Title: HIV LABORATORY CASE DEFINITION

The Public Health Laboratory Network has developed a standard case definition for the diagnosis of diseases which are notifiable in Australia. These pages contain the laboratory case definition for Human Immunodeficiency Virus (HIV) infection.

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PHLN Summary Laboratory Definition

Reporting
Confirmed cases should be notified to the local jurisdictional public health unit consistent with jurisdictional guidelines.

Laboratories should notify confirmed cases only. Current national Australian guidelines (see AHPPC SoNG guidelines below) recommend notification by clinicians of confirmed and probable cases.

Confirmed case

A confirmed case requires laboratory definitive evidence only.

Consistent with international guidelines (see for example the CDC website listed below), diagnostic confirmation of an AIDS-defining condition without laboratory-confirmed evidence of HIV infection, is insufficient to classify an individual as HIV-infected.

1.1 Condition:

- Human Immunodeficiency Virus infection (HIV), and the associated clinical condition of Acquired Immune Deficiency Syndrome (AIDS)

1.1.1 Definitive Criteria for Confirmed case in Children aged >18 months and Adults

- Detection of HIV antibody on HIV antibody or antigen/antibody combination assays that have repeatedly reactive results on screening assay; AND
  Positive results on confirmatory or supplemental assay – this may be a western blot or line probe immunoassay (the latter is currently not available, but may be available in future on the Australian Register of Therapeutic Goods (ARTG)) OR

- HIV isolation from a patient sample OR

- Positive p24 antigen assay, including neutralisation in antigen only assays on two separate specimens (one of which may include the initial screen on Ag/Ab assay) in the absence of a diagnostic western blot.

Positive result on nucleic acid tests (NAT) either on qualitative, or a detectable quantity on quantitative assays, are part of testing for corroboration of difficult diagnostic assessments. Nucleic acid tests such as PCR are not currently validated for formal confirmation of a diagnosis of HIV infection. However, they are useful supplemental tests to assist in diagnosis (*from Requirements for Laboratory Testing for Human Immunodeficiency Virus and Hepatitis C Virus (3rd Edition), the National Pathology Accreditation Advisory Council (NPAAC) comments on Standard 10.1*). In practice, in some cases the loss of indeterminate western blot and NAT with treatment may impact on diagnoses, especially if follow up samples are not received.

1.1.2 Definitive Criteria for Confirmed Case in Children aged <18 months

- Detection of HIV-1 nucleic acid (RNA or DNA) or detection of HIV-1 p24 antigen confirmed by neutralisation (HIV-1 p24 antigen must be from a child >1 month) in one blood sample, excluding cord blood AND confirmed in a second blood sample collected on a different date OR

- HIV isolation from a patient sample

1.1.3 Suggestive Criteria for Probable Case in Children aged <18 months

- Detection of HIV-1 nucleic acid (RNA) or detection of HIV-1 p24 antigen confirmed by neutralisation (HIV-1 p24 antigen must be from a child >1 month) in one blood sample, excluding cord blood AND no subsequent negative HIV virological or antibody test. The age at diagnosis is determined by the time of blood sample collection OR

- Detection of HIV nucleic acid as proviral DNA using a qualitative NAT assay (currently this assay is not registered on the ARTG).

1.1.4 Probable Case in Children aged >18 months and Adults

A probable case requires Laboratory suggestive evidence OR Clinical evidence:

Laboratory suggestive evidence:

1. Detection of HIV-1 nucleic acid (RNA or DNA) OR

2. A positive antibody or Ag/Ab screen and an indeterminate HIV-1 western blot with a glycoprotein band OR

3. Detection of HIV-1 p24 antigen, confirmed by neutralisation OR

Clinical evidence including:
Well documented evidence of a previous HIV diagnosis

Note:

a) Age at diagnosis is determined by the time of blood sample collection

b) Additional information to distinguish newly acquired HIV infection should be collected routinely for surveillance purposes, and includes evidence of a negative or indeterminate HIV antibody test and/or a seroconversion illness occurring within the 12 months prior to diagnosis]

1.1.5 Special Considerations and Guide for Use

- Some rapid HIV tests have been registered on the ARTG for use at point-of-care (POC) in order to increase uptake of testing and increase diagnoses. They are not confirmed positive until definitive criteria for a confirmed case as outlined above are obtained.
- Dissociation between HIV antigen/antibody tests and HIV NAT assays can occur in seroconverters (HIV NAT positive/antibody negative) or late stage AIDS (HIV NAT positive/antibody negative), as discussed further below in section 3.

1.1.6 Links to related documents

AHPPC SoNG – Series of National Guidelines for response to HIV:

National Pathology Accredition Advisory Council (NPAAC) 2013.

