specificity of measurement. Depending on the antigen-antibody combination, the assay is called direct ELISA, indirect ELISA, sandwich ELISA or competitive ELISA. Multiplex methods (detection of several analytes in one test) are still under development and current assay are limited to few clinical applications using a completely different technology.

Direct ELISA

Direct ELISA was simultaneously developed in 1971 by Engvall and Perlmann and by Van Weemen and Schuurs. Direct ELISA method is suitable for determining the amount of high molecule-weight antigens. 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 an enzyme to allow quantification.

The steps of direct ELISA follow the mechanism below:

- A buffered solution of the targeted antigen is added to each well (usually 96-well plates) of a microtiter plate, where it is given time to adhere to the plastic. Then, the plate is washed.
- A solution of nonreacting protein, such as bovine serum albumin, casein or fish gelatin, is added to each well in order to cover any plastic surface in the well which remains uncoated by the antigen. Then, the plate is washed.
- The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well. Then, the plate is washed.
- A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.

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- The higher the concentration of the primary antibody present in the serum, the stronger the color change. A plate reader is used to give quantitative values of the optical density.

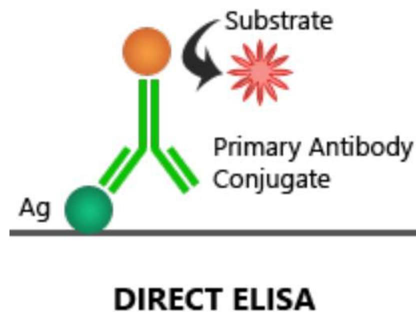

Figure 1: Schematic Direct ELISA [4]

Finally, the direct ELISA technique is typically used when the immune response to an antigen needs to be analyzed.

Advantages:

Direct ELISA was developed in order to take place of radioimmunoassay and avoid potential health issues. It is the pioneer technique of ELISA assay and is a quick method compared to other ELISA tests. Another limited advantage is that there is no cross-reactivity from the secondary antibody as no secondary antibody is used in direct ELISA but this specificity also brings limitations.

Disadvantages:

A major disadvantage of the direct ELISA is that the method of antigen immobilization is not specific. When a complex sample is used as the source of antigen, all proteins in the sample may adsorb on the microtiter plate well. If the concentration of the targeted analyte is low in sample, it may be possible that it will not adsorb on the plate surface du to the competition of other proteins. The sandwich or indirect ELISA provides a solution to this issue, by using a "capture" antibody specific for the tested antigen to pull it out of the mixture. A second consequence of this lack of specificity is the potential high background as all proteins bind to the microplate surface

Another disadvantage is that the primary antibody must be conjugated, which is more expensive than for secondary antibody. In addition, the bound between the enzyme and the antibody may influence

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the immunoreactivity of the antibody, reducing the sensitivity of the assay. It should be noted that not all the primary antibodies can possibly be enzyme labeled, thus this type of the assay is limited.

Indirect ELISA

The technique was developed in 1978 by Lindström and Wager, who were inspired by the direct ELISA method [5]. Indirect ELISA is a two-step ELISA involving two binding process of primary antibody and labeled secondary antibody. Like for direct ELISA assays, the antigen is immobilized to the surface of the microplate. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody, in a two-step process. However, this may lead to nonspecific signals because of cross-reaction that the secondary antibody may cause. In the indirect ELISA test, the sample antibody is sandwiched between the antigen coated on the plate and an enzyme-labeled, anti-species globulin conjugate. The addition of an enzyme substrate chromogenic reagent causes color to develop. This color is directly proportional to the amount of bound sample antibody. The more antibody present in the sample, the stronger the color development in the test wells. This ELISA format is often used to determine the concentration of antibodies in serum. It is therefore an ELISA test that can be used in the food industry, but it is very useful for developing ELISA tests that will be used for the detection / quantification of food contaminants.

