



# LABORATORY DIAGNOSIS OF HIV INFECTION: IMMUNOASSAYS

Compiled by Dr Steven Miller

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## Background

Accurate laboratory diagnosis of HIV infection is essential to identify people who can benefit from antiretroviral therapy, to reassure people who are uninfected, and to reduce transmission.<sup>1</sup>

Testing involves a combination of assays (“immunoassays”) with high sensitivity and specificity to maximise diagnostic accuracy; specific rules are applied to resolve discrepancies.<sup>2</sup> Immunoassay technology has improved substantially over time. Tests previously used detected the presence of antibodies against HIV only. These have been replaced with 4<sup>th</sup> **Generation HIV-1 and -2 antigen/antibody immunoassays.**

## Evolution of HIV Immunoassay Technology

Generation	Antigen used	Factor detected	Sensitivity and Specificity	Window period (interval between infection and a reactive result)
1 <sup>st</sup>	Lysed whole virus (HIV-1 only)	IgG	Poor sensitivity & specificity. Misses antibodies against HIV-1 subtype O.	6 – 8 weeks
2 <sup>nd</sup>	Synthetic peptide or recombinant antigen with / without lysed virus (HIV-1 & -2)	IgG	Improved sensitivity & specificity. Better detection of antibodies against HIV-1 subtype O.	Up to 40 days
3 <sup>rd</sup>	Synthetic peptide or recombinant antigen (HIV-1 & -2)	IgG and IgM	Increased sensitivity in early infection. Specificity good.	17 – 28 days
4 <sup>th</sup>	Synthetic peptide or recombinant antigen (HIV-1 & -2)	IgG, IgM and p24 antigen	Allows test to become reactive prior to seroconversion. Specificity good.	14 – 21 days

## Markers of HIV Infection and Time to Detection with Current Tests

- HIV nucleic acid can be detected (via Qualitative PCR or Viral load assays) approximately 10 – 14 days after infection and increases thereafter.
- p24 antigen is detectable approximately 4 – 10 days thereafter, but is detectable for a short time only due to the rapid formation of immune complexes.
- IgM antibodies can be detected 3 – 5 days later than p24 antigen.
- IgG antibodies can be detected after a variable interval following detection of IgM and remain detectable lifelong.

## Terminology

*Eclipse period:*

Period after infection when ALL laboratory tests are non-reactive.

*Window period:*

Time between infection and the first laboratory test becoming reactive.

*Acute HIV infection:*

Period between the detection of HIV nucleic acid and IgM/IgG antibody.

*Established/Chronic infection:*

Time from the appearance of IgG antibody onwards.

## Testing Protocol<sup>3</sup>

### Children >18 months of age, adolescents and adults

- Initial Screening is done using a 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassay: if the sample is Reactive, the laboratory will automatically perform a
- Confirmatory 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassay.

### Neonates and infants up to 18 months of age

- HIV-1 Qualitative PCR (p24 antigen is a far less-sensitive investigation, and may be undetectable despite established infection).
- Antibody tests are of little/no clinical utility as they cannot distinguish between foetal/neonatal antibody and maternal antibody that has crossed the placenta. They can only establish whether an infant has been exposed to HIV.

## Reporting of Immunoassay Results

- **Reactive:** Both the initial and confirmatory 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassays have detected HIV antibody and/or p24 antigen. The values obtained reach or exceed the threshold stipulated by the assay. Since the tests do not distinguish between antibody and antigen, the term “Reactive” is a more scientifically accurate term than “Positive”.
- **Non-reactive:** Neither HIV antibody nor p24 antigen have been detected by a 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassay.
- **Indeterminate:** No definite conclusion can be drawn regarding the result. Either there is a lack of agreement between the results of the two 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassays, or reactivity is minimal and does not reach the threshold stipulated by the assay.

## Limitations of Immunoassays

The positive and negative predictive value of any immunoassay depends on the seroprevalence rate of the particular disease in the patient population being tested. No diagnostic test or testing protocol is completely accurate in all cases of HIV infection. Rare instances have been reported of people who are persistently antibody negative despite a positive PCR test.<sup>4</sup> False-positive test results can occur for several reasons including sample mix-up or mislabelling, auto-immune disease, multiple prior blood transfusions, and infection with other retroviruses.<sup>5,6</sup>

False-negative test results have been described in people with delayed seroconversion, immune dysfunction due to a defective humoral immune response and, rarely, in individuals treated very early in the course of their infection with potent antiviral agents. This is postulated to result in reduced antigenic stimulation leading to a poor antibody response.<sup>7</sup>

## Specimen Collection and Submission

Freshly-collected blood samples yield the most accurate HIV results. Specimen volume influences the laboratory's ability to perform the recommended testing protocol.

The following blood samples should be submitted for 4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassay testing:

- Children older than 18 months: a single 5 mL SST tube (clotted blood).
- Adolescents and adults: a single well-filled 7 – 10 mL SST tube (clotted blood).

The patient's name, date and the signature of the phlebotomist must be written on all tubes.

4<sup>th</sup> Generation HIV-1 and -2 antigen/antibody immunoassay testing will NOT be performed on children less than 18 months of age. Only in the rare situation in which an infant's HIV exposure status needs to be evaluated (rather than a formal diagnosis being required), will a Rapid HIV test be performed. EDTA blood must be sent for an HIV-1 Qualitative PCR in HIV-1 infection is suspected clinically.

## Follow-up Samples

A separate, freshly-collected sample should be submitted in the following situations:

- To confirm a Reactive result on an initial test; the follow-up sample may be collected and submitted immediately on receipt of the initial laboratory report.
- To determine the significance of an Indeterminate result; the second sample should be drawn 10 – 21 days after the first.
- To confirm a Non-reactive result if there has been a potential risky exposure in the 6 weeks prior to the initial test. If the person has used pre-exposure or post-exposure prophylaxis; the second sample should be drawn at least 14 days after completion of the pre-exposure or post-exposure prophylaxis.

## References:

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