Revised Surveillance Case Definitions for HIV Infection Among Adults, Adolescents, and Children Aged <18 Months and for HIV Infection and AIDS Among Children Aged 18 Months to <13 Years --- United States, 2008, December 5, 2008 / 57(RR10); 1-8. Prepared by Eileen Schneider, Suzanne Whitmore, M. Kathleen Glynn, Kenneth Dominguez, Andrew Mitsch, Matthew T. McKenna, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (www.cdc.gov/mmwr/pdf/rr/rr5710.pdf)

Updated as CDC Revised Surveillance Case Definition for HIV infection- United States, April 11, 2014 / 63(RR03); 1-10 Prepared by Richard M. Selik, Eve D. Mokotoff, Bernard Branson, S. Michele Owen, Suzanne Whitmore, H. Irene Hall, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC.http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm?s_cid=rr6303a1_e

WHO Guidelines for HIV Diagnosis and Monitoring of Antiretroviral Therapy. 2009. (www.Searo.WHO.int/linkfiles/Publications_SEA-HLM-382.pdf)

Hoy J, Lewin S, Post JJ, Street A, editors. HIV Management in Australia: a guide for clinical care. Darlinghurst: Australasian Society for HIV Medicine; 2009

(www.ashm.org.au/images/Publications/Monographs/HIV_Management_Australasia/hiv-management-mono_WHOLE.pdf).

2. Introduction

Human Immunodeficiency Virus (HIV) has two known subtypes - HIV-1 and HIV-2, of which HIV-1 causes the overwhelming majority of infections in Australia, where the only known reservoir is humans. The viruses are enveloped plus- stranded RNA viruses within the family retroviridae. HIV-1 was isolated from a patient with an early stage of the newly recognized AIDS and reported in 1983, then HIV-2 was reported in 1986. HIV-1 is subdivided into four genetic groups M, O, N and P while HIV-2 has eight subtypes (A- H).

HIV-1 has been reported globally, while HIV-2 has remained limited primarily to West Africa and to migrants from West Africa. Both HIV-1 and HIV-2 are most commonly transmitted horizontally sexually, with vertical transmission considered to be the most common method of transmission in developing countries. Infection may occur through parenteral means including inoculation of infected blood, reuse of contaminated needles, transfusion of infected blood products, and transplantation of infected tissues. The natural history of infection is divided into three phases: Acute retroviral syndrome occurs four to six weeks post exposure, 40 to 70% of those infected present with fever, maculopapular rash, oral ulcers, lymphadenopathy, malaise, weight loss, arthralgia, night sweats, or pharyngitis, with associated high HIV viral load. The second clinical phase of infection is clinical latency, which is usually several years without symptoms. The third phase is Acquired Immune Deficiency Syndrome (AIDS) where the patient presents with different opportunistic infections, characterized virologically by high viral loads and low CD4 T- cell counts, typically < 200 cells/mm^3.

The diagnosis of HIV is commonly accomplished via detection of HIV antibody using a test that includes antigen and antibody targets, with verification using a subsequent confirmatory or supplemental test. The assays detecting antigen and antibody (Ag/Ab “combo” tests), which are now licensed for blood and tissue screening, are currently the predominant assays used in patient screening/testing. Qualitative NAT assays when available may be used to diagnose and characterise viraemia although currently they are not definitive criteria for diagnosis when used alone. Quantitative viral load assays measure the quantity of HIV-1 RNA in plasma or in whole blood as surrogate prognostic markers, to monitor therapy, and to determine infectiousness.

The majority of Australian laboratories now operate using instrument-based assays, though some may continue to use microtitre-based assays for screening. All Australian laboratories operate under quality management systems monitored with quality assurance programs (from the Royal College of Pathologists of Australia Quality Assurance Program - RCPAQAP or NRL – National Serology Reference Laboratory) to regularly check performance. Clinical syndromes most commonly identifying AIDS in an HIV-infected individual globally include *Pneumocystis jirovecii* pneumonia, HIV wasting syndrome and oesophageal candidiasis. However in Australia, earlier identification and treatment have changed this spectrum considerably in recent years.

HIV is a notifiable disease in all Australian states and territories, with the mechanisms of notification being similar, although not identical, in all jurisidictions.

3. Tests

3.1 Test Regulation

The system for regulation of *in vitro* diagnostic devices ("tests" in simple language) in Australia is risk based. HIV is considered to be a disease that poses high risk to individuals and to the public, and therefore, tests for its diagnosis are assessed in the highest risk class, class 4. This means that HIV tests for diagnosis are assessed with highest stringency in the assessments undertaken by the Therapeutic Goods Administration (TGA). Tests that are used for monitoring or characterising HIV infection once it has been diagnosed are in a lower risk class (Class 3) from a regulatory perspective. Once a test is assessed as appropriate for its intended use in Australia, it is registered on the Australian Register of Therapeutic Goods (ARTG).

