AGRIALLIS
Science for Agriculture and Allied Sector: A Monthly e Newsletter

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

SEROLOGICAL ASSAY TECHNIQUES FOR INDEXING PLANT VIRUSES

Article Id: AL202183

[1]Rajesh S* and [1]Varanavasiappan S

[1]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India

Email: biotekrajesh@gmail.com

Serological methods play a pivotal role for the diagnosis, detection, and identification of plant viruses. Conventional assay procedures for plant viruses require bioassay using an indicator plant, determination of the host range, symptomatological observations, and transmission studies through host-vector relations. Physical assay procedures require studying the virus particle morphology using electron microscopy. With the advent of antibody-based detection procedures, molecular detection of plant pathogens has undergone a rapid change over in the past three decades. ELISA, DBIA, TBIA are among the few techniques that are widely deployed for screening procedures for plant pathogen detection.

Virus indexing procedures are using a single assay may provide adequate information on the identity of a virus; however, a combination of methods is generally needed that are specific, sensitive, and inexpensive for virus detection. Due to the progress in biochemistry, molecular biology, and immunology, accurate, rapid, and less labour-intensive methods of virus detection have been developed. There are various immune-molecular diagnostic techniques currently available in the field of virology, and these are Protein based techniques which include precipitation/agglutination tests, enzymes linked immunosorbent assay (ELISA), dot immunoblotting assay (DBIA), and Tissue blot immunobinding assay (TBIA). A comprehensive review on the same has been reported (Abd El-Aziz, 2019)

Serological assay for rapid plant pathogen detection is based on the recognition of the antigen with antibodies produced against them. The demonstration that glutaraldehyde cross-linked enzyme-antibody conjugates retained both the specificity of the IgG molecule and the catalytic properties of the enzyme revolutionized the use of the enzyme-linked immunosorbent assay (ELISA) as a rapid and highly sensitive serological method for qualitative and quantitative analysis of virus infection. Most often, the antibodies are raised in

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

the lab model organisms or any type of organism that has got a circulatory system. These antibodies are polyclonal in nature. Specificity of antibodies can be expected in a monoclonal antibody based production system that uses hybridoma technology.

Serological Assay Techniques

1) Enzyme Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay is a commonly used analytical biochemistry assay that is a plate-based technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. Most of the economically important and widespread plant viruses can now be characterized by this method (Koenig, 1978). The stepwise workflow of ELISA is provided in Fig.1.

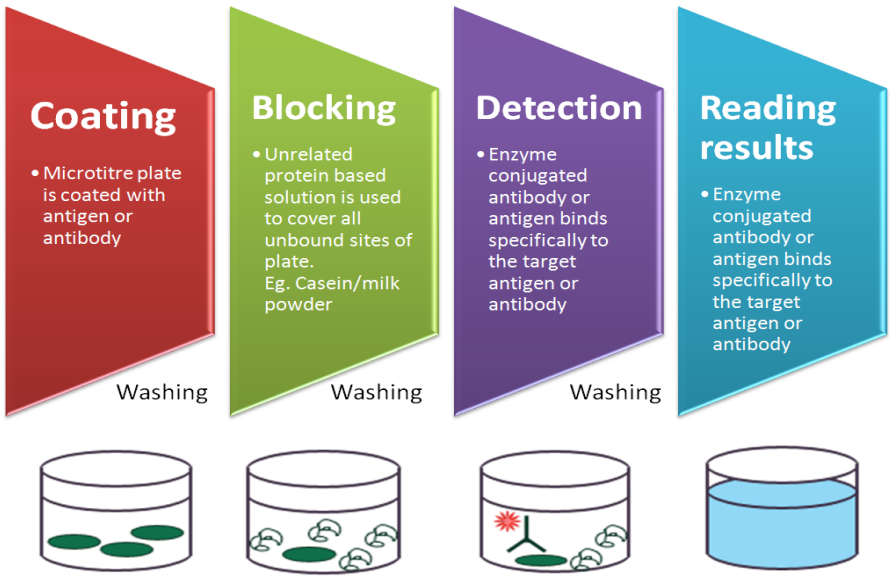

Fig. 1: Steps in ELISA

Advantages

1. Choice of varieties of labeled secondary antibodies commercially is a big plus for this method
2. This is a versatile method since primary antibodies can be made in one species, and the same labeled secondary antibody can be used for detection.

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

3. Maximum immunoreactivity of the primary antibody is retained.

4. Increased sensitivity because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody that allows signal amplification.

Disadvantages

1. Possibilities of cross-reactivity with the secondary antibody resulting in nonspecific signal.

2. An extra incubation step is required in the assay procedure.

Types of ELISA

ELISA tests are classified into different types based on how the analytes and antibodies are bonded and used (Fig.2).

