B. Sc (P) Life Science III year Semester VI

DSE-1 Analytical Techniques in Plant Sciences

Practical- Study of ELISA

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Enzyme-Linked Immunosorbent Assay (ELISA)

1. The technique of **Enzyme-Linked Immunosorbent assay** (ELISA) was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden in 1971.

2. ELISA is one of the most sensitive and commonly used tests for the detection and quantification of antigens, antibodies, peptides, proteins and hormones. There are variety of modifications of this test and these are collectively known as enzyme immunoassay (EIA).

3. In the basic technique of ELISA, the antigens from the samples are attached to a surface. Then high-affinity antibody is applied over the surface. The specific antigen binds to this primary antibody and the unbound antibodies are removed during washing step. The antigen-enzyme complex is then subjective to competitive binding of an enzyme-linked secondary antibody.

4. The enzyme-linked secondary antibody specifically combines with the antigen-primary antibody complex. Finally, the substrate for the enzyme is added. The subsequent reaction produce a detectable colored product indicating presence of antigen, hence confirming positive test for the particular antigen.

5. The traditional ELISA involves Chromogenic reporters and substrates that produce some kind of observable color change to indicate the presence of antigen. Advance ELISA techniques use fluorogenic, electrochemiluminescent and quantitative PCR reporters to generate quantifiable signals.

6. To conduct ELISA test polystyrene microtiter plate, usually 96-well plate, is used. The antigen/antibody are adhered to the well surface through charge interactions.

7. The general experimental strategies employed in ELISA can be primarily classified into two categories-

    a. Direct ELISA

    b. Indirect ELISA

8. In Direct ELISA only one antibody is used. The buffered solution of sample containing **antigen** is added to wells of microtiter plate. Then, the **enzyme conjugated antibody** is added, which binds specifically to the test antigen. The substrate for this enzyme is then added, the intensity of color indicates the concentration of antigen.

9. In indirect ELISA two antibodies are used. The wells of microtiter plate are coated with **antigen** followed by addition of specific **primary antibody** which binds specifically to the test antigen. An **enzyme-linked secondary antibody** is now added which is specific for primary antibody. On addition of substrate, the presence of color and its intensity indicate positive test for test-antigen and its quantity as well.

10. The technique of ELISA has many applications in the clinical diagnosis and research field. Some applications are-

i. Detection of presence of antigen or antibody in a sample

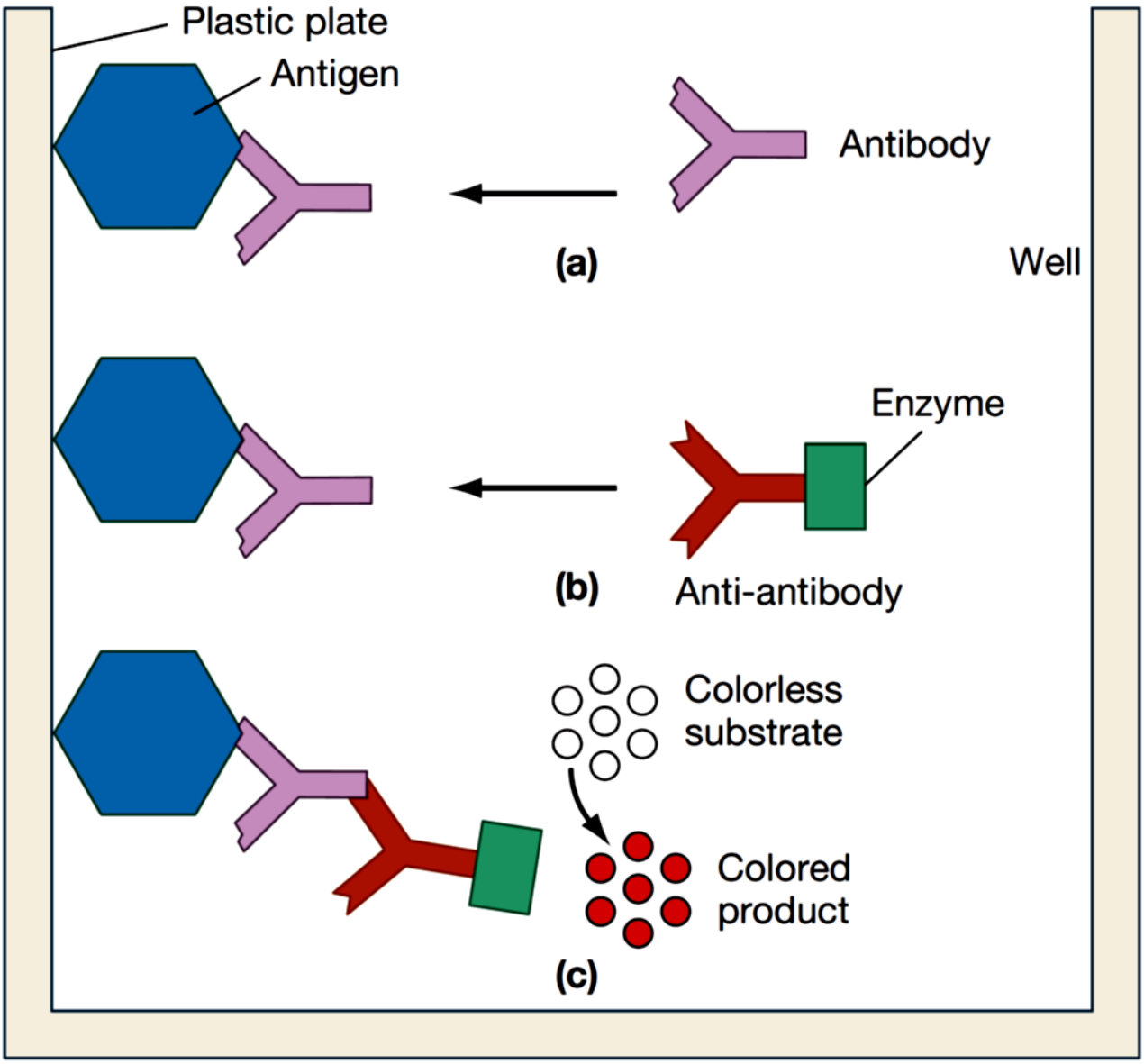
ii. Detection of serum antibody for past exposure to disease, e.g.- Lyme disease, trichinosis, HIV, etc.

iii. Detection of antigens for different diseases like- Herpes, Syphilis, etc.

iv. Detection of potential food allergens in food industry, like milk, eggs, walnuts, hazelnuts, etc.

v. Detection of antigens like- pregnancy hormones, drug allergens, GMO.

vi. Determination of antigenic determinants for vaccine development.



Enzyme-linked immunosorbent assay (ELISA) (a) Antigen bound to a well of a plastic plate reacts with the antibody (primary antibody) being detected. (b) In one form of ELISA (indirect), an anti-antibody (secondary antibody) is then added, which is conjugated with an enzyme. (c) A substrate specific to the

enzyme is then addaed. If this enzyme has bound to the original antibody, it can catalyze a reaction, converting the colorless substrate into a color product. Such ELISA tests are done routinely as an initial test to detect HIV in blood samples.

Sources:

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