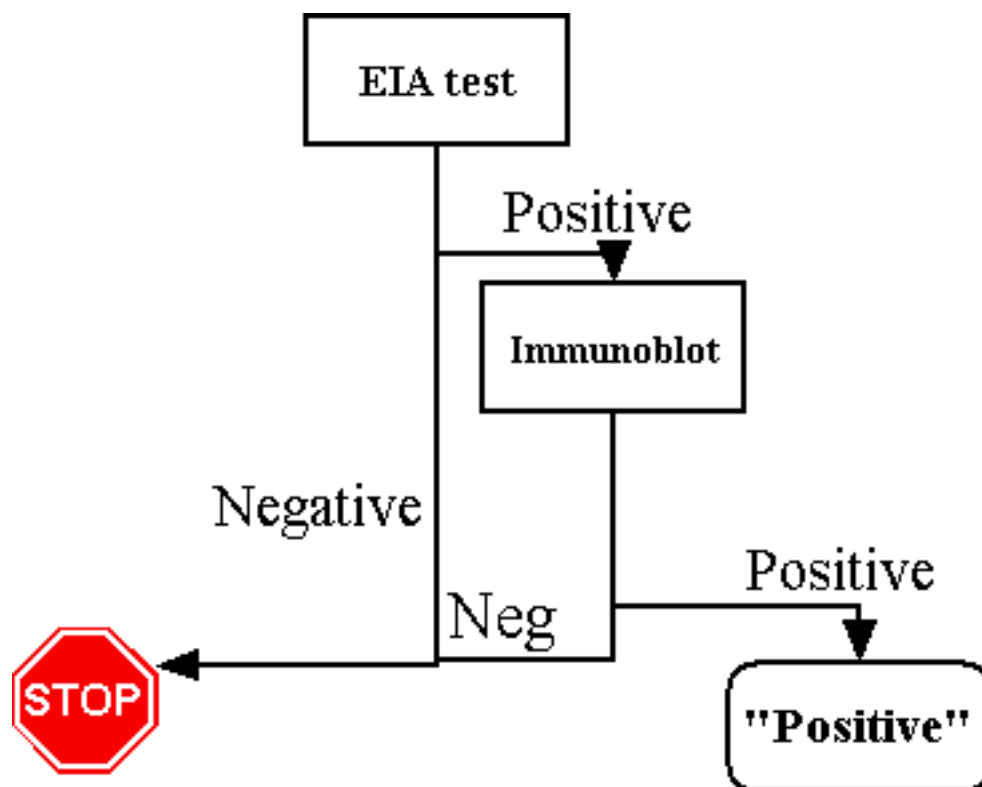


# HIV Antibody Testing at HUP

HIV blood antibody testing is used to diagnose HIV-1 infection by using a two-tiered testing protocol. First, a screening test for HIV-1, and in some cases HIV-2, antibodies is performed, using an enzyme immunoassay ("EIA") or immunochromatographic card assay. If positive, then a second test is done, using immunoblot technology. The immunoblot is also sometimes referred to as a "Western" blot. If both the EIA and the immunoblot are positive, then the overall test result is termed positive. Two-tiered testing is performed with two goals in mind, to detect all those who have HIV antibody, and to reduce as much as possible the chance of falsely-concluding that HIV antibodies are present when they in fact are not. The EIA or card test is highly sensitive, but not very specific. However when a second test is performed on EIA-positive specimens, the combination of both tests being positive is very specific. For example, in one [study](#) of 290,000 asymptomatic Minnesota blood donors donating 630,000 blood units, the false positive rate when both the screening and confirmatory assays were positive was 0% per donation (95% confidence interval 0% to 0.0006%).



## EIA test

Two types of HIV screening assays are used at HUP, the Abbott HIV-1, HIV-2 enzyme immunoassay and the OraQuick HIV-1/2 immunochromatographic card assay. The Abbott assay is performed five days per week, M-F, and is the routine screening assay performed except in special circumstances. The [OraQuick](#) card test is performed only for the following emergency situations: needle-stick incidents in staff and students being seen by Occupational Health, and for emergent listing of solid-organ transplant patients, and previously untested women in labor ("walk-ins"); this test is available seven days per week with a short (hours) turn-around-time. Both tests have nearly equivalent sensitivity and specificity for HIV-1/2. One large [study](#) of pregnant women found that the OraQuick test was much more specific than EIA testing in this population. The OraQuick test gives either a positive or negative visually read result, whereas the Abbott assay result is read by a spectrophotometer; the higher the specimen optical density, the more likely it is that the test result is truly positive. OraQuick positive tests are retested later using the Abbott assay, but an immunoblot is done on all OraQuick positive sera regardless of the EIA result. At HUP, about 74% of all positive Abbott EIA tests are confirmed to be positive by immunoblot, which reflects both the specificity of the screening assay and the pre-test probability of HIV infection in those being tested.

