

Elisa test for hiv: limitations



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The full name of ELISA is called the Enzyme-Linked ImmunoSorbent Assay (5). ELISA is used for diagnosing numerous different conditions. As Virtual Medical Centre stated that it is commonly requested if it is suspected you have been exposed to viruses such as HIV and Hepatitis B or C, or bacteria and parasitic infections such as Toxoplasmosis, Lyme disease and Helicobacter pylori (7). Other uses of the ELISA include detection of prohibited drugs such as cocaine and methamphetamines (7). ELISA is also a very sensitive immunoassay and is usually the first test performed on potentially Human Immunodeficiency Virus (HIV)-infected blood (4, 8-9). This test involves an enzyme and antibody or antigen (5). Results can be positive or negative.

How the test is performed

The goal of an ELISA is to find out if a particular protein is present and how much does it contain in a sample (3). There are a few ways for performing an ELISA. Here is one of the commonly used methods and the one significant to detecting HIV antibodies:

ELISAs are performed in 96-well plates which allow high throughput results (2-3). The bottom of each well is coated with prepared HIV protein to which will bind the antibody (diagram 1) (2-3).

Entire blood is allowed to clot and the cells are centrifuged out to obtain the clear serum with antibodies which is called the primary antibodies (2). The serum is incubated in a well, and each well contains a different serum (diagram 2) (2-3).

The antibodies of the patient in the serum. If the patient is HIV positive, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate (2).

The serum is removed after a period of time, and weakly adherent antibodies are washed off with a series of buffer rinses (9). A secondary antibody is added to each well to detect the bound antibodies (diagram 3) (2-3).

Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies (2).

An enzyme such as peroxidase or alkaline phosphatase is attached to the secondary antibody (3). These enzymes can metabolize colourless substrates called chromagens into colour products (diagram 4) (2, 9).

After an incubation period, the secondary antibody solution is removed and weakly adherent ones are washed off as before (3). The final step is the adding up of the enzyme substrate and the production of colour product in wells with secondary antibodies bound (3, 9).

When the enzyme reaction is complete, the entire plate is placed into a plate reader and the optical density is determined for each well (3). The amount of colour produced is correspondent to the amount of primary antibody bound to the proteins on the bottom of the wells (diagram 5) (9).

Limitations of this test

A negative aspect of the ELISA is the occurrence of false positive or false negative result. As the Virtual Medical Centre and Stowell stated an example such that the antibodies induced by a recent flu injection can cause a

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positive result and so although ELISA is an important diagnostic test for HIV, in various cases it is usually followed up by a confirmatory test (7, 9). False positives may also occur if you have an underlying condition such as Lupus or rheumatoid disease (7). However, a negative result does not always mean there is no infection as some antibodies are not produced at once following infection (9). The Virtual Medical Centre reported that the antibodies to HIV do not appear in blood until 6 weeks after exposure to the virus so it is recommended that if the individual suspect he or she has been exposed then the test should be repeated after 6 weeks to 6 months (7).

Other assays are routinely performed to detect HIV

There are a number of tests besides ELISA can find antibodies or genetic material (RNA) to the HIV virus. These tests include the most common examination used that is a Western blot test. A positive ELISA test is always followed by a Western blot test and a positive Western blot confirms an HIV infection (6). A negative Western blot test means the ELISA test was a false positive test (4, 6). However, negative tests do not rule out HIV infection (1, 4). There is a period called the window period-the time between HIV infection and the appearance of anti-HIV antibodies that can be measured, thus a negative HIV ELISA and Western blot will not rule out HIV infection (6, 8). In addition, the Polymerase Chain Reaction (PCR) finds either the RNA of the HIV virus or the HIV's DNA in white blood cells infected with the virus (8). The PCR test is very useful to find a very recent infection, determine if an HIV infection is present when antibody test results were uncertain, and screen blood or organs for HIV before donation (8). However, PCR test is not done as regularly as antibody assay because it requires technical skill and expensive

equipment (4, 8). In addition, the Indirect Fluorescent Antibody (IFA) test also detects HIV antibodies (8). It is used to confirm the results of an ELISA just like a Western blot test. But it is more expensive than a Western blot test and not frequently used (8).