Potential of Enzyme-linked Immunosorbent Assay for Detecting Viruses, Fungi, Bacteria, Mycoplasma-like Organisms, Mycotoxins, and Hormones

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Abstract

Enzyme-linked immunosorbent assay (ELISA) is one of the most widely employed serological tests. For example, the double antibody sandwich (DAS-ELISA) technique has been successfully employed for the detection of various rhizobia, to assess the quality of inoculum, and to assess the establishment of the inoculated rhizobial strains in soil. This technique can also distinguish between different strains of Rhizobium. *ELISA has successfully been adapted to direct antigen coating (DAC-ELISA) and protein A coating (PAC) procedures for the detection and assay of several peanut viruses. DAC-ELISA is a simple and useful tool for virus detection in field surveys and in seed samples. Both DAC- and PAC-ELISA are more convenient to use for the detection of large numbers of antigens than DAS-ELISA. More recently, a competitive ELISA procedure has been developed to detect up to 2 ng aflatoxin B_1 in groundnut seed samples.*

Introduction

Nakane and Pierce (1966) coupled low molecular weight antibodies to produce enzymatically active immunological conjugates, and visualized the immune reaction with histochemical-staining techniques. Utilizing this principle, enzyme immunoassays, of the type popularly referred to as enzyme-linked immunosorbent assay (ELISA), were developed by Engvall and Perlmann (1971) and van Weeman and Schuurs (1971). Subsequently, several modifications were introduced to the ELISA test procedures. ELISA is currently by far the most widely used test in serological diagnosis. It permits de tion of antigens, of widely varying size and m. ology, and is adaptable to a wide range of plant extracts and conditions. This paper discusses the uses of various forms of ELISA at ICRISAT for the detection and/or quantification of peanut viruses, mycoplasma-like organisms (MLOs), *Rhizobium* strains, and aflatoxin B_1.

Detection of Peanut Viruses

Most workers use the double antibody sandwich form of ELISA (DAS-ELISA) described by Clark and Adams (1977) for the detection of plant viruses. In this procedure the solid phase (often wells of polystyrene or polyvinylchloride microtitre plates) was coated with immunoglobulins (Ig), which were mainly immuno gamma globulins (IgG). Partially purified Ig were prepared from antisera by precipitation with either ammonium sulfate or sodium sulfate. Test samples were then added and the Ig trapped the virus antigen. Enzyme-labeled antivirus Ig were added, which attached to the trapped virus antigens (Fig. 1). Finally, a suitable substrate was added to produce colored hydrolysates, thus permitting visual scoring and quantitative measurement, by colorimetry, of the amounts of antigen trapped. The DAS-ELISA procedure is known to be highly specific and often detects closely related strains (Koening 1978). The main disadvantage of this test is

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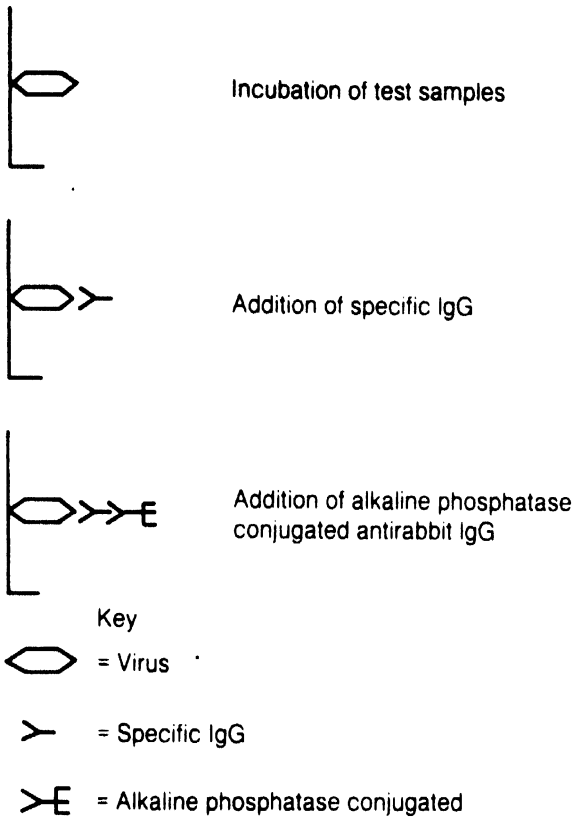