## Immunoblot test

The Bio-Rad (formerly Genetic Systems) HIV-1 immunoblot is used as the confirmatory test at HUP. This test utilizes a plastic membrane onto which electrophoresed HIV-1 proteins have been transferred. When reacted with patient serum, and then washed, antibody in the serum that reacts with the proteins is detected by use of an enzyme-conjugated secondary antibody to human globulins. The end result is a series of darkly colored lines on the membrane. The identity of the lines is determined by comparison with positive control strips. A positive result is one in which the patient's serum

reacts with two of the following three proteins: GP160/120 (counted as one protein band, even though it constitutes two distinct bands), GP41, and P24. Thus positive results could be any one of the following three combinations: 160/120 + 41, 160/120 +24, or 41 + 24. An indeterminate result is the presence of any visible band on the blot, and a negative result is the absence of any visible band on the blot. Of note, this test will be called negative in HIV-2 infection, using the above criteria. HIV-2 infection results in cross-reacting antibodies to some HIV-1 proteins (gag and pol, but not env), so a typical finding of HIV-2 infection is the presence of multiple positive bands, including P24, but without GP41 or GP 160/120 reactivity. If a HIV-2 like pattern is seen on the immunoblot then the serum is sent to a reference laboratory for a HIV-2 specific immunoblot. Examples of immunoblots are shown in this [link](#).

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## False-positive tests

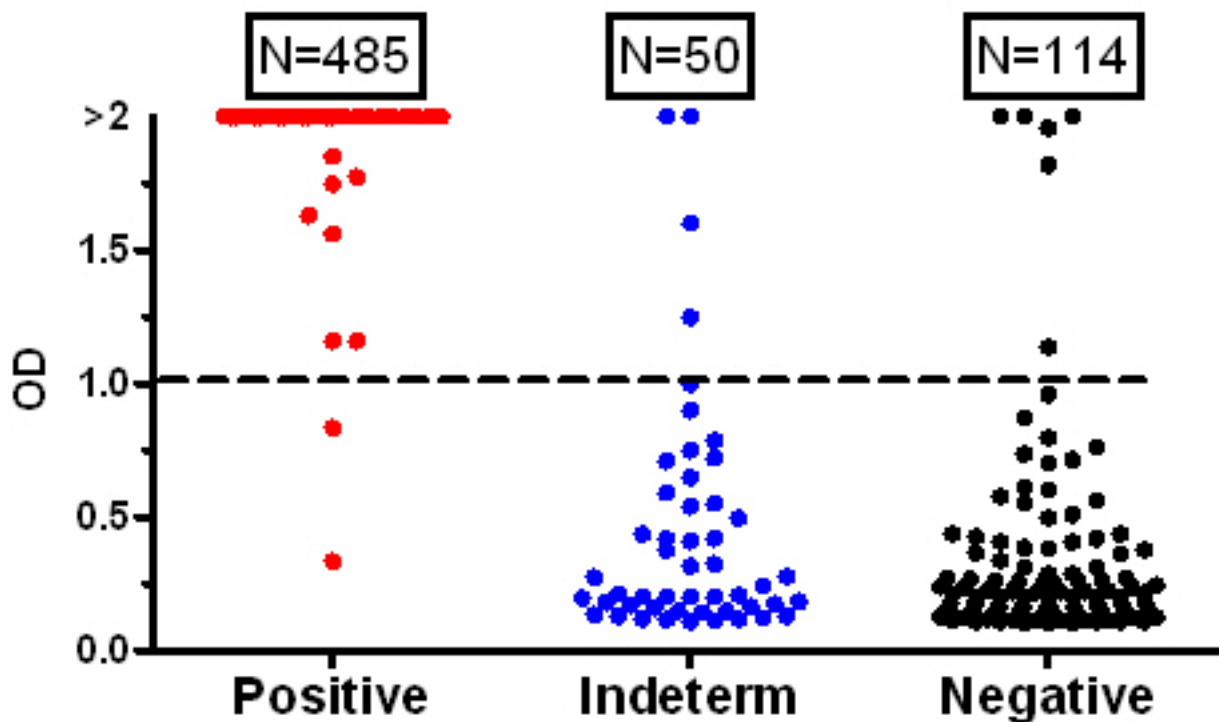
Both the EIA/card assays and the immunoblot assays are relatively [non-specific](#) when not used in the two-tier testing method. This means that a positive EIA/card assay by itself can not be interpreted as being positive for HIV antibodies, until confirmed by a positive immunoblot assay. For example, in one [study](#) of asymptomatic Italian blood donors, only about 13% of donors with repeatedly-reactive EIA tests were confirmed to be HIV-infected on the basis of positive immunoblot. However, in a high risk population the false-positive rate can be much lower, as low as [1-3%](#). There are [multiple possible causes](#) of a false-positive EIA/card test.

