

## TEXT SUPPLEMENT (Ch 16): Tests for HIV Infection

### ELISA and Western Blot Tests for Detection of Antibodies to HIV

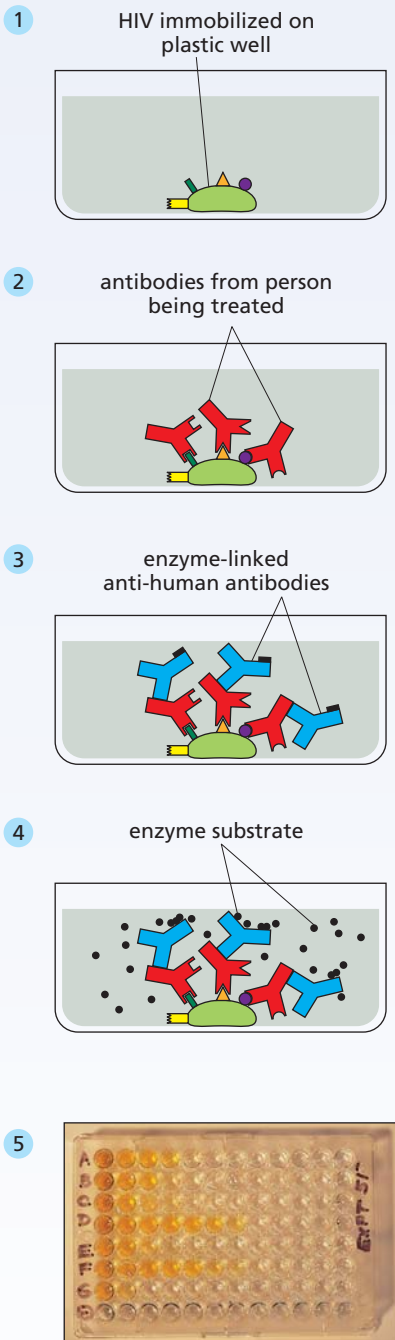
The ELISA and the Western blot test use immunological techniques to detect HIV-specific antibodies in a person's blood and thus are called immunodiagnostic techniques. In the ELISA (Enzyme-Linked ImmunoSorbent Assay), laboratory-grown HIV particles are immobilized onto a surface, generally small wells made of a plastic designed to bind protein molecules tightly (1). The rest of the plastic is coated with other proteins to block any non-specific binding of proteins used later in the assay. The immobilized virus particles then act as binding sites for specific antibodies: they are exposed to blood serum from the person being tested, and if that person's serum contains antibodies whose specific binding sites match molecules on the virus, the antibodies bind to the immobilized virus (2). There are many other antibody molecules in the person's blood that do not match any HIV molecule; these do not bind. The wells are then rinsed, removing any unattached antibody molecules. The viral molecules and any specific anti-HIV antibodies bound to them are so tightly attached that they do not wash away. Anti-HIV antibodies are then detected by a second antibody to which an enzyme has been attached (3). The binding sites on the enzyme-linked antibody match amino acid sequences on human antibody molecules; they bind, not to the plastic or to the HIV, but to any human antibodies present. Again any unbound antibody is washed away, then enzyme substrate is added (4). Enzymes are proteins that catalyze biochemical reactions; the enzymes used in these tests cause a color change in the medium. A well in which the medium has changed color (from clear to yellow in this photo, part 5) is thus a well in which the blood plasma used in the test contained

antibody specific for HIV, an initial bit of evidence that the person is HIV positive.

Rows A to G are sera from different people. Many dilutions of each serum are tested, with wells to the right in each row being the most dilute. The yellow color thus decreases from left to right across a row; the more wells that are yellow in a row, the more antibody was present in the sample. The bottom row is a negative control sample with no anti-HIV antibody.

If an ELISA suggests the presence of anti-HIV antibodies, the Western blot test is done. The viral proteins are separated by using a technique called electrophoresis (1) (see Figure 3.8, p. 73). The separated proteins are then transferred out of the gel onto special paper that has a high affinity for proteins (2). The paper is then exposed to blood serum from the person being tested. Specific antibodies bind, but in this case they bind not to the whole virus but to some individual viral protein. The serum may contain specific antibodies that bind to some viral proteins but may lack antibodies to other viral proteins (3). Unbound antibody is rinsed away and bound antibody is detected, as in the ELISA, with enzyme-linked antibody that binds to all human antibodies (4). If the enzyme-linked antibody finds human antibody to bind to, the enzyme makes dark bands on the paper where the blood contained antibody specific for that viral protein; where there is no specific antibody, no band appears (5). If antibody to specific proteins is present, the person has tested HIV positive (also referred to as seropositive, because the test is done on serum, the liquid part of the blood after blood has been allowed to clot).

## ELISA TEST



## WESTERN BLOT TEST

