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Immunoanalysis – Part 2: Basic Principles of ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) is a commonly used technique for the determination of known analytes (for example, GMOs, food allergens, pesticides, herbicides, PCBs and veterinary drug residues). ELISAs are routinely used in scientific research, veterinary medicine, environmental and agricultural applications, and in healthcare. The fundamental principle of the ELISA is that the target analyte (the antigen) is recognised with high specificity by antibodies, which are proteins produced by the immune system. The immune system of animals produces antibodies in response to the presence of antigens. These antibodies can recognise and bind to the antigens, the labelling of the bound antibody forms the basis of the detection.

The history of the ELISA

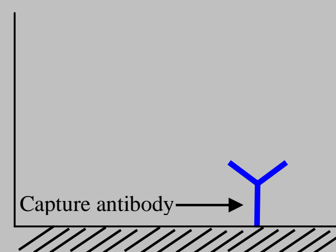
The format of the ELISA was developed in the 1960s independently at the same time by two research groups; Peter Perlmann and Eva Engvall at Stockholm University, and the Dutch research group of Anton Schuurs and Bauke van Weemen. The assay was based on the underlying principles of conventional radioimmunoassay, with the key difference that the antibodies are labelled with an enzyme, rather than radioisotopes.

Typical Steps of an ELISA

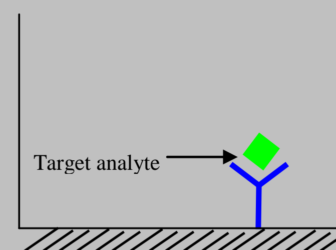
The success of an ELISA assay is dependent upon the underlying level of immunoreactivity of the capture and detection antibodies to the target analyte. Box 1. shows one format of the assay, which uses two separate antibodies: the first to recognise and bind the target analyte, the second to detect the bound target. In Step 1, one of the antibodies is applied to the well of a microtitre plate: this is known as the capture antibody. The capture antibody binds to the plate via passive adsorption and this step is often performed at 4°C overnight. A blocking solution (typically milk protein (casein), bovine serum albumin or fish gelatin) is applied. These proteins adhere to any vacant sites on the plastic surface of the well that are not occupied by capture antibodies thereby minimising the effect of non-specific binding by other reagents to the plate surface during subsequent incubation steps. Excess blocking agent is removed and the plate is rinsed before addition of the test sample (wash steps are incorporated between all incubation steps to minimise the background signal due to non-specific binding).

Box 1. A Typical Direct-Detection ELISA.

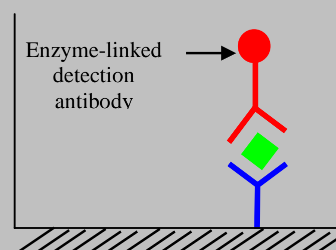
Step 1
Immobilise the capture antibody and block vacant sites.



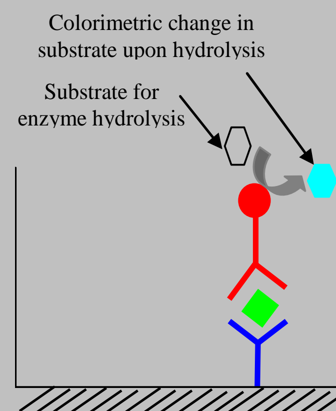
Step 2
Apply test material.



Step 3
Challenge with the enzyme-linked detection antibody.



Step 4
Apply substrate that is hydrolysed by the enzyme, resulting in a colorimetric change and measure the absorbance.



