Lab Investigation

Elisa\Rapid tests

Who should undergo the HIV test?

The HIV test should be undertaken by people who have been exposed to situations which are considered risky in transmission of HIV infection. HIV is mainly transmitted through three routes namely;

- Sexual
- Parenteral
- From an HIV infected mother to her baby

Considering these routes of transmission, the HIV test should be Undertaken by the following category of people;

1. Those persons who have more than one sex partner
2. Those persons who have a sexually transmitted disease.
3. Those persons who indulge in needle sharing for intravenous drug use
4. Those persons who have received transfusion of a blood unit which was not tested for HIV
5. Those children who are born to an HIV-infected women
6. Sex partners of an HIV- infected person/
7. Rape victims
8. Health care workers who have received an accidental needle stick injury while working from a person known to be HIV- infected or a person with unknown HIV status.
9. Persons suffering from diseases associated with AIDS

Why Should you Be tested?

Pros

- If you know you are HIV-positive, you can take advantage of immune system monitoring and early treatment and intervention.
- By taking the test, you can find out whether or not you can infect others.
- Regardless of the result, testing often increases your commitment to overall good health habits.
- If you test negative, you may feel less anxious after testing.
- Women and their partners considering pregnancy can take advantage of treatments that potentially prevent transmission of HIV to the baby.

Cons:

- If you test positive, you may show an increase in anxiety and depression.
- When testing is not strictly anonymous, you risk job and insurance discrimination. You can prevent this by ensuring that you test at an anonymous testing site.

Whether or not to take the antibody test is an extremely personal decision. We cannot make that decision for anyone. We can only advise you of the implications of a positive and a negative result in your life. The decision is yours.

Q. Should I Get Tested?
Ans. For some people taking the HIV antibody test can be a scary decision. Some people get tested every six months, even if they practice safer sex. No matter the reasons, taking the HIV antibody test can be a good idea. Sometimes taking the test is a way to make a new found commitment towards safer practices.

One thing that is important to remember is that getting tested for HIV will not change your HIV status, just tell you whether or not you have it. With all the new treatments available finding out your HIV status early on can extend your life.

To find out if you are at risk for HIV, ask yourself the following questions:

- Have you had unprotected vaginal, oral or anal sex (e.g., intercourse without a condom, oral sex without a latex barrier)?
- Have you shared needles to inject street drugs or steroids or to pierce your skin?
- Have you had a sexually transmitted infection (STI) or unwanted pregnancy?
- Have you had a blood transfusion or received blood products

Would I know whether I was infected with HIV if I got tested straight away?

Infection with HIV has no specific symptoms. The only way you can find out for sure if you are infected with HIV is by taking the HIV antibody test.

The HIV antibody test looks for antibodies to the virus in a person's blood. For most people these antibodies take 3 months to develop. In rare cases, it can take up to 6 months. It would be extremely uncommon to take longer then 6 months to develop detectable antibodies.

Getting tested before the 3 month period is up may result in an unclear test result, as an infected person may not have developed antibodies to HIV yet. So it is best to wait for at least three months after the last time you were at risk before taking the test.

It is also important that you are not at risk of further exposures to HIV during this time period. Most importantly you should continue to practice safe sex and not share needles.

What is an HIV antibody test?

An antibody test is one type of HIV test. This test shows whether a person has been infected with HIV. This test looks for HIV antibodies in a person's

blood. When HIV enters a person's body, special chemicals are produced. These are called antibodies. Antibodies are the body's response to an infection. So if a person has antibodies to HIV in their blood, it means they have been infected with HIV

What does the HIV test involve?

It is recommended that you get the HIV test done at a health clinic or at a HIV/AIDS voluntary counselling and testing (VCT) site. When you attend to get tested, you will see a doctor, trained counsellor, a nurse or some other health professional in private. He or she will explain what the test involves and what the result means.

