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accelerating drug development. exactly.

Enzyme-Linked Immunosorbent Assay (ELISA)

Application

The ELISA method is a biochemical technique commonly used to detect antibodies in serum. Performing this test typically involves a set-up step of immobilizing antigens on the surface of wells in microtiter plates made of specially prepared plastic. Test serum is incubated in the wells, allowing antibodies to bind to the antigen. ELISA testing is simple to perform and requires only commonly-available technology, making it useful in a laboratory animal diagnostic setting.

Assay

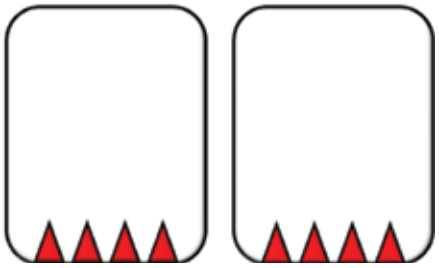


Figure 1: An ELISA is routinely performed in a 96-well, polystyrene, flat-bottom microtiter plate. Each well is coated with antigen ( ) specific to an infectious agent of interest. In addition to the antigen-coated well, an adjacent well can be coated with a tissue control to detect serum sample-related, non-specific binding. The tissue control is usually prepared from the host system in which the infectious agent is propagated and does not contain any material from an infectious agent.

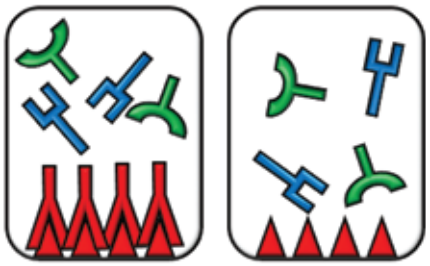


Figure 2: Diluted serum samples are added to individual wells and incubated. Ideally, bound antibodies are antigen-specific ( ). In practice, however, they may bind non-specifically (or). To decrease the possibility of non-specific protein-protein or protein-plastic interactions, a blocking buffer is used to dilute the serum and only high-purity antigens are used.

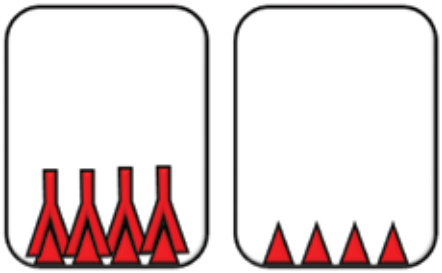
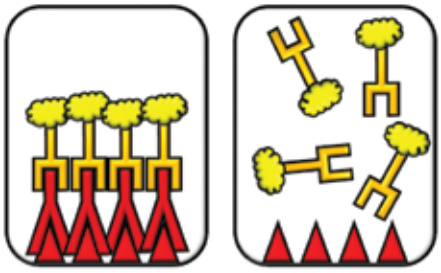
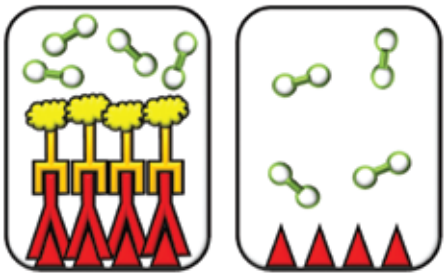


Figure 3: Unbound antibodies are removed by washing the test plate.



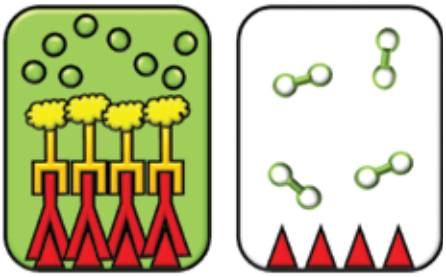
Positive Sample Negative Sample

Figure 4: An enzyme-conjugated, species-specific, anti-immunoglobulin ( ) (e.g. horseradish peroxidase labeled anti-species IgG) is added to the plate. The anti-immunoglobulin binds to the antigen-antibody complexes. Following incubation, the test plate is washed *(not shown)* to remove excess anti-immunoglobulin.



Positive Sample Negative Sample

Figure 5: A chromogenic enzyme substrate ( ) is added to each well in order to detect the labeled antigen-antibody complexes.



Positive Sample Negative Sample

Figure 6: A subsequent colored reaction develops ( ). The rate of this reaction is proportional to the amount of antibodies from the specimen which have attached to the antigen in a well. Color intensity can be assessed visually or spectrophotometrically with an ELISA reader. The data can then be transformed to give a 'result' of positive, negative or indeterminate.

Advantages

Depending on sample volume, ELISA reagents can be less expensive than bead-based antibody detection methods (e.g., Multiplexed Fluorometric ImmunoAssay® (MFIA®)). Can screen large numbers of samples at once and can be adapted for automation. Simple technology allows generation of novel ELISA plates by individual laboratories.

Disadvantages

Generates large quantities of liquid and plastic waste. Use of poor-quality antigen can result in non-specific reactions and false positive/negative results. Limited to testing one antigen per well. Assay controls are in separate wells from test samples of interest. As with all serologic tests, it does not detect the infectious organism; only provides a historical indication of infection (antibodies). As they do not produce antibodies, ELISA is not suitable for use with immunodeficient animals.