Figure 1. Principle of the double antibody sandwich (DAS) form of enzyme-linked immunosorbent assay (ELISA).

that it requires preparation of different Ig conjugates for each virus to be tested. To overcome this disadvantage we have tested several "indirect ELISA" procedures for the detection of three peanut viruses. Rabbit Fc specific Ig prepared in goats and conjugated with alkaline phosphatase was utilized in these procedures. A single conjugate could be utilized with antisera for different viruses. We describe below the two indirect-ELISA procedures that we have been able to apply successfully for the detection of several peanut viruses. They facilitated both the detection of a broad range of serologically related viruses, and the utilization of high dilutions of crude antisera.

For the direct antigen coating procedure (DAC-ELISA), the method developed by Mowat (1985) was used with minor modifications (Hobbs et al. 1987b). In the first step, plant extracts prepared in a carbonate buffer were applied directly to the wells. In the second step, diluted unfractionated antiserum was added. Ig attached to virus antigens were detected by the addition of enzyme conjugates of rabbit Fc specific Ig prepared in goats (Fig. 2). This method is by far the simplest of all the ELISA test procedures and can be completed within 3 hours. The main disadvantage of DAC-ELISA is that adsorption of the viral antigen to the well surface depends to some extent on the host components present in the plant extracts (Mowat 1985). We found DAC-ELISA to be very useful for virus detection in disease surveys (Reddy 1986, Hobbs et al. 1987b) since plant extracts could be used for coating plates without the necessity of using Ig for trapping viral antigens. The procedure has also been successfully used for detecting peanut mottle virus (PMV) in groundnut seed, thus facilitating screening of groundnut germplasm for seed-transmitted PMV. Small portions of cotyledon from 25 individual seed were mixed, ground in approximately 2.0 mL of carbonate coating buffer, and added to a well of an ELISA plate. As 8 plates, each with 96 wells, could be processed in a single day, we were able to test over 16 000 seeds per day for PMV presence.

Since host components tend to adsorb to the well surface, DAC-ELISA is not suitable for quantitative estimation of viruses present in crude plant extracts. DAC-ELISA is not suitable for investigating serological relationships, unless the virus is present in high concentration in crude extracts, and antisera having few, or no, antibodies against plant host components, are available. As a result we tried various indirect ELISA procedures described by Barbara and Clark (1982), van Regenmortel and Burck-

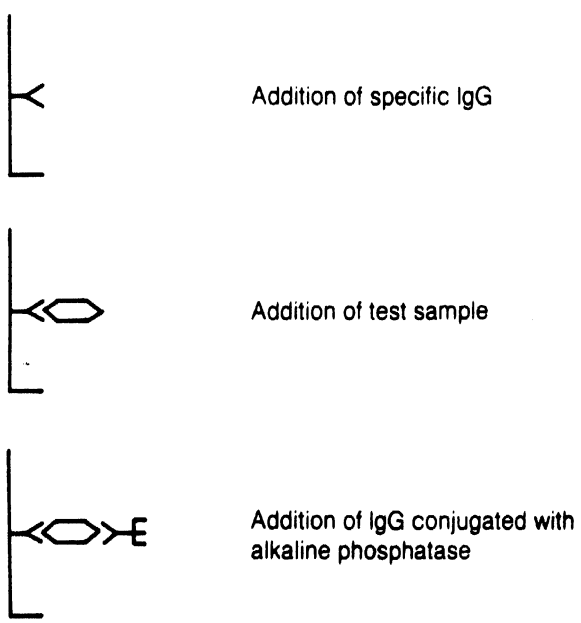

Figure 2. Principle of the direct antigen coating (DAC) form of enzyme-linked immunosorbent assay (ELISA).