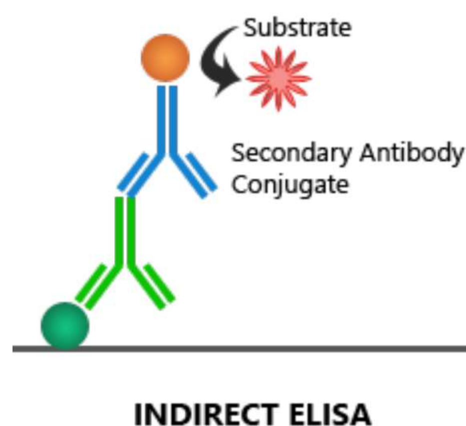

Figure 2: Indirect ELISA [4]

The use and meaning of the names "indirect ELISA" and "direct ELISA" differ in the literature and on web sites depending on the context of the experiment. When the presence of an antigen is analyzed,

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the name "direct ELISA" refers to an ELISA in which only a labelled primary antibody is used, and the term "indirect ELISA" refers to an ELISA in which the antigen is bound by the primary antibody which then is detected by a labeled secondary antibody. In the latter case a sandwich ELISA is clearly distinct from an indirect ELISA. When the "primary" antibody is of interest, e.g. in the case of immunization analyses, this antibody is directly detected by the secondary antibody and the term "indirect ELISA" applies to a setting with two antibodies.

Advantages:

Indirect ELISA resolve several issues of direct ELISA methodology. The first one is an increase of sensitivity du to the use of a secondary antibody. Indeed, more than one labeled secondary antibody can bind the primary antibody leading to a signal amplification. The second is a better flexibility as several primary antibodies can be used with the same secondary antibody, as long as the are produced form the same specie. Morover, the use of a secondary-antibody conjugate avoids the expensive process of creating enzyme-linked antibodies for every antigen one might want to detect.

Disadvantages:

Among its disadvantages is the possibility of cross-reactivity of secondary antibody to the adsorbed antigen, which could increase background noise. Besides, indirect ELISA assays take longer to run than direct ELISAs since an additional incubation step for the secondary antibody is required.

Sandwich ELISA

Sandwich ELISA (or sandwich immunoassay) is the most commonly used format in food diagnostic. 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 (called **capture antibody**). The other antibody is conjugated and facilitates the detection of the antigen (**detection antibody**). If the detection antibody used is unlabeled, a secondary enzyme-conjugated detection antibody is required. This is known as an indirect sandwich ELISA.

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The steps of a sandwich ELISA follow the mechanism below:

- A surface is coated with a known quantity of capture antibody.
- Any nonspecific binding sites on the surface are blocked using a non-reactive protein solution.
- The antigen-containing sample is applied to the plate and captured by antibody. The plate is washed to remove unbound antigen.
- A specific antibody is added and binds to antigen (hence the 'sandwich': the antigen is stuck between two antibodies).
- In case of indirect sandwich ELISA, enzyme-linked secondary antibodies are applied as detection antibodies. The plate is washed to remove the unbound antibody-enzyme conjugates.
- An enzyme substrate is added to be converted by the enzyme into a color signal.
- The absorbance or fluorescence or electrochemical signal (e.g., current) of the plate wells is measured to determine the presence and quantity of antigen.

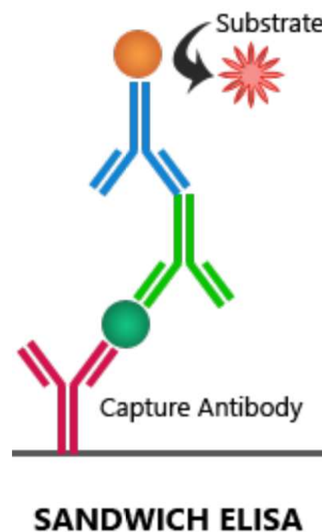

Figure 3: Presentation of a Direct Sandwich ELISA [4]

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Sandwich ELISAs are particularly suited to the analysis of complex samples, since the antigen does not need to be purified prior to the assay yet still delivers high sensitivity and specificity. Sandwich ELISA tests are also used to assess the reactivity of a serum to a given antigen, to allow the selection of the best antibodies for future commercial applications.