Normally a small sample of blood will be taken from your arm, sent to a laboratory and tested. The test is always strictly confidential and only goes ahead if you agree. Your personal doctor will not be told about the test without your permission. Depending on the test used, it can take anything from a few days to a week or longer to get the result back.

What is a rapid test?

A rapid HIV test is also an antibody test. The advantage of a rapid test is that you do not have return to get your test result. The test results from a rapid test are usually available in approximately 30 minutes. Rapid tests are single-use, and do not require laboratory facilities or highly trained staff. This makes rapid tests particularly suitable for use in resource-limited countries.

What is Window Period?

The "window period" is the time it takes for a person who has been infected with HIV to *seroconvert* (test positive) for HIV antibodies."Antibodies generally appear within three months after infection with HIV, but may take up to six months in some persons."

What does window period mean?

- Many people will have detectable antibodies in three or four weeks. Most seroconvert at 3 months. Very, *very* rarely (i.e., only a few cases *ever*), a person could take six months to produce antibodies.
- One may be anxious to be tested soon after an encounter which one perceive to be risky You may have "heard" that AIDS/HIV can take years to be detectable. Here's the clarification: *AIDS*, or the clinical symptoms that define it, takes many years to develop after exposure. *HIV* -- the virus that causes AIDS -- is usually detectable within three months after exposure, and does not cause symptoms in most people.

How do I interpret the results of antibody test?

A **positive** result means:

- You are HIV-positive (carrying the virus that causes AIDS).
- You can infect others and should try to take precautions to prevent doing so.

A **positive** result does *NOT* mean:

- You have AIDS.
- You will necessarily get AIDS.
- You are immune to AIDS, even though you have antibodies.

A **negative** result means:

- No HIV antibodies were found in your blood at this time.

A **negative** result does *NOT* mean:

- You are not infected with HIV (you may still be in the "window period").
- You are immune to AIDS.
- You have a "resistance" to infection.
- You will never get AIDS. You may wish to consider avoiding unsafe activities to protect yourself.

An **indeterminate** result (which is rare) means:

- The Western Blot (WB) result is unclear. The entire HIV test must be repeated with a new blood sample, usually several weeks after the first blood test.

- Indeterminate results usually occur if the test is performed just as the person begins to seroconvert.

Should I do Western Blot?

The Western Blot is an antibody test, is one of the tests used to confirm the diagnosis of HIV infection. A positive Western Blot result is synonymous with HIV infection and the attendant risk of developing AIDS. However, a number of concerns were raised around the specificity, reliability and reproducibility of the Western Blot test.

The Western Blot should not be used to confirm and validate the results of the ELISA test since the Western Blot and ELISA tests are based on the same antibody reaction mechanism. As with the ELISA test, another concern over the use of the Western Blot test is its non-specific positive reaction to a number of diseases (including tuberculosis, a variety of parasitic infections and other viral infections) in the absence of HIV infection. The antigens used in the Western Blot test may be similar or identical to other human proteins, and hence the results of the Western Blot may thus not provide an indication of HIV infection.

Can the diagnosis of HIV infection be ascertained by doing ELISA for HIV-1 & -2 alone or is the PCR test necessary for detection?

The Polymerase Chain Reaction (PCR) is an advanced test which can detect the presence of HIV even if it is present in a small quantity in the blood. Generally the ELISA test for HIV-1 &-2 might require up to three months for becoming positive after the entry of HIV in the body. However, the PCR can detect the presence of HIV in the blood much earlier. This test can reduce the window period considerably. One must remember that the PCR can be false positive as well as false negative. Hence, one needs to be cautious in its interpretations. The PCR ideally should be undertaken when the ELISA test for HIV-1 & -2 is negative but the history is strongly suggestive of exposure and there is evidence that the patient has developed symptoms of acute primary HIV infection. Irrespective of the PCR result the confirmation should to be done by undertaking the ELISA test. Since ELISA is essential for confirmation of the PCR results, one may choose to undertake ELISA for HIV-1 & -2 instead of the PCR. Besides, the PCR is a costly test.

