



HIV Laboratory Testing: a Resource for Health Professionals

Table of Contents

Overview.....	2
Window Periods and HIV Testing	2
Current HIV Testing System at Provincial Public Health Reference Laboratory	3
Detection of HIV types	4
Need for a repeat specimen if a confirmed HIV result.....	4
False Reactive and Indeterminate Results.....	4
Testing Advice for Clients after a Potential Exposure to HIV	5
HIV testing related to blood and body fluid exposures.....	6
Additional HIV Testing Technologies under Evaluation.....	6
Diagnosis of maternal and neonatal HIV infection.....	7
Test Result Turn Around Times	7
Interpreting Provincial Public Health Reference Laboratory HIV Result Reports	7
Contact Information	8
Figure 1: Schematic Representation of the Appearance of Biological Markers of HIV Infection and Window Periods of HIV Tests.....	9
Table 1: Characteristics of HIV Tests Currently in use at Provincial Public Health Reference Laboratory (June 2010)	10

Please Note: Laboratory testing technologies for HIV will change over time. The online version of this resource will be updated as new HIV laboratory tests are introduced. Please refer to www.bccdc.ca for the most current version of this resource document.



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Overview

This resource document provides up to date and comprehensive information for health professionals and other individuals conducting or providing advice about Human Immunodeficiency Virus (HIV) testing.

This document includes information about the types of HIV tests available, window period information for specific HIV tests, and additional information that may be required to provide appropriate advice to clients undergoing HIV testing. The primary focus of this document is on laboratory-based serologic testing conducted at the PHSA Provincial Public Health Reference Laboratory. Some information about point of care (rapid) HIV testing is provided; for further information on point of care testing please refer to the provincial guidelines for point of care HIV testing (see references for links).

If you have any questions regarding the interpretation of a client's HIV test results, or to determine if additional tests are indicated given your client's history of recent exposures or risk events, please contact the Provincial Public Health Reference Laboratory at 1-877-747-2522 and ask to speak to a medical or clinical virologist.

Window Periods and HIV Testing

In HIV testing, the window period refers to the time interval between the point when a person is infected and the point when laboratory tests can detect HIV infection. Understanding the window period of HIV tests is important for health care providers to provide appropriate information to clients during pre- and post-test counseling, when interpreting HIV test results, and when discussing testing or re-testing with clients following an event or potential exposure to HIV.

The most common HIV tests used are based on the detection of three biological markers of HIV infection which appear at different times following HIV infection (Figure 1): viral RNA, p24 antigen (a viral core protein), and HIV-specific antibodies. For more information about the window periods for specific HIV tests, please refer to Table 1.

Progress in HIV testing technologies continues to result in tests with shorter window periods. A potential benefit of the shorter window period is the reduction in the time interval for testing following a risk event or exposure, which can reduce client anxiety. Additionally, earlier diagnosis of HIV infection benefits both individuals and populations in the following ways:

- Individuals who are diagnosed with HIV can be connected earlier to HIV-related primary care.
- Persons who are aware they are infected with HIV will often take steps which will prevent HIV transmission to others.

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- Earlier testing can diagnose individuals in the acute phase of their infection, within the first 4-6 weeks after infection, when a person often has a very high viral load and there is a greater likelihood of transmitting HIV to others compared with individuals in later stages of HIV infection.

While maximum window periods for specific HIV tests are difficult to estimate, under the standard testing algorithm at the Provincial Public Health Reference Laboratory it is estimated that greater than 95% of individuals will show detectable antibodies to HIV by 4 to 6 weeks, with greater than 99% having sero-converted by 3 months (as detected by Western Blot).

When providing client counselling about window periods in relation to HIV testing it is important to note that window period estimates are typically based on averages or statistical projections from studies of a small number of sero-converting individuals (typically among repeat blood donors). Window periods are estimates – there is considerable individual variation with some individuals having shorter or longer than average window periods.