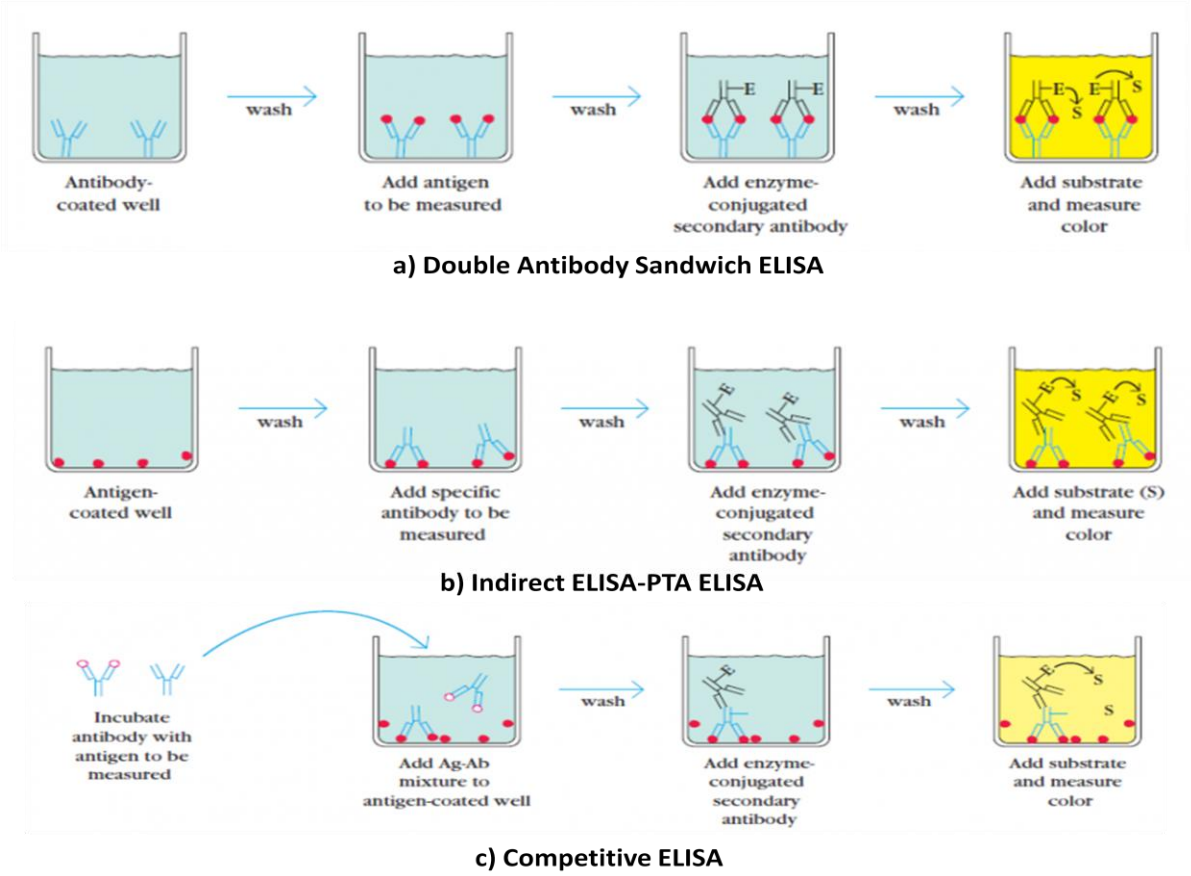

Fig. 2: Types of ELISA

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

i) Double Antibody Sandwich ELISA (DAS-ELISA)

The direct ELISA procedure involves the binding of antibodies [immuno gamma globulin (IgG) fraction of antiserum] to the well surface of the microtitre plate, which further captures the virus in the test sample (Fig.2a) . The captured virus is then detected by antibody-enzyme conjugate kept for a particular incubation followed addition of color development reagents (substrate). This procedure is also referred to as Double Antibody Sandwich ELISA (DAS-ELISA).

ii. Indirect ELISA - Double Antigen coated (DAC)/Plate Trapped Antigen ELISA

In the case of an indirect form of ELISA, Antibodies raised in two different animal species are alternatively immobilized with the virus in the wells of the ELISA plate. The indirect ELISA detects the presence of antibodies in a sample. The antigen for which the sample must be analyzed adheres to the wells of the microtitre plate. The primary antibody present in the sample bind specifically to the antigen after the addition of the sample (Fig. 2b).