3. 2 Serological Tests

The serological tests for establishing HIV infection in an individual are referred to as either screening or supplemental (confirmatory) tests. Samples are tested using assays registered on the ARTG (currently Enzyme Immunoassays - EIA, chemiluminescent microplate immunoassay - CMIA) before supplemental assays are performed. Supplemental testing can include additional immunoassays, but, in Australia, always includes a western blot. Serological testing for HIV antibody is undertaken for primary diagnosis, screening of blood products, organ and tissue donor screening, management of untested women when pregnant, in labour and delivery, evaluation of occupational exposure to blood or body fluid, and epidemiological purposes. There are many commercial serology assays available in Australia, some of which are shown in Table 1.

Table 1: Some HIV immunoassays registered on the ARTG

Assay Name and Methodology	In use in Australia?	Diagnostic Type
Abbott ARCHITECT HIV Ag/Ab Combo CMIA	YES	Screening
Abbott Prism HIV Ag/Ab ChLIA	YES	Screening
BioMerieux VIDAS HIV DUO Ultra	YES	Screening
BioRad Access HIV Combo HIV1/HIV2 CMIA	YES	Screening
Bio-Rad Genscreen HIV-1 Antigen assay	YES	Supplemental
BioRad Genscreen HIV-1/2 V2 EIA	YES	Screening
Bio-Rad NEW LAV BLOT I WB (Western blot)	YES	Supplemental
BioRad Genscreen ULTRA HIV Ag/Ab	YES	Screening
DiaSorin LIAISON XL Murex HIV Ab/Ag CMIA	YES	Screening
MP Diagnostics HIV BLOT 2.2 WB	YES	Supplemental
Ortho-Clinical Diagnostics VITROS anti-HIV 1+2 CMIA	YES	Screening
Roche Elecsys HIV Combi PT ECLIA	YES	Screening
Siemens ADVIA Centaur HIV Ag/Ab CMIA	YES	Screening
Siemens ADVIA Centaur HIV 1/O/2 Enhanced CMIA	YES	Screening

3.2.1 HIV-1 and HIV-2 Antigen and Antibody screening tests

Following infection with HIV, virus core protein (p24 antigen) appears in the blood within two to three weeks, about the same time as seroconversion illness occurs in 40-48% of

affected individuals. Antigenemia and viraemia may precede the appearance of antibody by up to two weeks. In some individuals, some serological assays show delayed seroconversion (George, 2014).

Early screening tests were designed to detect antibody to HIV. The HIV combo tests, are a fourth generation immunoassay, so named because they detect both HIV antigen and antibody simultaneously in serum and plasma (Ly, 2004).The recombinant antigens used in the assays allow antibody to HIV 1 and HIV 2 to be detected. A commonly-used commercial assay is the Abbott Architect HIV Ag/Ab Combo, based on using paramagnetic microparticles and chemiluminescent reaction to detect antibody against gp41 or 36 or p24 antigen. This so-called chemiluminescent microparticle immunoassay (CMIA) uses recombinant antigens and synthetic peptides. These assays referred to as combo tests, are now used for screening in most centres and have been shown to detect infection earlier than tests that detect antibody alone (Ly, 2004). Assays are easily performed, robust, automated and have high throughput with short turnaround time.

Most clinicians now accept that a negative combo test six weeks after an exposure nearly always excludes HIV infection. The occurrence of a prolonged second diagnostic window with certain 4th generation HIV assays has to be considered.[3]False positive results can occur rarely with screening tests, and hence results should be reported as reactive rather than positive. Most manufacturers of HIV screening assays recommend samples that are initially reactive should be retested in duplicate in the same assay to confirm the result and this is part of NPAAC guidelines (NPAAC 2009). Specimens reactive on retesting are referred to as repeat reactors. Repeat reactors on combo assays are retested using an antibody and a p24 antigen-specific assay to determine if the reactivity is due to the presence of antigen alone, suggestive of recent infection. Samples reactive on p24 antigen assays should be retested after neutralisation before the results are reported. It is recommended that all newly diagnosed patients have a repeat test on a second blood sample (AHPCC SoNG, 2009).