Current HIV Testing System at Provincial Public Health Reference Laboratory

At the Provincial Public Health Reference Laboratory, the first test applied to a blood specimen is an antibody test, a **3rd generation enzyme immunoassay (EIA) test**.¹ This test has high sensitivity and is used as a screening test. To confirm or to rule out HIV infection, any degree of reactivity on EIA testing leads to a series of further tests including:

- 4th generation EIA test:** This test identifies specimens that are positive for p24 antigen or HIV antibody, but does not distinguish between them. The 4th generation EIA test has a shorter window period compared to 3rd generation EIA tests and is used as a supplemental test in the standard HIV test protocol (i.e., performed when the 3rd generation EIA test used for screening is reactive).
- Western Blot test:** The Western Blot test is considered the gold standard for confirmation of HIV infection. Specimens that are reactive on both EIA screening tests and on Western Blot are considered to be confirmed HIV-positive. The EIA-Western Blot combination is estimated to have an overall sensitivity of 99.9% and specificity of 99.9%.
- Individual RNA Nucleic Acid Amplification Test (NAAT):** If there is a weak signal on EIA testing and the Western Blot is non-reactive or indeterminate, an

¹ Note that a 4th generation EIA test is used as the initial screening test for specimens sent to the Victoria General Hospital Laboratory for HIV testing. The PHSA Provincial Public Health Reference Laboratory is planning to switch to a 4th generation EIA test as the initial screening test as well and this document will be updated at that time.

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individual RNA NAAT is performed. A negative RNA NAAT result can rule out HIV infection.

If viral RNA is detected, then the result is suggestive of acute HIV infection. The RNA NAAT test has a lower detection limit of 40 HIV-1 RNA copies/mL of EDTA plasma. During acute HIV-1 infection, RNA copy numbers greatly exceed this threshold.

Note: individuals with HIV infection who are receiving adequate antiretroviral therapy will likely have a negative RNA NAAT, even in the presence of a positive Western Blot.

Detection of HIV types

The majority of HIV infections detected in British Columbia are HIV-1 infections. To date in BC, only one HIV-2 case has been confirmed, likely acquired in Africa, and no group O infections have been identified. The 3rd generation EIA assay used is able to identify all known subtypes of HIV-1, including group O, and HIV-2 infections. Confirmation of HIV-2 infection, other HIV-1 variants such as group N or group O and indeterminate HIV serology may be complex. If suspected, please consult with the medical or clinical virologist at Provincial Public Health Reference Laboratory.

Need for a repeat specimen if a confirmed HIV result

If the client is identified as being HIV-positive for the first time, it is important that a repeat sample be submitted to confirm the diagnosis. This will help to eliminate the low possibility of error that might result from specimen handling errors such as mislabeling of submitted specimens. Repeat testing is particularly recommended when the client's history suggests a low risk for exposure to HIV or the client does not undergo subsequent viral load testing as part of HIV primary care follow-up.

False Reactive and Indeterminate Results

Since a large percentage of submitted specimens are from individuals at low risk for HIV infection a falsely reactive 3rd or 4th generation EIA test can occasionally occur. In BC, it is estimated that approximately 0.5% of all HIV screening tests or about 5 per 1000 individuals screened are falsely reactive.

Falsely reactive screening test results can occur due to non-specific or cross-reacting antibodies in the client sample (for example, some individuals may produce an antibody to latex or other compounds used in EIA testing). False reactive results can cause a lot of anxiety to the client. Individuals with falsely

4

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reactive screening tests usually do not change their serological pattern over time and confirmatory tests, including RNA NAAT on the initial specimen, will help rule out HIV infection.

Individuals who are newly HIV-infected will typically demonstrate evidence of complete sero-conversion to a reactive Western Blot over 1-2 months. Therefore, if the initial EIA result and RNA NAAT is suggestive of early stage HIV infection, follow-up samples will be requested as required to confirm HIV infection by the Western Blot test.

Occasionally, the Western Blot is indeterminate (i.e. not positive but not negative either). An indeterminate Western Blot may occur when a client is in early stages of sero-conversion, if the client has advanced HIV infection at diagnosis, or if the client has cross-reacting antibodies that are not HIV-related. Most indeterminate Western Blot results fall into this latter category and typically, an RNA NAAT will help to confirm or rule out HIV infection.

