ELISA Methods

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Some Serology Basics

Antigen

Molecule or molecular structure (virus, bacteria, fungi, peptides, toxins…) that triggers an immune response in the host

Antiserum:

Blood serum containing antibodies to a specific antigen

Antibodies

Group of serum proteins called ***immunoglobulins (Igs)*** that recognize and bind to a particular antigen with very high ***specificity***

Epitopes

Part of antigen that interacts with an antibody

Polyclonal Antibodies

Collection of immunoglobulin molecules that react against a specific antigen, each identifying a different epitope

Monoclonal Antibodies

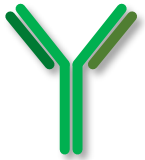
Monospecific antibodies that are identical copies made by one type of immune cell, each identifying only one specific epitope

ELISA (Enzyme-linked Immunosorbent Assay)

Replaced Radio-Immunoasssays in the early 1970s

ELISA Plate 96-wells

Primary Antibody (AB)



Enzyme-linked Secondary or Primary Antibody

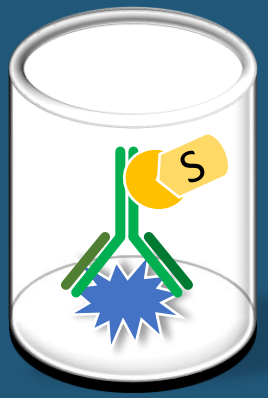
Enzyme-Substrate

Antigen (AG) to be analysed

Antibody Capture ELISA

1. Adhesion of AG to well
2. Binding of labelled primary AB to AG
3. Enzymatic reaction with substrate

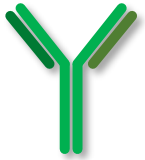
- Only one AB is needed
- Fast and easy
- Low specificity
- Low sensitivity due to lack of signal amplification
- Labeling of primary AB necessary



ELISA (Enzyme-linked Immunosorbent Assay)

ELISA Plate 96-wells

Primary Antibody (AB)



Enzyme-linked Secondary or Primary Antibody

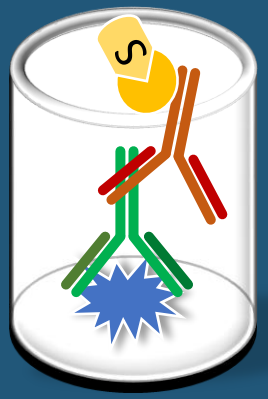
Enzyme-Substrate

Antigen (AG) to be analysed

Indirect ELISA

1. Adhesion of AG to well
2. Binding of primary AB to AG
3. Binding of secondary (anti-species) AB to primary AB
4. Enzymatic reaction with substrate

- No labeling of primary AB necessary, all binding sites are free
- A whole range of secondary ABs available
- Higher sensitivity due to signal amplification
- Possible cross reactivity through secondary AB



ELISA (Enzyme-linked Immunosorbent Assay)

ELISA Plate 96-wells

Primary Antibody (AB)

Enzyme-linked Secondary or Primary Antibody

Enzyme-Substrate

S

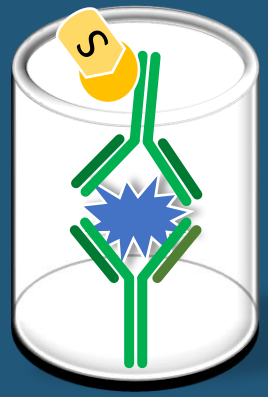
Antigen (AG) to be analysed

Most commonly used in plant pathogen detection

(Double or Triple) Sandwich ELISA: DAS/TAS

1. Adhesion of primary AB to well
2. Binding of AG to primary AB
3. Binding of labelled AB to AG on second binding site
4. Enzymatic reaction with substrate

- Sensitive, high specificity
- Antigen without purification can be used
- High signal amplification
- Antigen needs two binding sites for ABs



How to produce an Antiserum for Plant Disease Diagnostics (Polyclonal A...

CC/NUMBER 33
AUGUST 13, 1990

This Week's Citation Classic®

Clark M F & Adams A N. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses.
J. Gen. Virol. 34:475-83, 1977.
[East Malling Research Station, Maidstone, Kent, England]

A new serodiagnostic method for plant viruses, enzyme-linked immunosorbent assay (ELISA), was described. Morphologically different plant viruses were detected in purified preparations and in unclarified extracts of infected plants. Virus concentration in the samples was proportional to the colour intensity of the enzyme-hydrolysed substrate. [The *SCI*® indicates that this paper has been cited in over 925 publications, making it the most-cited paper from this journal.]

ELISA and the Plant Virus

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the Nuffield Institute of Comparative Medicine, London. Alister was intrigued by the possibility of applying the method to plant pathogens and readily agreed to collaborate in some trials with our plant viruses. Plum pox virus was the obvious first choice while arabis mosaic virus, a pathogen of strawberries and hops, was selected as a representative isometric virus. Initial tests carried out in Alister's laboratory were spectacularly successful, so Tony and I embarked on a comprehensive evaluation of the method.

The technology was fairly straightforward. The problems we had to face were more conceptual in nature. Serology was regarded by many plant virologists as more of an art form than a scientific technique, interesting as an adjunct to infectivity tests and determining physicochemical properties but not really trusted as a mainstream diagnostic tool. To bring about a radical change in attitudes would require that this new procedure be universally applicable, reliable, and extremely

Advantages and Limitations of ELISA Methods

- Robust technique – high reproducibility
- Requires only little equipment
- High throughput screening
- Fast and cheap sample preparation
- Assays can be automated
- ABs and Kits are commercially availabe for many important pests

- Time consuming development of new Abs for emerging pests (1-3 years)
- Best performance with plant viruses – often not suitable for bacteria and fungi (low specificity)
- Due to lack of reference standards – only limited quantification possible
- Sensitivity lower than molecular methods

When to use ELISA?

- Routine screening of plant material
- Analysis of suspicious plant material and identification of pests
- Diagnostic method with good confidence in positive samples

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Do‘s

Dont‘s

Link to a video : https://youtu.be/HidQJUBGPwc