Advantages:

Without the first layer of "capture" antibody, any proteins in the sample (including serum proteins) may competitively adsorb to the plate surface, lowering the quantity of antigen immobilized. Use of the purified specific antibody to attach the antigen to the plastic eliminates a need to purify the antigen from complicated mixtures before the measurement, simplifying the assay, and increasing the specificity and the sensitivity of the assay. The key advantage of a sandwich ELISA is its high sensitivity; it is 2-5 times more sensitive than direct or indirect ELISAs. Sandwich ELISA also delivers high specificity as two antibodies are used to detect the antigen. It also offers flexibility since both direct and indirect methods can be used.

Disadvantages:

The advantages bring with them a few disadvantages; if a standardized ELISA kit or tested antibody pair is not available, antibody optimization has to be worked out since it is important to reduce cross-reactivity between the capture and detection antibodies. Besides, this strategy is not a good fit for small antigen as it might be difficult to find several binding sites on them. For example, partially hydrolysed samples can stay undetectable with this methodology.

Competitive ELISA

The competitive ELISA is perhaps the most complex of all the ELISA techniques. However, each of the above assay types can be adapted to a competitive format. The competitive ELISA is predominantly used to measure the concentration of an antigen or antibody in a sample by detecting interference in an expected signal output. Essentially, sample antigen or antibody competes with a reference for binding to a limited amount of labeled antibody or antigen,

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respectively. The higher the sample antigen concentration, the weaker the output signal, indicating that the signal output inversely correlates with the amount of antigen in the sample.

The steps for this ELISA are somewhat different from the first assays:

- Unlabeled antibody is incubated in the presence of its antigen (sample).
- These bound antibody/antigen complexes are then added to an antigen-coated well.
- The plate is washed, so unbound antibodies are removed. (The more antigen in the sample, the more Ag-Ab complexes are formed and so there are less unbound antibodies available to bind to the antigen in the well, hence "competition".)
- The secondary antibody, specific to the primary antibody, is added. This second antibody is coupled to the enzyme.
- A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.
- The reaction is stopped to prevent eventual saturation of the signal.

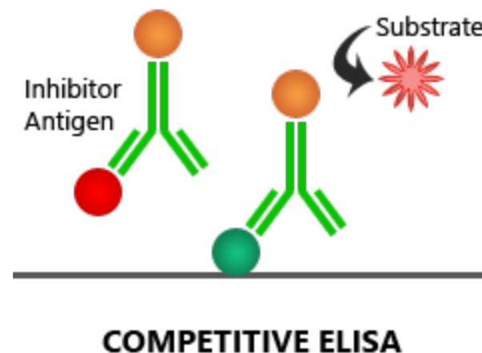

Figure 4: Direct Competitive ELISA [4]

Some competitive ELISA kits include enzyme-linked antigen rather than enzyme-linked antibody. The labeled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The less antigen in the sample, the more labeled antigen is retained in the well and the stronger the signal. This strategy allows the production of coated plate in advance by using unlabeled primary antibody for the coating.

Advantages:

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The main advantage of Competitive ELISA is that it fits for crude or impure samples and the matrix effect is reduce compared to sandwich ELISA. It also possible to detect small analytes with this assay. This ELISA is commonly used when only one antibody is available for the antigen of interest in food diagnostic.

Disadvantages:

As competitive ELISA are adaptation of the others ELISA methods, this assay presents the same limitations than the format it is based on.

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References

1. Engvall & Perlmann (1971). Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry, 8(9): 871-874.

2. Aydin (2015). A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides, 72: 4-15.

3. Van Weemen & Schuurs (1971). Immunoassay using antigen-enzyme conjugates. FEBS letters, 15(3): 232-236.

4. Aryal (2017). Available at: https://microbenotes.com/enzyme-linked-immunosorbent-assay-elisa/

5. Lindström and Wager (1978). IgG Autoantibody to Human Serum Albumin Studied by the ELISA-Technique. Scandinavian Journal of Immunology, 7(5): 419-425.

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