Testing Advice for Clients after a Potential Exposure to HIV

For early reassurance, a client can be tested as early as 6 weeks following a possible risk event or exposure to HIV, with testing repeated at 3 months if negative. Waiting three months to get tested after a possible exposure to HIV was a common recommendation in the past; however, the use of current HIV test technologies with shorter window periods has made waiting for three months unnecessary.

Regardless of the timing of the possible exposure, those clients more likely to be infected with HIV should be offered HIV testing at the time of presentation. This includes clients who have a partner who is known to be HIV positive, have an exposure that is at high risk for HIV transmission, or who have symptoms of seroconversion illness.

Seroconversion illness typically presents like an early influenza-like illness and occurs in 50-90% of newly infected people. Symptoms may include fever, myalgia, fatigue, nausea or vomiting, pharyngitis, headache, or lymphadenopathy.

If the initial HIV test at presentation is negative, a baseline negative HIV status has been established. Another HIV test can be performed 2-3 weeks following the exposure; many individuals infected with HIV will have detectable HIV antibodies by this time. For these clients, writing “4th generation HIV EIA” or “p24 antigen” on the laboratory requisition form will ensure a test is performed that has the best capacity to detect early HIV infection.

The benefits to testing earlier after a possible exposure to HIV are:

- Fewer missed opportunities to diagnose clients who are infected with HIV as there is a chance that the client may not return for testing. Sending a client away without testing may be construed by a client that testing is not really necessary.
- Infection with HIV may have occurred during an earlier exposure to HIV.

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- Earlier testing can help if clients are anxious about their HIV status following a potential exposure to HIV. An early negative result at 6 weeks, which is likely to remain HIV negative at three months, may help to reduce anxiety.
- If HIV is detected at an earlier stage, there is a greater possibility to prevent HIV transmission through detection of acute HIV infection, and steps taken by clients to prevent HIV transmission upon awareness of their HIV status.

Clinical judgment remains important in HIV testing. If you receive a negative or indeterminate result for a client who you consider to have a high likelihood of having an HIV infection, you may contact a medical or clinical virologist at the Provincial Public Health Reference Laboratory to review the case and to determine if additional tests are indicated.

HIV testing related to blood and body fluid exposures

Testing of specimens from source clients during the management of an occupational blood and body fluid exposure will currently undergo both 3rd and 4th generation EIA testing. Please refer to provincial guidelines for management of blood and body fluid exposures (see references for links).

Additional HIV Testing Technologies under Evaluation

The Provincial Public Health Reference Laboratory at the BCCDC, in partnership with the STI/HIV Division, is currently evaluating the use of pooled RNA NAAT testing in diagnosing acute HIV infection. Pooled RNA NAAT testing is currently under evaluation as part of a CIHR team grant focused on the use of the test at clinics accessed by gay men for HIV testing and is currently performed only from specimens at these clinics.²

Pooled RNA NAAT testing, currently used by Canadian Blood Services to screen donated blood, pools specimens from individuals with non-reactive 3rd generation EIA tests. Pools are then tested using RNA NAAT testing. If any pool is positive and viral RNA is detected, then the individual specimens are tested to identify the RNA positive specimen using individual RNA NAAT.

This test identifies people who have acute HIV infection and are RNA positive, but still antibody negative. In STI clinics where pooled RNA NAAT testing has been added to standard HIV testing, the number of individuals newly diagnosed with HIV infection has increased by between 3 and 11%. The window period of pooled RNA testing is approximately 10 to 12 days.

² CIHR Team in the Study of Acute HIV Infection in Gay Men. For more information about this study please see www.acutehivstudy.com



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Anti-HIV-1 Western Blot Reactive
HIV-1 Quantitative NAAT HIV-1 RNA detected
Interpretation: Findings indicate HIV infection.

c) Probable early/acute HIV infection (sero-conversion phase):

Anti-HIV-1&2 EIA Reactive
HIV-1 & 2 Ab/Ag EIA Reactive
Anti-HIV-1 Western Blot Indeterminate or Non-reactive
HIV-1 Quantitative NAAT HIV-1 RNA detected
Interpretation: Findings are suggestive of acute HIV infection. Please submit a follow-up EDTA blood in 1-2 weeks to confirm infection.

d) Indeterminate serologic results with NAAT testing for resolution of status:

Anti-HIV-1&2 EIA Reactive
HIV-1 & 2 Ab/Ag EIA Reactive (or Non-reactive)
Anti-HIV-1 Western Blot Indeterminate, Non-reactive or Non-specific reactivity
HIV-1 Quantitative NAAT No HIV-1 RNA detected
Interpretation: No evidence of HIV infection.