What is the difference between an Anonymous and a Confidential Test?

Anonymous and Confidential use the same testing method. The only difference is one does not have your name attached to the results.

Anonymous antibody testing is available at Anonymous Test Sites in most California counties. Anonymous testing means that absolutely no one has access to your test results since your name is never recorded at the test site.

Confidential antibody testing means that you and the health care provider know your results, which may be recorded in your medical file.

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If I test HIV negative, does that mean my partner is HIV negative too?

No. Your HIV test results reveals only your HIV status. Your negative test result does not indicate whether or not your partner has HIV. HIV is not necessarily transmitted every time there is an exposure. Therefore, you taking an HIV test should not be seen as a method to find out if your partners is infected.

CD4

What Are T-Cells?

T-cells are a type of lymphocyte (white blood cell). They are an important part of the immune system. There are two main types of T-cells. **T-4 cells**, also called CD4+, are "helper" cells. They lead the attack against infections. **T-8 cells** (CD8+) are "suppressor" cells that end the immune response. CD8+ cells can also be "killer" cells that kill cancer cells and cells infected with a virus.

Researchers can tell the T-cells apart by specific proteins on the cell surface. A T-4 cell is a T-cell with CD4 molecules on its surface. This type of T-cell is also called "CD4 positive," or CD4+.

Why Are T-Cells Important in HIV?

When HIV infects humans, the cells it infects most often are CD4+ cells. The virus becomes part of the cells, and when they multiply to fight an infection, they also make more copies of HIV.

When someone is infected with HIV for a long time, the number of CD4+ cells they have (their T-cell count) goes down. This is a sign that the immune system is being weakened. The lower the T-cell count, the more likely the person will get sick.

There are millions of different families of T-cells. Each family is designed to fight a specific type of germ. When HIV reduces the number of T-cells, some of these families can be totally wiped out. You can lose the ability to fight off the particular germs those families were designed for. If this happens, you might develop an opportunistic infection (see Fact Sheet 500).

What Factors Influence a T-Cell Count?

The T-cell value bounces around a lot. Time of day, fatigue, and stress can affect the test results. It's best to have blood drawn at the same time of day for each T-cell test, and to use the same laboratory.

Infections can have a large impact on T-cell counts. When your body fights an infection, the number of white blood cells (lymphocytes) goes up. CD4+ and CD8+ counts go up, too. Vaccinations can cause the same effects. Don't check your T-cells until a couple of weeks after you recover from an infection, or after you get a vaccination.

How Are the Test Results Reported?

T-cell tests are normally reported as the number of cells in a cubic millimeter of blood, or mm^3. There is some disagreement about the normal range for T-cell counts, but normal CD4+ counts are between 500 and 1600, and CD8+ counts are between 375 and 1100. CD4+ counts drop dramatically in people with HIV, in some cases down to zero.

The ratio of CD4+ cells to CD8+ cells is often reported. This is calculated by dividing the CD4+ value by the CD8+ value. In healthy people, this ratio is between 0.9 and 1.9, meaning that there are about 1 to 2 CD4+ cells for every CD8+ cell. In people with HIV infection, this ratio drops dramatically, meaning that there are many times more CD8+ cells than CD4+ cells.

Because the T-cell counts are so variable, some doctors prefer to look at the T-cell percentages. These percentages refer to total lymphocytes. If your test reports CD4+% = 34%, that means that 34% of your lymphocytes were CD4+ cells. This percentage is more stable than the number of T cells. The normal range is between 20% and 40%. A CD4+ percentage below 14% indicates serious immune damage. It is a sign of AIDS in people with HIV infection.

What Do the Numbers Mean?

The meaning of CD8+ cell counts is not clear, but it is being studied.

The CD4+ cell count is a key measure of the health of the immune system. The lower the count, the greater damage HIV has done. Anyone who has less than 200 CD4+ cells, or a CD4+ percentage less than 14%, is considered to have AIDS according to the US Centers for Disease Control.