The immunoblot test can not be used as a screening test, as there is a high rate of indeterminate reactions, even in low risk populations. Of 298 EIA-negative blood donors tested with the Bio-Rad test, 32 (11%) had indeterminate results, and none positive results (Bio-Rad test product insert). Of 176 high risk people with EIA-negative tests, 28 (16%) had indeterminate results (Bio-Rad product insert). In contrast, all 172 EIA-positive patients with AIDS had a positive Bio-Rad blot result. Indeterminate

immunoblot results can be falsely positive in both [high](#) and [low](#) risk populations, even if these people are followed for several years. Some of the same [causes](#) for false-positive EIAs can cause false-positive immunoblots. Remember that the chance of a false-positive is quite low if both the EIA and immunoblot are positive.

The chance of a EIA being truly or falsely-positive can be roughly estimated for the Abbott EIA performed at HUP. The higher the test optical density (OD) , the more likely the test will be confirmed by immunoblot, and the lower the optical density the less likely the test will be confirmed by immunoblot. Of note these data are specific for the HUP patient population, and may not apply for other patient populations. The results of 649 consecutive sera tested by immunoblot at HUP from Jan 2004 to April 2007 are shown below. 98.9% of patients with an EIA OD  $\geq 2.0$  had a positive immunoblot result, and 98.7% of those with an OD  $< 1.0$  had negative or indeterminate immunoblots. Thus the chances of an immunoblot being positive are very small if the OD is less than 1 and quite high if the OD is 2 or greater. For all those with a positive screening EIA test result (OD $>0.1$ ), 75% were immunoblot positive.

## HIV OD Sorted by Immunoblot Result



Note that 98.9% (95%ci= 97.6-99.7%) of specimens with  $OD \geq 2$  were blot pos, and that 98.7% (95% ci= 95.4-99.8%) of specimens with  $OD \leq 1$  were blot neg or indeterm

### False-negative tests

The EIA/card tests can be falsely-negative in very early HIV infection, for the first 4-12 weeks of infection. For example, patients with the acute HIV infection syndrome frequently have negative antibody tests. After this "window" period, false-negative tests in patients with HIV-1 infection are exceedingly rare. Patients with HIV type O infections may have negative EIA

**tests, although many HIV-1/HIV-2 EIAs detect a variable number of such patients; infections with this HIV type are very rare in the US. Patients who don't make antibody, due to immunosuppression or intrinsic humoral immune defects, may have negative assays. An unusual cause of false-negative tests is massive transfusion before testing. Not all test kits are known to detect HIV-2 antibodies; the Abbott assay used at HUP is known to detect these antibodies. Technical errors can result in false-negative tests, as can glove powder.**

**Immunoblot tests can be falsely-negative in early HIV disease, usually with indeterminate rather than negative results (that is after the EIA test has become positive). Patients with HIV-2 infection can also have false-negative immunoblots, although as stated above there is often cross-reaction with some HIV-1 antigens resulting in a stereotypical pattern. If you have a EIA-positive, immunoblot negative or indeterminate, patient who may have acquired HIV infection in West Africa, or from a West African, call the laboratory to ask about sending the serum for a HIV-2 blot. Also, if you have a West African patient with immunodeficiency disease and negative EIA/blot, call the laboratory to inquire about HIV type O testing.**

**Depending on the clinical circumstances, there are several options available when a HIV test is negative, and there is a high pre-test probability of disease. One option is to repeat the test one to three months later. If the patient is suspected of having early HIV infection, and the EIA is positive and immunoblot indeterminate, then repeating the immunoblot with serum collected 2 to 4 weeks later usually results in a positive immunoblot if the patient truly has HIV infection. Repeat serologic testing is recommended in all cases of EIA-positive, blot-indeterminate results, 1 to 3 months later. If the immunoblot remains indeterminate after 3 to 6 months, then it is very unlikely that it will become positive in the future absent new infection. Molecular methods are better for diagnosing the acute HIV syndrome, in particular viral quantitation - as the viral load should be quite high in this setting.**

**revised by Paul H. Edelstein, Apr 2007**

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