If follow-up serology is required, it will be requested on the initial report. When follow-up testing has ruled out HIV infection, results of individual tests will be reported together with the following interpretation statement:

No significant changes from samples collected on DD/MMM/YYYY. No evidence of HIV infection.

Contact Information

If you have any questions regarding the interpretation of a client's HIV test results, or to determine if additional tests are indicated given your client's history of recent exposures or risk events, please contact the Provincial Public Health Reference Laboratory at 1-877-747-2522 and ask to speak to a medical or clinical virologist.

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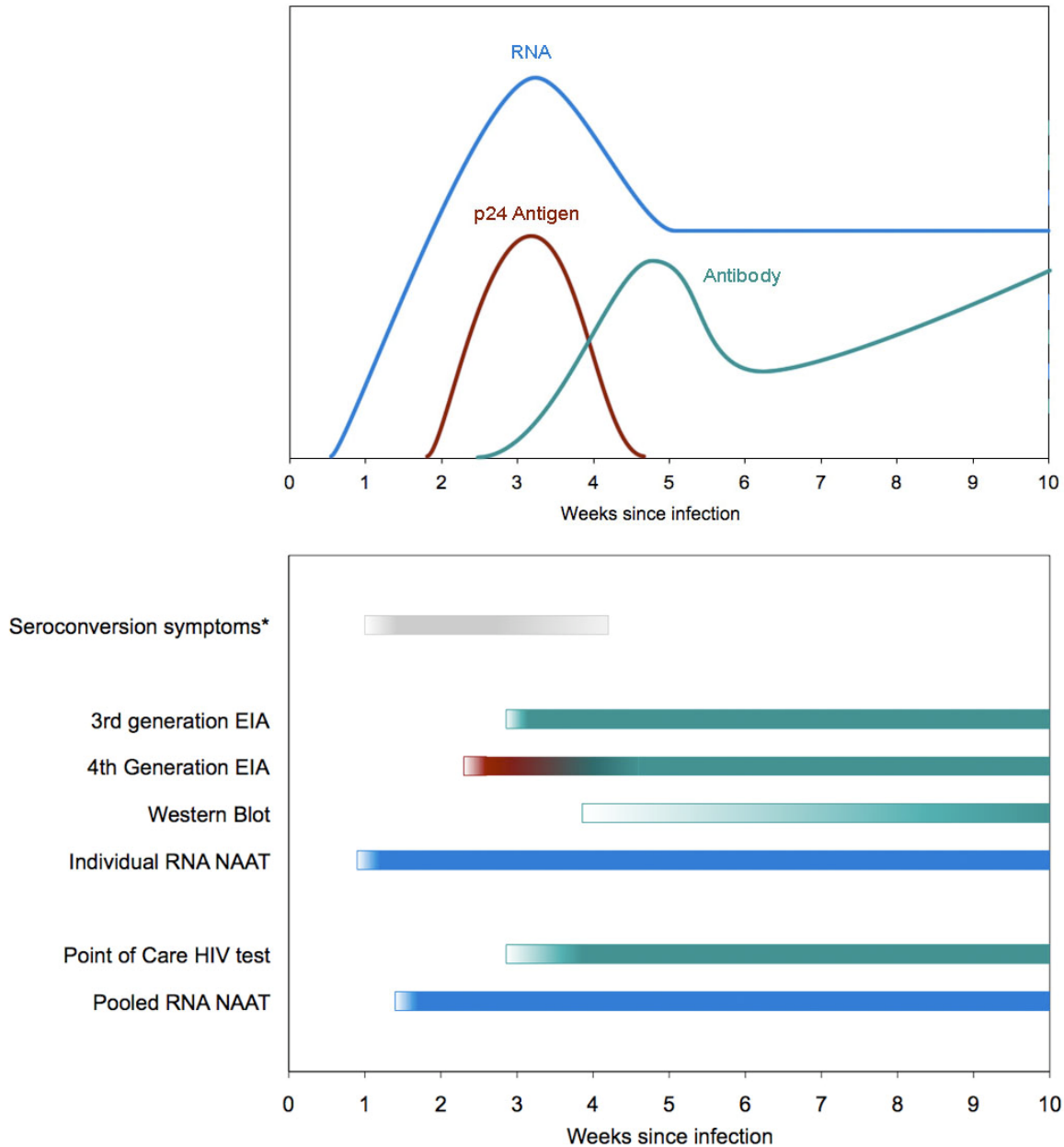
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Figure 1: Schematic Representation of the Appearance of Biological Markers of HIV Infection and Window Periods of HIV Tests