CD4+ counts are used together with the viral load to estimate how long someone will stay healthy. See Fact Sheet 125 for more information on the viral load test.

> CD4+ counts are also used to indicate when to start certain types of drug therapy

Viral load / PCR Testing

What Is Viral Load?

Viral load testing is the direct measurement of the amount of HIV present in the blood. Several different tests identify and measure the genetic material resulting from virus infection, either RNA or DNA. These tests are also called nucleic acid tests. The laboratory procedure used to test for the genetic material of HIV is called the Polymerase Chain Reaction (PCR) test. PCR viral load testing is usually done to allow doctors to track how active HIV is in a person's body to help make antiviral treatment decisions.

A person with an "undetectable" viral load is still infected with HIV and can still infect others. Viral loads measure the amount of virus in the blood; most transmission is sexual and sexual fluids may contain measurable virus even when blood virus is undetectable. Generally, however, the higher one's viral load the more likely one is to transmit the virus and the lower one's viral load the less likely one is to transmit the virus.

How Are Changes in Viral Load Measured?

Repeat tests of the same blood sample can give results that vary by a factor of 3. This means that a meaningful change would be a drop to **less than 1/3** or an increase to **more than 3 times** the previous test result. For example, a change from 200,000 to 600,000 is within the normal variability of the test. A drop from 50,000 to 10,000 would be significant. The most important change is to reach an undetectable viral load.

Viral load changes are often described as "log" changes. This refers to scientific notation, which uses powers of 10. For example, a 2-log drop is a drop of 10^2 or 100 times. A drop from 60,000 to 600 would be a 2-log drop.

What Do the Numbers Mean?

There are no "magic" numbers for viral loads. We don't know how long you'll stay healthy with any particular viral load. All we know so far is that lower is better and seems to mean a longer, healthier life.

U.S. treatment guidelines suggest that **anyone with a viral load over 100,000 should be offered treatment.**

Some people may think that if their viral load is undetectable, they can't pass the HIV virus to another person. This is not true. **There is no "safe" level of viral load.** Although the risk is less, **you can pass HIV to another person even if your viral load is undetectable.**

Q. On viral load tests, what is considered a high viral load and what is considered a low one? What are these tests used for?

Ans. Viral load tests measure how much of the HIV virus is in the bloodstream. They are very new tests and can be very expensive A result below 10,000 is considered a low result. A result over 100,000 is considered a high result. The primary use of these tests is to help determine how well a certain antiviral drug is working. If the viral load is high, your physician may consider switching you to another drug therapy. The viral load tests are best used if trends in results are compared over time. If the viral load increases over time, then the drug treatment may need to be changed. If the viral load goes down over time, antiviral treatment may be working for you. So rather than just taking 1 test, a series of viral load tests gives much more useful information. Of course, antiviral therapy must not be determined by this test alone. Other tests (like CD4 cell counts) are also important indicators as to how well antiviral therapy is working. It is presently not known what a test result between 10,000 and 100,000 means. That's why trends in viral load tests are of much greater value. Current guidelines (see Fact Sheet 404) suggest measuring baseline (pre-treatment) viral load. A drug is "working" if it lowers viral load by at least 90% within 8 weeks. The viral load should continue to drop to less than 50 copies within 6 months. The viral load should be measured within 2 to 8 weeks after treatment is started or changed, and every 3 to 4 months after that.

P24/PCR

When Is Viral Load Testing Inappropriate?

The vast majority of people concerned about HIV infection ***do not*** need viral load testing. In nearly all cases, this is ***not a test we recommend for assessing one's HIV status***.

