HIV attacks a type of immune system cell called the **T–helper** cell. This cell carries on its surface a protein called CD4, which HIV uses to attach itself to the cell before gaining entry.

The T–helper cell plays an important part in the immune system by helping to **co–ordinate all the other cells to fight illnesses**
. A major reduction in the number of T–helper cells can have a serious effect on the immune system.

HIV causes many T–helper cells to be damaged or destroyed. As a result, there are fewer cells available to help the immune system to fight illnesses.

The CD4 test measures the number of T–helper cells in your blood. The more cells you have per cubic millimetre of blood, the stronger is your immune system. The stronger your immune system, the better your body can fight illnesses. A low CD4 count does not mean that you will certainly become ill, but it makes it more likely.

HIV, our Immune System and Antibodies

Antigens are large molecules (usually proteins) on the surface of cells, viruses, fungi, bacteria, and some non–living substances such as toxins, chemicals, drugs, and foreign particles. The immune system recognizes antigens and produces **antibodies** that destroy substances containing antigens. After getting the HIV infection, our immune system starts producing antibodies to HIV. These antibodies are the source for testing the HIV infection.

The dangerous "Window period".

If you become infected with HIV, it usually takes between three weeks and **two months** for your immune system to produce antibodies to HIV. This period may extend upto
6 months
in some cases(5%). If you think you were exposed to HIV, you should wait at least for two months before being tested. You can also test right away and then again after two or three months. During this "window period" an antibody test may give a negative result, but you can transmit the virus to others if you are infected.

Antiretroviral therapy during the window period can delay the formation of antibodies and extend the window period **beyond 12 months**.

Different Types of HIV Tests.

Antibody test results for HIV are accurate more than 99.5% of the time. Before you get the results, the test has usually been done two or more times. The **first test** is called an "EIA" or **"E LISA"**
test.
Before a positive ELISA test result is reported, it is confirmed by another test
called a
"Western Blot."

ELISA

The **ELISA test**, or the enzyme immunoassay (EIA), was the first screening test commonly

employed. It has a high sensitivity.

The test proceeds by the general ELISA method: the person's serum is diluted 400 fold and applied to a plate to which HIV antigens have been attached. Some of the antibodies in the serum may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. Then a specially prepared "Secondary antibody" an antibody that binds to human antibodies is applied to the plate, followed by washes. This secondary antibody is chemically linked in advance to an enzyme. Thus the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. As the ELISA results are reported as a number, the most controversial aspect of this test is deciding the "cut off" point between positive and negative.

Western blot

The **Western blot test** uses the general Western blot procedure. HIV infected cells are opened and the contained proteins are entered into a slab of gel to which a voltage is applied. Different proteins will move with different velocities in this field, depending on their size, while their electrical charge is leveled by a substance, called sodium lauryl sulfate. Once the proteins are well separated, they are transferred to a membrane and the procedure continues similar to ELISA: the person's diluted serum is applied to the membrane and antibodies in the serum may attach to some of the HIV proteins. Antibodies which do not attach are washed away, and enzyme linked antibodies with the capability to attach to the person's antibodies first detect to which HIV proteins the person has antibodies.

OraQuick

Oraquick is an antibody test that provides results in 20 minutes. The blood, plasma or oral fluid is mixed in a vial with developing solution, and the results are read from a sticklike testing device.

Orasure

Orasure is an HIV test which uses mucosal transudate from the tissues of cheeks and gums. It is an antibody test which first employs ELISA, then Western Blot.

There is also a **urine test** it employs both the ELISA and the Western Blot method.

Antigen tests

The **p24 antigen test** detects the presence of the p24 protein of HIV (also known as CA), a major core protein of the virus. Monoclonal antibodies specific to the p24 protein are mixed with the person's blood. Any p24 protein in the person's blood will stick to the monoclonal antibody and enzyme–linked antibody to the monoclonal antibodies to p24 causes a color change if p24 was present in the sample.

This test is now used routinely to screen blood donations , thus reducing the window to about 16 days. It is not useful for general diagnostics, as it has very low sensitivity and only works during a certain time period after infection before the body produces antibodies to the p24 protein.

Nucleic acid based tests

Viral load tests detect pieces of HIV genetic material. They show up before the immune system manufactures antibodies. Also, in early 2002, the FDA approved “Nucleic acid testing.” It is similar to viral load testing. Blood banks use it to screen donated blood.

The viral load or nucleic acid tests are generally not used to see if someone has been infected with HIV because they are much more expensive than an antibody test. They also have a slightly higher error rate.

Nucleic acid based tests amplify and detect a 142 base target sequence located in a highly conserved region of the HIV gag gene. Since 2001, donated blood in the United States, has been screened with nucleic acid based tests, shortening the window to about 12 days. Since these tests are relatively expensive, the blood is screened by first pooling some 10–20 samples, testing these together, and if the pool tests positive, each sample is retested individually. A different version of this test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV–1 infected patients.

In the **RT–PCR test**, the viral RNA is extracted from the patient’s plasma and is treated with reverse transcriptase so that the RNA of the virus is transcribed into DNA . The polymerase chain reaction (PCR) is applied, using two primers thought to be unique to the virus’s genome. After the PCR amplification process is completed, which takes some time, the resulting amplified segments bind to specific oligonucleotides bound to the vessel wall and are then made visible with a probe that’s bound to an enzyme. The amount of virus in the sample can be quantified with sufficient accuracy to detect three fold changes.

In the **Quantiplex bDNA** or **branched DNA test** plasma is centrifugated to concentrate the viruses, which are then opened to release the RNA. Special oligonucleotides are added which bind to viral RNA and to certain oligonucleotides bound to the wall of the vessel. In this way, viral RNA is fastened to the wall. Then new oligonucleotides are added which bind at several locations to this RNA; and other oligonucelotides which bind at several locations to those oligonucleotides. This is done to amplify the signal. Finally, oligonucleotides that bind to the last set of oligonucleotides and that are bound to an enzyme are added; the enzyme action causes a color reaction which allows to quantify the viral RNA in the original sample. Monitoring the effects of antiretroviral therapy by serial measurements of plasma HIV–1 RNA with this test has been validated for patients with viral loads greater than 25,000 copies per millilitre.

Children born to HIV–positive mothers may have false positive test results for several months because mothers pass infection–fighting antibodies to their newborn children. Even if the children are not infected, they have HIV antibodies and will test positive. Other tests, such as a viral load test, must be used.