Online Training Curriculum

Confirmation Methods for Food contaminants

Principles of ELISA

Introduction to ELISA techniques

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Overview

Presentation

ELISA plays a major role in food industry. It is the main technique used for the identification of food allergens such as milk, eggs, peanuts, tree nuts, soya, etc. Moreover, it is also used to confirm the authenticity of food products, preventing public health issues and potential economic losses. For example, ELISA can be employed for the detection of melamine in milk. It is also an essential technique for quality control of fish, milk (as well as their sub products), genetically modified foods, irradiated foods, or other harmful food components that can be transferred to human, such as bovine spongiform encephalopathy. However, the production of ELISA kits for food industry applications remains difficult because test calibration requires the use of standards representative of a wide variety of food products. Consequently, ELISA methods are often validated for very specific purposes.

Principle and terminology

Antibody-antigen interaction

Enzyme-linked immunosorbent assay (ELISA) is a quick analytical method commonly used in medicine, food sciences, agriculture, etc. It is an immunoassay method, and like other types of immunoassays, it relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions. All the ELISA methods use:

- **an antigen:** defined as any molecule that elicits the production of antibodies when introduced into a body. Each antigen can present **one or several epitopes. An epitope** is defined as the part of the antigen that is targeted by an antibody;
- **an antibody**: protein produced by the body in response to antigenic stimuli to neutralize the antigen. Several kinds of antibodies can be used in an ELISA test:
    - **Monoclonal antibodies**: They are produced by identical immune cells which are all clones belonging to a unique parent cell. They are clones and target a unique epitope.

- **Polyclonal antibodies**: They are a mix of all the monoclonal antibodies produced by several immune cells. As the monoclonal antibodies produced by each immune cell can target a different epitope, polyclonal antibodies can target different epitopes for one antigen.

Why is it called Enzyme linked immunosorbent assay?

A sorbent is defined as a material used to absorb or adsorb liquids or gases. In an ELISA test, depending on the ELISA method that is used (see lesson 2), it is either the antigen or the antibodies that are immobilized to a solid surface and used as sorbent. As both are used in immunoreactions, it is called **immunosorbent**. An ELISA assay is typically performed in a multi-well plate which provides the solid surface to immobilize the immunosorbent.

To allow the detection and quantification of molecules using the antibody-antigen interaction, ELISA techniques use antibody conjugated with a molecule amenable for detection such as an enzyme (enzyme-linked). The complex antibody-enzyme is called **conjugate**. In practice, the number of enzymes that are used in commercial kits is limited. The main enzymes used in ELISA tests are the Horseradish peroxidase (HRP) or the Alkaline phosphatase (ALP). A substance is the added to react the enzyme to produce a colored product. This substance is called **chromogen**. In fact, the chromogen is a substrate of the enzyme used in the conjugate. For example:

- OPD (o-phenylenediamine dihydrochloride) turns amber to detect HRP
- TMB (3,3',5,5'-tetramethylbenzidine) turns blue when detecting HRP
- ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) turns green when detecting HRP
- PNPP (p-Nitrophenyl Phosphate, Disodium Salt) turns yellow when detecting ALP

In some rare cases, the substrate is bound to the antibody to form the conjugate and the chromogen is the enzyme.

Different steps

All the different ELISA methods contain similar steps

- **Adsorption**: Adhesion of antibodies or antigen (immunosorbent) from a liquid to a microplate. It is almost always already done in commercial kits.
- **Incubation**: Period of time to let a reaction to occur. It is important to standardize and interpret the results of a test. Several incubation times exist in an ELISA assay depending on the step (adhesion of the immunosorbent, interaction between the antibody and the antigen, reaction between the enzyme and its substrate...)
- **Washing**: The simple flooding and emptying of wells with a buffered solution to separate bound from un-bound reagents in ELISA. Washing is a crucial step when performing an ELISA to obtain results with high precision.
- **Stopping**: Addition of adding a solution to stop the action of an enzyme on a substrate. It is usually an acid or a base. The solution stop denatures all proteins including the antibodies and thus stops the reaction.Nevertheless it is recommended to read the microtiter plate directly after addition of stop solution or at least within the time stated in the instructions for use. A large delay may still cause a shift of the absolute values measured.
- **Reading**: The spectrophotometric measurement of color developed during the assay. The wavelength depends on the substrate used during the reaction between the enzyme and its substrate.