- Why shouldn't someone use a PCR test to get quicker HIV test results?
  Answer: Because the test was not designed for that purpose, it could easily report either false positive or false negative, it is much more expensive than antibody testing and any PCR result must be followed by an antibody test after the appropriate interval in order to be meaningful.
- Can someone get a PCR test to diagnose HIV faster than antibody screening?
  Answer: Yes, if they are willing to pay for it.
- Does it give them a true report of their HIV status?
  Answer: NO! They will still need an antibody test later, after waiting through the window period.

In many cases, people who want PCR testing to determine their HIV status are highly concerned/anxious. They are unwilling to wait through a three to six month window period and take an antibody test. They may have heard that PCR testing will indicate HIV status sooner than antibody testing

What is a p24 antigen test?

A p24 antigen test is a type of HIV test. It is primarily used to screen the blood supply but in some places it is used for testing for HIV. The p24 antigen is a protein that is part of the HIV. Early in the infection, it is produced in excess and can be detected in the blood serum by a commercial test. The p24 test can detect HIV infection before the HIV antibody test can and it is recommended you take the test 2-3 weeks after a risk exposure. Therefore, p24 antigen test is used in diagnosing HIV early in the course of infection.

If a p24 test becomes negative after being positive earlier, does it men
mean that the person has been cured of HIV infection?

No, **p24 is a core protein of the virus. Once HIV enters the body, it I found in high concentration in the blood. The p24 test is found to be positive at this time. After about two to six months, the virus prefers to go into hiding in the lymph nodes. At that time the p24 test is likely to become negative. However, this is a normal course of HIV disease progression. It does not mean that the patient has been cured or that the body has been able to suppress the infection completely. If a PCR for HIV is done at this time it will reveal that the HIV is still present in the body. This anomaly is mainly due to the fact that the qualitative test for p24 antigen is crude and when the antibodies against the p24 protein are produced by the body, they bind to it. Such antibody bound form of the p24 antigen is not detected unless a procedure is done on the sample for dissociating the complex or a better test is used for detecting of the evidence of infection.**

Pediatric HIV diagnosis

Q. Can a baby have the HIV test?

Ans. Yes, but it will not necessarily show whether the baby is infected. This is because the test is for HIV antibodies and all babies born to mothers with HIV are born with HIV antibodies. Babies who are not infected lose their antibodies by the time they are about 18 months old. However most babies can be diagnosed as either infected or uninfected by the time they are 3 months old by using a different test, called

a PCR test. The PCR test is more sensitive than the HIV test, and is not used in the standard HIV testing of adults. The PCR test looks for the presence of HIV itself, not antibodies.

Q. Are all pregnant women tested?
Ans. Pregnant women are not automatically tested for HIV. In some ante-natal clinics the test is offered and in others women have to ask for it. All pregnant women can have an HIV test. A woman will never be tested without her consent. If a woman is not sure what the arrangements are at her ante-natal clinic, she can ask her doctor or midwife about an HIV test.

Q. What happens if a woman has a positive test result?
Ans. When a woman has a positive test result she should be able to plan with a doctor or midwife what happens next and arrange to have follow-up checks. She will be offered special medical care to reduce the risk of her baby being infected.

Some pregnant women with HIV decide to have their baby. Others choose to have a termination. The decision to terminate a pregnancy is very personal and difficult. Someone who has a termination needs time to grieve for the loss of their baby. Someone who is HIV positive also needs to think about how it will affect decisions about pregnancy in the future.
Q. Are all babies born to women with HIV also HIV positive?
Almost all babies born to mothers with HIV are born with antibodies against the virus, so they do test HIV seropositive at birth. Mothers transfer antibodies age\ainst diseases across the placenta, including HIV antibodies. What this means is that newborns who have an HIV-positive mother will test positive on an HIV antibody test, even if the baby doesnot have the virus. The mother's antibodies disappear after a few months, when the infant is capable of producing his or her own antibodies. If an infant is infected with HIV, their own antibodies replace the mother's and they will continue to test HIV positive. However, infants who are not themselves infected will usually begin HIV seronegative within first year of the birth process.
Q. Can a woman who gets HIV while she is pregnant give the virus to the fetus?
Yes. The chances are higher as the viral load is higher immediately after infection.