Rapid (point-of-care) assays that detect HIV specific antibodies are available internationally (Den 2003, Stevens 2014). Saliva, urine, other body fluids or test analytes may be used with some rapid tests to allow people to self-sample. These assays require less technical expertise to perform and are more commonly used in resource limited settings (Stevens, 2014). They are increasingly used in Australia and overseas in specific settings, including post-exposure prophylaxis management or pre-transplant screening in developing countries, and in specific populations to increase test uptake in at-risk populations (Ward, 2013). Individuals reactive in a rapid test must undergo conventional laboratory testing test as rapid tests may show inferior specificity compared with laboratory based tests. (Den 2004, Ly 2014).

3.2.2 HIV Supplemental assay- Western Blot

During the immune response to HIV, antibodies to the various antigens of HIV appear in serum after different times. These different antibodies can only be identified using the western blot (WB) assay. The WB assay should only be performed on sera that are reactive on a screening assay.

Western blot assays detect antibodies in patient sera to a number of different viral proteins. These viral proteins (gag, pol, env protein) are separated into bands of distinct molecular weight using protein gel electrophoresis. After transfer (blotting) to a solid material (such as

nitrocellulose strips), proteins that are reactive with specific HIV antibodies in serum can be identified after the addition of enzyme labelled conjugate and reaction with substrate.

The development of a positive WB usually takes approximately six weeks from infection although the evolution of the blot pattern is affected by early primary treatment with anti-retroviral drugs. WB patterns of reactivity detected during the immune response that do not meet the criteria for an interpretation of "positive" (often with missing bands) are referred to as indeterminate. Some of these patterns can also be seen in uninfected individuals. In 1992 the Australian National HIV Reference Laboratory in collaboration with state reference laboratories published a classification of the positive, negative and indeterminate WB patterns (Dunn 1995). This classification has been used since that time throughout Australia and New Zealand providing a reliable uniform system of reporting western blot results as shown in Table 2 (Healey 1992), until the introduction of the new in vitro diagnostic device (IVD) legislation governing Class 4 IVDs, which includes HIV WB. Previous to the IVD framework, the NRL WB interpretation results were regarded as negative if there were no reaction of the patient`s serum with any protein bands. A positive result was defined by detection of antibodies to all of the three main groups of HIV proteins –one envelope (gp 160, or gp120, or gp 41), plus 3 other viral specific bands from the gag (p24) and polymerase (p66 or p51) series. HIV western blot-indeterminate patterns were grouped into four groups (1 to 4) according to the antibodies detected allowing a significant percentage to be reported as anti-HIV-1-negative (group 1, group 2). Indeterminate groups 3 and 4 WB patterns were seen early in the immune response, although they could be seen in uninfected individuals as well. Conversely, with disease progression, gag antibodies may become undetectable and the WB pattern may revert from positive to indeterminate group 4. Therefore the interpretation of an indeterminate group 4 result can only be made by considering clinical history and clinical findings.

Since introduction of the IVD legislation, use of the indeterminate (IND) groupings may be useful, but if using them results in an interpretation different from that recommended by the manufacturer of the ARTG licensed WB assay, then data must be available to validate the accuracy of the result. Where those data are not available, laboratories cannot use the NRL criteria instead of manufacturers' criteria. In patients with repeatedly reactive screening and indeterminate WB results, follow up samples should be tested. Any positive WB should be confirmed by testing a second sample.

There is an RCPA initiative underway to standardise HIV Western blot interpretations, as well as review of the criteria by reference laboratories. This has come about due to most Australian reference laboratories using NRL interpretive criteria established for their own in-house Western blot in 1992. The RCPA initiative surveyed HIV reference laboratories around Australia and found two different commercial western blot kits are in use predominantly. The interpretive criteria differ between the manufacturer's instructions and those of the NRL, and laboratories are using one of these criteria or a hybrid combination of the two. The manufacturer of the kit most commonly used around Australia does not have subcategories of indeterminate western blots. It is possible for laboratories to have categories of higher risk indeterminate western blots without conflicting with the manufacturer's instructions. It may be clinically very useful to distinguish between western blot indeterminate categories in terms of risk. Basically the higher risk indeterminate blot will have a glycoprotein band present

(two bands needs for positive), as outlined in Table 2. Group 1 and group 2 indeterminate western blots are reported negative when these NRL criteria are applied.

Table 2: Western blot results classification by commonly used laboratory criteria, outside of TGA-licensed manufacturer's recommendations in ARTG registered western blot kits

Positive	gp 160/41 plus three or more gag/pol bands
IND4*	gp 160/41 plus not more than two gag/pol bands
IND3*	p24 ± other bands, no gp 160/41 present
IND2*	p17/18 ± other bands, no gp 160/41 and no p24 present
IND1*	Bands other than those listed above
Negative	No bands

IND = indeterminate

3.2.3 Suitable specimens

Serum samples are used routinely for standard HIV antibody and antigen determinations, although plasma samples are also acceptable. Dried blood spot can be used for diagnosis of children who are less than 18 months old. Urine, oral fluid as well as dried blood spot are approved for use in some rapid HIV antibody kits.[8]

The serum should be promptly separated from the clot/cellular elements and refrigerated at +2°C to +8°C. If the test is not performed within seven days, the serum specimens should be frozen at –20°C or lower. Serum can be transported either at room temperature if it is expected to be processed in four to six hours of transportation, or refrigerated in screw capped plastic jar, if transportation takes place within seven days of collection (NPAAC 2009).