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If the test sample contains the target analyte, this is bound by the capture antibody that is anchored to the plate (Step 2). After the incubation step with the test sample, the plate is washed prior to the addition of the enzyme-linked detection antibody (Step 3). The detection antibody is conjugated with an enzyme which is commonly either horseradish peroxidise, alkaline phosphatase or β-*D*-galactosidase. These enzymes are proteins that catalyse the hydrolysis of a chromogenic substrate, such as 3,3',5,5'-tetramethylbenzidine or 2,6-dichlorophenolindophenol, which undergoes a colorimetric change that is measurable using a spectrophotometric plate reader at specified wavelengths (Step 4). With the catalysis of the traditional chromogenic substrates the reaction is terminated by the addition of a stop solution prior to measuring the absorbance of each of the wells of the microtitre plate.

Indirect detection

In addition to the direct detection method, as illustrated in Box 1, ELISAs may utilise an indirect detection approach. In this instance, as illustrated in Figure 1, the secondary antibody used will recognise and bind to the appropriate species-specific sub-class of the antibodies (or immunoglobulins). It is the secondary detection antibody that is conjugated to the enzyme, as opposed to the primary antibody.

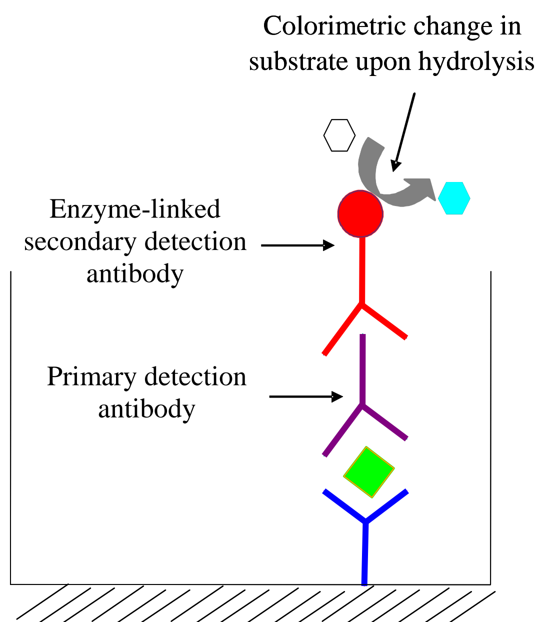

Figure 1. A Typical Indirect-Detection ELISA. (Same symbols as in Box 1.)

This method offers versatility because a single enzyme labelled secondary detection antibody may be used for all assays involving the use of single host species of primary antibodies. The sensitivity of the ELISA may also be enhanced as the secondary antibody tends to be polyclonal, and this affords signal amplification via the recognition of multiple epitopes on the primary antibody.

Signal output and assay performance

Recently, ELISAs have moved away from the use of chromogenic substrates that give the detectable colour change, to fluorogenic substrates (for example, acetyl-3,7-dihydroxyphenoxazine). Enhanced chemiluminescence reagents are also used to emit light as the measure of the signal output. The fluorogenic and luminometric methods are more sensitive, and are reported to offer femtogram-level sensitivity with some recently developed commercially available proprietary substrates. Fluorogenic and luminometric substrates may also extend the linear detection range in comparison to the conventional chromogenic substrates.

Target analyte quantification is facilitated through either internal or external standardisation, with the use of calibrants. ELISAs are often undertaken using duplicate or triplicate sample measurements where a coefficient of variation of around 10% is not uncommon for commercially available ELISA kits. Although kits are readily available for many common analytes, ELISAs may be constructed with relative ease if the antibodies to the analyte of interest can be commercially sourced. The success of ELISA is dependent upon the quality of the antibodies available. ELISA is popular because the technique is amenable to automation, and does not require the use of expensive dedicated equipment.

Further Reading

- J Crowther. *The ELISA Guidebook.* Humana Press; 2nd Ref. Ed edition, 2000. ISBN-10: 0896037282.
- B Alberts, A Johnson, P Walker, J Lewis, M Raff and K Roberts. *Molecular Biology of the Cell.* Garland Publishing Inc, US; 5th Ref. Ed edition, 2008. ISBN-10: 0815341067.

This Technical Brief was drafted by Dr Susan Pang (LGC Ltd.) on behalf of the Analytical Methods Committee.

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