The solution is washed to remove unbound antibodies, and then enzyme conjugated secondary antibodies are added. The corresponding substrate for the enzyme is added to quantify the primary antibody through a color change. The concentration of primary antibodies present in the serum directly correlates with the intensity of the color.

iii. Triple Antibody Sandwich ELISA (TAS-ELISA)

A lot of similarity with DAS-ELISA is there, but an additional step is involved by adding a monoclonal antibody (MAb) followed by the addition of enzyme-conjugated species specific antibody. This method was developed by Geering and Thomas (1996) for virus indexing of Banana bunchy top virus (BBTV), which is the preferred method when compared to dot immunobinding assays.

iv. Competitive ELISA

This ELISA is based on the competitive reaction between the sample antigen and antigen bound to the wells of the microtitre plate with the primary antibody (Fig. 2c). First, the primary antibody is incubated with the sample, which leads to the formation of Ag-Ab complex is then added to the wells that have been coated with the same antigens.

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

After incubation, unbound antibodies are washed off. The more antigens in the sample, the more primary antibodies will bind to the sample antigen. Ultimately there will be a smaller amount of primary antibody available to bind to the antigen coated on well. Secondary antibody conjugated to an enzyme is added, followed by a substrate to elicit a chromogenic signal. Concentration of color is inversely proportional to the amount of antigen present in the sample.

2) Immuno blotting

The immune blotting techniques use antibodies to identify the target viral proteins among a member of unrelated protein samples, and this involves the antigen-antibody specific reaction to identify the protein target.

Two major classes:

I. Dot Immunoblotting Assay (DIBA).
II. Tissue Immunoblotting Assay (TIBA).

a. **Dot Immuno blotting Assay (DIBA):**

Dot Immunoblotting Assay (DIBA) is widely used for the detection in both plants and vectors for routine diagnosis of plant viruses. DIBA can be considered a strong technique as comparative to ELISA because it requires only a crude specific antiserum for each of the viruses tested for, and therefore, DIBA can be used in poorly equipped laboratories.

It is mostly similar to ELISA, but in one aspect, it is different, and that is the plant extracts are spotted onto a membrane rather than in a microtitre plate or solid support used in ELISA. Here hydrolysis of the chromogenic substrate leads to visible colour precipitation, confirming the presence of virus in the sample.

b. **Tissue Immuno blotting Assay (TIBA):**

Tissue Immuno blotting Assay (TIBA) is mainly based on blotting of a target biological sample such as freshly cut edge of leaf blade, leaf, stem, tuber, root or an insect on to the membrane followed by detection with labeled antibodies (Fig.3; Lin *et al*., 1990). The disadvantage of DIBA and TIBA is the weak positive reactions are not visible sometimes for more sap colour development.

Volume 3 – Issue 9
Online ISSN: 2582-368X
www.agriallis.com

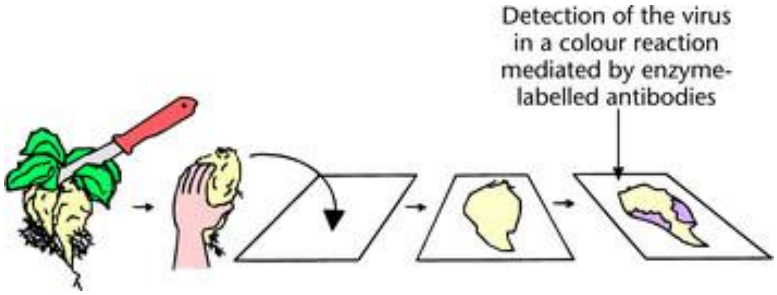

Fig. 3. Tissue Immuno blotting Assay

But in overall, both of these techniques have great handling and shipment of experimental membranes from one location to another location or one country to another country.

Conclusion

Serological procedures remain the mainstay in indexing program for plant viruses, although recent editions of PCR based techniques has taken upper hand due to the simplicity and versatility. The diagnosis of viral infections represents the most widely used application of serological investigations in all fields of virology. Serological cross-reactions are most reliable criteria for deciding on relatedness between viruses;, since viruses that are serologically related always share morphological and biochemical characteristics that place them in the same taxonomical group. Serological procedures are now widely adopted for plant virus indexing and certification programs that particularly complement other established procedures of virus indexing.

References

Abd El-Aziz MH. (2019) Three modern serological methods to detect plant viruses. *J Plant Sci Phytopathol.* 3: 101-106.

Koenig R. (1978) ELISA in the study of homologous and heterologous reaction of plant viruses. *J Gen Virol.* 40: 309-318.

Lin NS, Hsu YH, Hsu HT. (1990) Immunological detection of plant viruses and mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. *Phytopathology*. 80: 824-828.