3.2.4 Test Performance Characteristics

HIV screening assays including the combined Ag/Ab assays, were initially developed for use in donor screening, which demands maximum sensitivity. Consequently, although they have a have a high specificity (>99.8%), false positive and false negative results can occur infrequently. Laboratory confirmation of infection in Australia requires demonstration of a positive pattern of HIV specific antibodies using a western blot. Factors associated with false negative Ag/Ab (CMIA, ChLIA or EIA) results include testing during the window period, patients on immnuosuppresive therapies, post-exposure prophylaxis patient with concurrent hepatitis C infection and those who have advanced HIV infection, as well as laboratory errors (Murray 2009, George 2014).

Western blot indeterminate results in infected individuals are related to several factors such as early seroconversion, incomplete HIV-1 antibody evolution, cross- reactivity with HIV-2 proteins, and cross reactivity HIV-1 subtype O proteins.

Test specificity depends upon several factors, including pre-test probability, population prevalence and population incidence. The proportion of reactive results that are false are higher in low prevalence populations. The specificity of current antigen/antibody screening

tests is above 99.5%. Factors associated with false positive results include frequent blood transfusion, pregnancy, autoimmune diseases, vaccination, and laboratory errors of procedure or specimen handling.

Western blots can produce indeterminate results in uninfected individuals associated with multiple transfusions, hypergammglobulinemia, recent vaccination, advanced AIDS and autoimmune diseases (Murray 2010).

3.2.5 Suitable internal controls

Commercial kit and a third party low positive quality control and negative control should be used in every run as recommended by NPAAC, although they are not mandatory.[9] These are in addition to use of kit controls, us of which is mandatory.

3. 2.6 Suitable external quality assurance program (QAP) program

These–are provided by the Royal College of Pathologist of Australasia (RCPA) serology quality assurance program (RCPA-QAP) and the National Serology Reference Laboratory (NRL).

3.3 HIV Nucleic Acid Testing

In recent years molecular based methods for detection of viraemia have become available with qualitative NAT assays and quantitative molecular methods (such as end point and real time PCR, TMA) widely used for monitoring of viral load and response to antiviral drugs. The manufacturers of these commercial assays do not, in general, include diagnostic purposes in the assays' intended use and therefore they should be used only in conjunction with serology to diagnose primary HIV infection in line with the guidelines outlined above. Increasingly molecular tests (qualitative and quantitative) are requested by clinicians for early detection of HIV infection prior to full seroconversion, to facilitate early antiretroviral therapy (WHO 2009, Hoy 2009), and used in settings for diagnosis of infants. The majority of assays available are quantitative, measuring viral load, although a small number of qualitative assays are becoming available.

Quantitative viral load assays determine the amount of HIV-1 RNA circulating in the blood of an infected individual. Differences exist in the absolute copy number generated by different viral load assays. International units (IU/mL) are used to report results and allow comparison between different assays, although ideally, the same assays should be used to follow up an individual's viral load.

In Australia, some of the HIV NAT assays available in 2015 are listed in Table 3 and some characteristics of these tests shown in Table 4.-

Table 3: HIV NAT assays currently registered on the ARTG

Manufacturer	**Assay name**	**ARTG ID NO**
Roche Diagnostics	Cobas AmpliPrep/TaqMan HIV-1 Test	180220
Abbott Diagnostics	Abbott Real Time HIV-1	217841

Cepheid Holdings	Xpert HIV-1	259967
Qiagen	Artus HIV-1RG	210376
Siemens Health Diagnostics	Versant HIV-1 RNA 1.5 assay (kPCR)	185755

Table 4: Dynamic ranges and conversion factors for Molecular HIV RNA quantitation