The lower figure demonstrates the window periods for specific tests following HIV infection (time from HIV infection to the time a test detects HIV).

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Table 1: Characteristics of HIV Tests Currently in use at Provincial Public Health Reference Laboratory (June 2010)

Test	Description	Use	Estimated Window Period	Assay used	Name on test report
3 rd Generation EIA test	Enzyme Immunoassay which detects the presence of HIV antibodies.	Standard test protocol, as screening test (first step).	~3-4 weeks	Siemens ADVIA Centaur HIV-1/O/2 EIA	Anti HIV 1 and 2 EIA
4 th Generation EIA test	Enzyme Immunoassay which detects the presence of both protein p24 antigen and HIV antibodies.	Standard test protocol, as supplemental test following reactive 3 rd Generation EIA test.	~2-3 weeks	Abbot AxSym HIV Combo	HIV 1 and 2 Ab/Ag EIA
Western Blot	Immunoblot which detects HIV antibodies directed against specific HIV proteins. Interpreted according to Canadian consensus guidelines.	Standard test protocol, as confirmatory test. Considered to be gold standard for confirmation of HIV infection.	~4-6 weeks, may take up to 8 weeks for a positive result.	BioRad Genetic Systems HIV-1 Western Blot	Western Blot
Individual RNA quantitative NAAT	Detects viral RNA in plasma	Standard test protocol, to resolve indeterminate results following 3 rd Gen EIA and Western Blot	<1-2 weeks	Roche COBAS TaqMan HIV-1 RNA Test	HIV 1 Quantitative NAT
Pooled RNA	Detects viral RNA in plasma of individuals who without detectable antibody (negative on antibody tests).	In evaluation / research protocols.	10 to 12 days	Roche COBAS TaqMan HIV-1 RNA Test	Not applicable (a positive pool is confirmed by Individual RNA NAAT).

Note: The bioLytical INSTI™ HIV-1 Antibody Test Kit is the only licensed point of care HIV test in Canada. This product detects HIV antibodies, similar to the 3rd Generation EIA test. According to the product monograph, the INSTI™ HIV-1 Antibody Test Kit will detect HIV infection at the same time or up to one week after the time HIV infection is detected by a 3rd Generation EIA test.



References

Provincial Guidelines related to Point of Care (POC) HIV testing:

- Guidelines for Use of the POC HIV Test Kit: Information for the Health Care Professional in BC. CD Control Manual, Chapter 5, April 2007. [\[Link\]](#)
- Point of Care HIV Counselling Guidelines. CD Control Manual, Chapter 5, May 2007. [\[Link\]](#)
- Appropriate Use of Point of Care HIV Testing in BC. CD Control Manual, Chapter 5, December 2007. [\[Link\]](#)

Provincial Guidelines related to the Management of Blood and Body Fluid Exposures:

- Blood and Body Fluid Exposure Management. CD Control Manual, Chapter 1, March 2010. [\[Link\]](#)
- Accidental Exposure Therapeutic Guidelines. BC Centre for Excellence in HIV/AIDS, February 2009. [\[Link\]](#)

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