

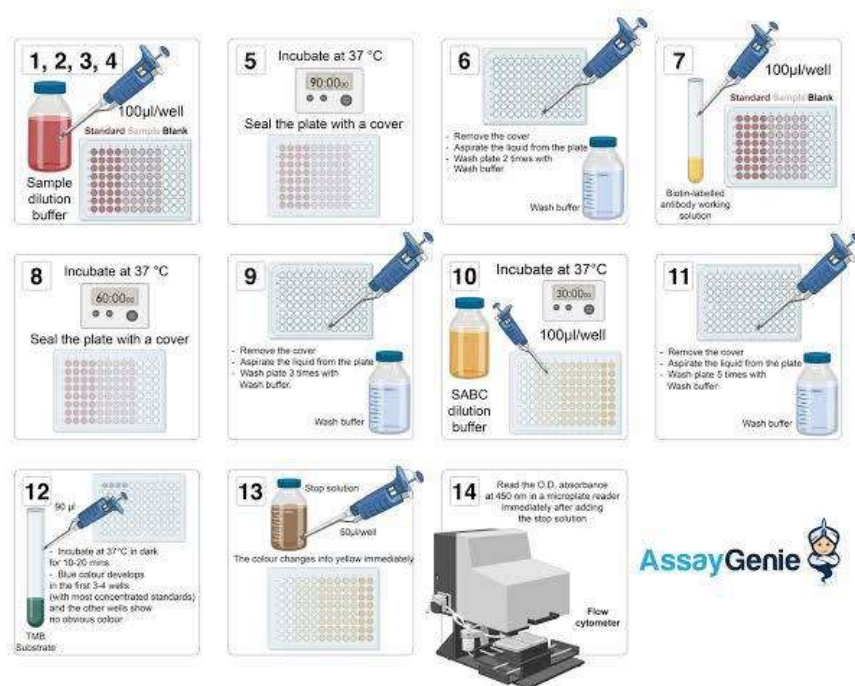
ELISA (Enzyme Linked Immunosorbent Assay) Testing in Blood Center

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ABSTRACT: ELISA tests are frequently used in blood centers to screen donated blood for infectious markers like HIV, Hepatitis B, and Hepatitis C.

Steps Of Elisa:



Material Required & Procedure :

ELISAs are performed in polystyrene plates, typically 96-well plates.

Depending on the ELISA type, testing requires a primary and secondary detection antibody, antigen, coating antibody & antigen, buffer, wash, and substrate/chromogenic.

The primary detection antibody is a specific antibody that only binds to the protein of interest. In contrast, a secondary detection antibody is a second enzyme-conjugated antibody that binds to a primary antibody that is not enzyme-conjugated.

There are four main general steps to completing an ELISA immunoassay. These steps are:

1. Coating (with either antigen or antibody)
2. Blocking (typically with the addition of bovine serum albumin [BSA])
3. Detection
4. Final read

Detection is carried out by adding a substrate that can generate a color. There are many substrates available for use in ELISA detection. However, the substrates most commonly used are horseradish peroxidase (HRP) and alkaline phosphatase (AP).

Between each of the above four steps is a “wash” of the plate using a buffer, such as phosphate-buffered saline (PBS) and a non-ionic detergent, to remove unbound material. The wells are washed two or more times during each wash step, depending on the specific protocol.

In a usual ELISA protocol, a serial dilution of concentrations is placed in the wells of the plate. After the results are measured, a standard curve from the serial dilutions data is plotted with a concentration on the x-axis using a log scale and absorbance on the y-axis using a linear scale.

Types of ELISA:

Direct ELISA

Both direct and indirect ELISAs begin with the coating of antigens to the ELISA plates.

Indirect ELISA

The steps of the indirect ELISA are identical to the direct ELISA, except for an additional wash step and the types of antibodies added after the buffer is removed. Indirect ELISA requires two antibodies: a primary detection antibody that sticks to the protein of interest and a secondary enzyme-linked antibody complementary to the primary antibody.

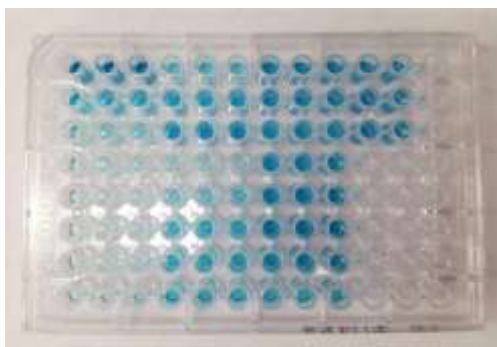
Sandwich ELISA

Unlike direct and indirect ELISA, the sandwich ELISA begins with a capture antibody coated onto the wells of the plate.

Competitive ELISA

The competitive ELISA tests for the presence of an antibody specific for antigens in the test serum.

Elisa Plate:



Result:

Once color changes are measured from the assay, the results are graphed either on paper or in software. Typically, the graph compares optical density to log concentration, which gives a sigmoidal curve. Known concentrations give the graph's standard curve, and measurement of unknowns can then occur when sample values are compared to the linear portion of the graphed standard curve.

Reference:

1. Elisa literature
2. Meta Version Introduce by what's app

3. https://www.google.com/search?q=steps+of+elisa&sca_esv=34b770eb4fc2a6ee&rlz=1C1CHBD_enIN906IN906&udm=2&biw=1366&bih=600&ei=1Uj-Z_ypFt-NnesPtLOxwQo&ved=0ahUKEwi86OaW_dmMAxXfRmcHHbRZLKgQ4dUDCBE&uact=5&oq=steps+of+elisa&gs_lp=EgNpbWciDnN0ZXBzIG9mIGVsSaXNhMgUQABiABDIKEAAYgAQYQxiKBTIFEAAAYgAQyBRAAGIAEMgQQABgeMgQQABgeMgQQABgeMgYQABgFGB4yBhAAGAUYHjIGEAAAYBRgeSlahJVCg8yRY9ZglcAF4AJABAjgBzgGgAa0PqgEGMC4xMy4xuAEDyAEA-AEBmAIOoAKwEcICCAAGIAEGLEDGIMBwgIOEAAAYgAQYsQMYgwEYigXCaggQABiABBixA8ICDRAAGIAEGLEDGEMYigWYAwwCIBgGSBwYwLjEzLjGgB4hPsgcGMC4xMy4xuAewEQ&scient=img#vhid=W-55jalrl0EdSM&vssid=mosaic
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