Manufacturer	Assay Name	Vol Used	Result	Interpretation	Dynamic Range	Conversion Factor	Insert Version
Roche Diagnostics	Cobas AmpliPrep/ TaqMan HIV-1 Test Cobas TaqMan HIV-1 Test with High Pure System	1000 – 1050 uL 500uL	Target not detected	HIV RNA not detected		1 IU/mL = 0.51 copies (1 copy = 1.96 IU)	2/2003 (pg.26&28), [Revision 4.0
			<40 copies/mL [Calculated copies/mL below range of assay]	HIV RNA detected, <40 HIV RNA copies/mL			
			>1.0 x 10^7 copies/mL [Calculated copies/mL above range of assay]	>6.9 x 10^7 HIV RNA copies/mL			
			Target not detected	HIV-1 RNA not detected			
	Cobas AmpliPrep/ TaqMan HIV-1 Test Cobas TaqMan HIV-1 Test with High Pure System	1000 – 1050 uL 500uL	<40 copies/mL [Calculated copies/mL below range of assay]	HIV-1 RNA detected, <47 HCV RNA copies/mL			
			>1.0 x 10^7 copies/mL [Calculated copies/mL above range of assay]	>1.0 x 10^7 HIV-1 RNA copies/mL			
			Not Detected	Target not detected			
			<40 copies/mL	Detected			
	Cobas AmpliPrep/ TaqMan HIV-1 Test Abbott RealTime HIV-1	1000 – 1050 uL 1mL	>1.0 x 107 copies/mL	> Upper limit of detection	20 – 1.0 × 10^7copies/ mL	1IU/mL = 0.6 copies (1 copy = 1.67 IU)	01/2013 (pg.1&25) Revision 8.0]
			Not Detected	Target not detected			
			<75 copies/mL	Detected			
	Cobas TaqMan HIV-1 Test with High Pure System	500uL 0.5mL	>1.0 x 107 copies/mL	> Upper limit of detection	47 – 1.0 x 10^7 copies/mL	1IU/mL = 0.61 copies (1copy = 1.64IU)	7/2006 (pg.5, 16&17 [Revision 3.0]
			Not detected	Target not detected			
			<150 copies/mL	Detected			
Siemens Healthcare Diagnostics	Versant HIV-1 RNA 1.5 Assay (kPCR)	0.5mL			37- 1.0 × 10^7copies/ mL		
Cepheid Holdings	Xpert HIV-1				40 – 1.0 × 10^7 copies/mL		
Abbott Diagnostics	Abbott RealTime HIV-1	1mL	Not Detected	Target not detected	40 – 1.0 x 10^7 copies/mL	1IU/mL = 0.58 copies (1 copy = 1.74IU)	2007 (pg 38-41)
			<40 copies/mL	Detected			
			>1.0 x 10^7 copies/mL	> Upper limit of detection			
		0.5mL	Not Detected	Target not detected	75 – 1.0 x 10^7 copies/mL		
			<75 copies/mL	Detected			
			>1.0 x 10^7 copies/mL	> Upper limit of detection			
		0.2mL	Not detected	Target not detected	150 – 1.0 x 10^7 copies/mL		
			<150 copies/mL	Detected			
			>1.0 x 10^7 copies/mL	> Upper limit of detection			

			>5.0 x 10^5 copies/mL	>5.0 x 10^5 copies/mL			
Qiagen	Artus HIV-1 RG-PCR Kit	1.5mL	Signal detected A.FAM	Analysis is positive: sample contains HIV-1 RNA	120 - 1.0 ×10^8 IU/mL	1 IU = 0.50 copies/mL	05/2011 (pg7&24)
			A.FAM no signal + signal Internal control	No HIV-1 RNA is detectable. Considered negative			

3.3.1 HIV NAT Testing- Roche COBAS Amliprep/ COBAS TaqMan HIV-1

This assay combines automated preparation of HIV-1 RNA purification using Ampliprep, and real- time PCR amplification and detection using COBAS TaqMan probe-based assays. It targets a conserved region in HIV-1 gag, using fluorescence labeled probes to detect the amplified products, with a dynamic range as in Table 4: 20 – 1.0 × 10^7 copies/mL.

3.3.2 Suitable Specimens

The viral load tests are most commonly performed on plasma, which is the validated specimen type for nucleic acid detection. For all assays, the plasma should be separated within six hours of collection, because delays in separation of plasma from cellular elements may lead to RNA degradation.

These assays have also been used on serum, CSF, cervical secretion, semen and dried blood spot where clinical need has been indicated, although this is outside the manufacturers and TGA guidelines as the assays are currently not validated for use in these specimens.

3.3.3 Test Sensitivity

Sensitivities for each assay vary slightly, although the lower limit of sensitivity is ~30-260 IU/mL For the Siemens Versant HIV-1 RNA and Cepheid Xpert HIV-1 assays, as the conversion factor is not available, the IU/mL is not known. The range for all assays in copies /ml is ~20-150- copies/mL, as shown in Table 4.

3.3.4 Test Specificity

Test specificity varies between assays with indicative specificities approaching 100%.