Q. Can an HIV-positive father pass on the virus during pregnancy if he has sex with the pregnant mother?
yes

Q. What are the possible advantages of testing for HIV in pregnancy?
Ans. If a pregnant woman has a positive test result there are now drugs that can reduce the risk of her passing HIV on to her baby in the womb or at birth. Delivery by elective Caesarean Section also reduces the risk of a baby becoming infected.

It is usually best for babies to be breast-fed. However, if a mother has HIV, beast-feeding will increase the risk of her baby becoming infected. If a pregnant woman has a negative test result this can be very reassuring.

What are the possible disadvantages of testing for HIV in pregnancy?
Ans. Some pregnant women feel that they could not cope with finding out that they have HIV and that they may have put their baby at risk.

A woman who is infected with HIV can still become pregnant and have a baby. Being pregnant will not increase her chances of developing AIDS. But, some doctors think that pregnancy will make a woman who already has AIDS more seriously ill.

If a woman's partner is not infected with HIV he is at risk of becoming infected if they have sexual intercourse without a condom. An HIV positive woman also has to consider how she will cope if her baby is infected with HIV. Some doctors think that a woman who has recently been infected, or a woman who has AIDS, is more likely to have an infected baby.

When should one test an infant born to an HIV-infected mother?

If the mother is infected with HIV, she would also have antibodies against HIV. These antibodies along with other antibodies will be transferred to a baby born to an HIV-infected mother. As the ELISA test for HIV infection is based on detection of antibodies to HIV, the test for HIV infection is based on detection of antibodies to HIV, the test shall be positive in all babies born to HIV-infected mothers. These antibodies generally disappear by the age of nine months. For the sake of diagnosis of HIV-infection in a child born to an HIV-infected mother, a positive ELISA after the age of 18 months is considered as confirmatory. If the ELISA is negative in the intervening period, it is indicative of HIV-uninfected status of the child.

When to do PCR test for diagnosis of HIV infection in infants?

Single DNA PCR test between 6 and 12 weeks of age
If DNA PCR positive, repeat test to confirm positive DNA PCR test
If DNA PCR negative, HIV antibody at ≥12 months (or 3 months post-cessation of breast feeding)

Child < 18 months of age
–DNA PCR (or RNA PCR) positive on *two* samples
•Child > 18 months of age
–HIV antibody positive
•Child of any age
–AIDS-defining illness
–DNA PCR (or RNA PCR) positive on *one* sample

How to diagnosis of acute retroviral syndrome?

The diagnosis of acute HIV-1 infection is based on the detection of HIV-1 replication in the absence of HIV-1 antibodies, as these are not yet present at this early stage of

infection. Different tests are available for diagnosis of acute HIV-1 infection. The most sensitive tests are based on detection of plasma HIV-1 RNA.

In a recently published study [30], all assays for HIV-1 RNA that were tested (branched chain DNA, PCR and GenProbe) had a sensitivity of 100 %, but occasionally (in 2 - 5 % of cases) led to false positive results. False positive results from these tests are usually below 2,000 copies HIV-1 RNA per ml plasma, and therefore far below the high titers of viral load normally seen during acute HIV-1 infection (in our own studies on average 13 x 10^6 copies HIV-1 RNA/ml with a range of 0.25 - 95.5 x 10^6 copies HIV-1 RNA/ml). Repetition of the assay for HIV-1 RNA from the same sample with the same test led to a negative result in all false positive cases. Measurement of HIV-1 RNA from duplicate samples therefore results in a sensitivity of 100 % with 100 % specificity. In contrast, detection of p24 antigen has a sensitivity of only 79 % with a specificity of 99.5 - 99.96 %. The diagnosis of acute HVI-1 infection must be subsequently confirmed with a positive HIV-1 antibody test (seroconversion) within the following weeks.