Detection and quantification

In ELISA, various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured using a substrate that changes color when modified by the enzyme. The optical density of the product formed after substrate addition is measured and converted to numeric values.

ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cut-off between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples.

All quantitative ELISA systems are calibrated by the use of standards. Therefore, the samples with unknown concentrations and a set of standards with known concentrations are analyzed in parallel

on one plate. The result will be a calibration curve (with the associated mathematic formula) built out of the measured OD values and the concentrations of the standard (Figure below). Based on this, the analyte concentration in the sample can be calculated.

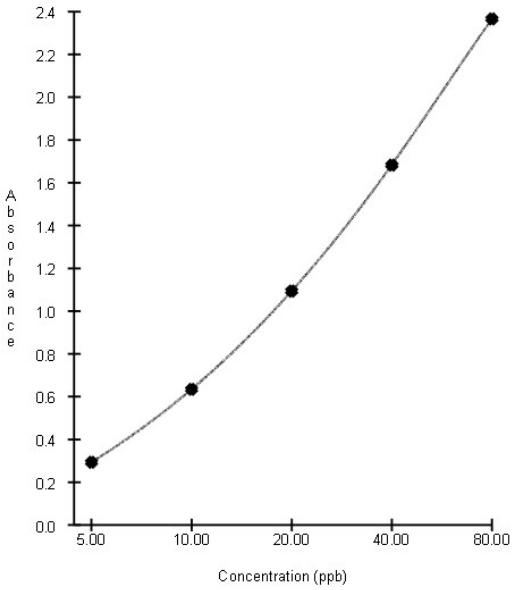

Figure 1: Example of standard curve.

The concentration of the analyte in an “unknown” sample can be determined by comparing the measured signal of the sample with the signal of standards containing known concentrations of the analyte. In ELISAs usually 5 - 7 standards are used to generate a standard curve covering the concentration range of interest.

It is advised, to dilute samples which are above the largest standard and to repeat the analysis until the result is within the concentration range of the standard curve. In general, extrapolation for values above the standard curve are not reliable. The further the sample is below the lowest or above the highest standard, the bigger the uncertainty of the calculated concentration is.

Advantages and disadvantages of ELISA

Advantages:

- High sensitivity and specificity: it is common for ELISAs to detect antigens at the picogram level in a very specific manner due to the use of antibodies.
- High throughput: commercial ELISA kits are normally available in a 96-well plate format. But the assay can be easily adapted to 384-well plates.
- Easy to perform, protocols are easy to follow and involve little hands-on time.
- Quantitative: it can determine the concentration of food contaminants in a sample.
- Possibility to test various sample types: food or biological samples among others.

Disadvantages:

- Temporary readouts: detection is based on enzyme/substrate reactions and therefore readout must be obtained in a short time span.
- A single analyte: the use of antibodies means that a single analyte (or sometimes a group of analyte) can be targeted per ELISA test. If you want to detect several food contaminants, you will need different ELISA tests.
- Number of samples: There is need of a large number of samples to avoid waste of buffer and antibodies, coated wells (economic losses).

These are the general ELISA advantages and disadvantages. There are other advantages and disadvantages depending on the type of ELISA used. They will be detailed in the next class lesson.

Needed equipment

Pipettes

A pipette is used to transfer a precisely defined volume of a solution to e.g. the wells of a microtiter plate. Pipettes are crucial for results with a high precision. Therefore, special attention should be set on maintenance by experts and proper pipetting technique. Different kinds of pipettes exist:

- Single-channel pipettes with fixed volumes e.g. 50 µl,
- Single-channel pipettes (e.g. with variable volumes between 10 and 100 µl); used normally for samples and standards,
- Multistepper pipettes (with the possibility to pipette multiple times of a specific volume); used normally for addition of antibody or conjugate solutions,
- Multi-channel pipettes (with the possibility to pipette the solution in 8 or 12 cavities at the same time); used normally for washing steps or addition of antibody or conjugate solutions,
- Fully automatic machines (all pipetting and incubation steps are done automatically).

ELISA reader

The ELISA reader is a spectrophotometer which allows to measure the optical density (OD) and to calculate the concentration of your sample. Regular maintenance by experts is essential for exact and accurate results.

Incubator

In some tests an incubator is required to guarantee a stable temperature while the test is running. Sometimes, a seal or a protecting plate cover are necessary to prevent evaporation or contamination. This is rarely the case for food contaminants as food samples are tested at ambient temperature.