3.3.5 Suitable internal controls

High positive, low positive, and negative test kit controls must be used in every run. Internal QC samples should be used regularly to monitor assay performance.

3.3.6 Suitable External QAP

There are provided by the Royal College of Pathologists of Australasia (RCPA) Serology quality assurance program (SQAP) and the National Serology Reference Laboratory (NRL).

3.3.7 HIV Proviral DNA PCR

Although HIV-1 DNA PCR has been used as an investigational tool for more than decade, there is no test in any format registered by the FDA or TGA. This qualitative procedure is sensitive and can detect between 1 and 10 copies of HIV-1 proviral DNA per sample.[1] Because of the extremely high sensitivity of this assay, small amounts of background noise in

the environment or contamination during laboratory processing may result in amplification of products that can produce false positive reactions. All of the positive DNA PCR results require confirmation with a second PCR test on a separate specimen. Currently, the only recommended diagnostic use of HIV-1 DNA PCR is for the detection of infection in infant born to mothers infected with HIV-1, although this is not currently licensed in Australia. When used in this setting, the potential for false positive results should be recognised.

3.4 Anti- Retroviral (ARV) drug resistance testing

The replication rate of HIV is very high and there is no proof reading of the reverse trascriptase (RT) enzyme during replication, as a result, it prone to several mutations. Most of these mutations target RT and protease enzymes. The resistance profiles are assessed and detected using genotypic and less commonly using phenotypic methods. Ideally, it should be performed in HAART naïve patients before initiation of treatment and in cases of virological failure (i.e. decrease in the viral load by 1 log after 4 to 6 weeks of treatment) or incomplete viral suppression (undetectable viral load in 4 to 6 months of treatment).[7]

3.4.1 Genotypic HIV drug resistance testing

A genotypic assay provides an indirect measure of drug resistance because it is based on detection of the mutations known to be associated with resistance especially those which involve nucleotide sequence of RT and protease enzyme [2].

The assay includes extraction of the RNA and RT- PCR to amplify viral RNA genes that code for HIV RT and protease. Then, the sequence of the amplified target is analysed and compared to wild type virus sequence to determine any mutations. For this to be accomplished the HIV VL required to be > 1000 copies/ ml to detect mutations.[9] One limitation of this assay is that it only detects mutants comprising major fractions of patient virus; resistant virus must represent ~25% of the virus population meaning low frequency drug resistance mutations may be missed.[9]

3.4.2 Phenotypic HIV drug resistance testing

Phenotypic assays are much less frequently undertaken as they are highly labour-intensive. They assess the ability of the virus to grow in various concentration of a given ARV drug. These are currently only available in Australia in clinical trials. These assays involve the amplification of reverse transcriptase or protease, insertion of the sequences into a plasmid vector, then culturing a hybrid virus containing that vector in comparison with wild- type HIV.

3.4.3 Suitable Specimens

Plasma collected from EDTA or plasma separator tube can be used. The plasma must be separated from the cellular elements within 6 hrs of collection and frozen immediately.

3.4.4 Utility

Treatment may be altered in the knowledge that a patient is carrying a virus resistant to one or more ARV agents. This may lead to improved virological outcomes in HIV patients whose therapeutic choices have been guided by the use of resistance testing.

3.4.5 Suitable external QAP program

There are provided by National Serology Reference Laboratory (NRL) for the genotypic resistance testing only.

3.5 HIV Viral Isolation

Although a positive culture provides direct evidence of HIV infection, HIV culture is no longer used for routine diagnosis, as it has long turnaround time, low sensitivity and it is labour-intensive.[3]It was useful before the development of NAT in detecting infection in infants born to mothers with HIV infection. It is utilized primarily in research laboratories.[3]

In the assay, patient specimens are first cultured by mixing patient peripheral blood monocyte cells (PBMCs) with PBMCs from healthy donors stimulated with phytohemagglutinin and IL-2. Freshly stimulated donor cells must be added weekly, because HIV-1 causes cell death. In the second step, the presence of RT or p24 antigen released in the culture supernatant is assayed periodically.

3.5.1 Suitable specimens

Whole blood is most often used. It should be collected EDTA tube. It can also be cultured from plasma or PBMCs. For preparation of PBMCs, the blood should not be refrigerated or frozen, but instead should be kept at ambient temperature for no longer than four days.[9]

3.5.2 Utility

Viral isolation is not used for diagnosis routinely. The major role is in antiviral research and maybe used for maintenance and analysis of viral strains.

3.5.3 Suitable external QAP program

No program is available in Australia.

3.6 Point of Care Testing (POCT)

Point of care testing is discussed elsewhere in detail. The POCT assays are screening assays that then require confirmation by conventional testing. The assays available are changing rapidly and the Table 5 below indicates some assays that are already registered on the ARTG in 2015-2016.

Table 5: Some HIV rapid tests currently registered on the ARTG

ASSAY	USE	STATUS IN 2015
Bio-Rad Geenius HIV-1/2	Supplemental	On ARTG
Integrated Sciences OraQuick Advance Rapid HIV-1/2 Ab	Screening	On ARTG
Diasorin Murex HIV-1.2.0 ICT	Screening (including blood	On ARTG

	donors)	
Inverness Determine HIV-1/2 Ab ICT	Screening	On ARTG
Inverness Determine HIV-1/2 Ag/Ab Combo ICT	Screening	On ARTG
Uni-Gold HIV ICT	Screening	On ARTG

4. Diagnosis Of HIV In Neonates And Children < 18 Months Age

4.1 Infants

HIV infection in neonates or children infants < 18 months born to HIV-infected mothers cannot be diagnosed by serological tests due to the potential false positive results from passively acquired maternal antibodies, which may persist for more than one year.[5] Diagnosis in this period is usually established using molecular testing such as proviral HIV DNA testing on whole blood or dried blood spots, and HIV RNA NAT on plasma. These virological assays are considered to be the reference standard for diagnosis of HIV infection in children younger than 18 months. However, the proviral DNA assay is currently not registered for use in Australia, and the RNA NAT (either qualitative or quantitative) is not registered currently for diagnosis in the absence of antibody results. Testing of cord blood is not recommended as there is a chance of contamination of the cord blood by maternal blood which can lead to false positive results.[1]

In diagnosis of neonates, HIV DNA or RNA NAT must be tested at different time points as sensitivity of PCR increases with age of testing in infants.[12] Regimens include testing at three time points - within the first 14 days of life, at 1-2 months and at 3-6 months.[13]A definitive diagnosis of HIV-1 infection can be made on the basis of positive HIV-1 DNA or RNA assay results from two or more separate samples(sensitivity >98%).[1] HIV-1 infection can be presumptively excluded based on two negative virological test results, one obtained at greater than one months age and one obtained at four months of age, or two negative HIV-1 antibody test results from separate specimens at six months of age.[12] A confirmatory test to document the clearance of maternal HIV antibodies (seroreversion) is recommended at 18 months of age when ~ 100% of initially seropositive infants will have seroreverted.

In **c**hildren over 18 months of age, HIV can be diagnosed using positive HIV-1 antibody detection tests, both screening and confirmatory assays. HIV-exposed infants are usually formula-fed in Australia. However, if the infant is still breastfeeding from an infected mother, negative results need to be repeated at 4-6 weeks after cessation of breastfeeding to confirm that the infant is not infected. In addition to the molecular and HIV antibody tests, the Ultra-sensitive p24 assay (ELISA) is gaining the support to diagnose HIV in infancy. However, passive transmission from maternal blood may still make diagnosis difficult and uncertain in this setting.[1]

5. HIV Screening In Organ And Blood Donors

The screening of donors for HIV infection is a large and complex area. Traditionally HIV seropositive donors have not been utilized in transplantation, due to the known risk of transmission to the recipient. HIV-1 and HIV-2 serology results are required for all potential donors and HIV-1 NAT testing is increasingly also performed on organ donors, as it has been on blood donors for many years. Western blot testing should be obtained for confirmation of any positive screening test for either HIV-1 or 2 as for standard confirmation of HIV infection. In the potential living donor with increased risk factors for HIV exposure but negative HIV serology, a molecular viral test should be obtained, as these tests become positive prior to the development of a positive antibody test. When available, NAT for HIV is also desirable for deceased donors with potential exposures identified in their social history.[12] A recent report of HIV transmission from an antibody-negative organ donor underscored the risk of transmission if the donor is in the 'window' period after infection but prior to development of anti-HIV antibody. Although previously considered a contraindication to transplantation, HIV seropositivity in the recipient is receiving renewed attention.[12] Now that many patients with HIV on highly active antiretroviral therapy (HAART) regimens are living longer and are far less immunocompromised, in some cases it is end-stage organ failure rather than HIV that is the survival-limiting condition.[12]

6. Snomed- Clinical Terms (Ct) Terminology

SNOMED Code System Concept Code	86406008
Read Code	X70M6
Code System Concept Name	Human immunodeficiency virus infection (disorder)
Code System Preferred Concept Name	Human immunodeficiency virus infection
Concept Status	Published
Concept Status Date	09/01/2015
ICD10 codes	B210 B211 B201 B200 B219 B202 B209 B208 B205 B204 B24X B206 B230 B218 B232 B220 B227 B221 B231 B212 B203 B222 B207 B213 B217 B238

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