Micropathology Ltd
Tel 24hrs: +44 (0) 24 - 76 – 323222
Fax / Ans: +44 (0) 24 - 76 - 323333
University of Warwick Science Park, Venture Centre, Sir William Lyons Road, Coventry CV4 7EZ
Website: www.micropathology.com E-mail: info@micropathology.com

HIV test methods and applications user information sheet - Version: 6.0. Index: S - 1578. Printed: 09-Mar-2023 12:07

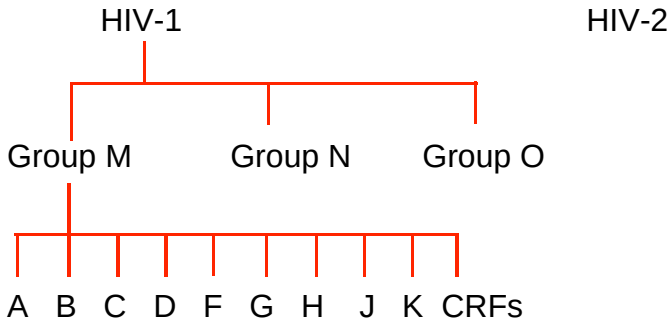
Human Immunodeficiency Virus (HIV)

HIV is a lentivirus that belongs to the Retroviridae family and is characterised by a single stranded RNA genome.

There are two types of HIV: HIV-1 and HIV-2 and they appear to cause clinically indistinguishable AIDS. Transmission of both types can occur by sexual contact, through blood-to-blood contact, vertically from mother to child, and by breastfeeding. Worldwide, the predominant virus is HIV-1 and generally when people refer to HIV, without specifying the type of virus, they will be referring to HIV-1.

HIV-2 is uncommon, concentrated in West Africa but also found in countries with immigrants from this region. There are around 230 cases of HIV-2 infection in the UK. HIV-2 infections are characterised by a very low viral load burden (as compared to HIV-1 infections) and a greater likelihood of remaining undiagnosed until AIDS develops in the infected individual. However, progressive disease is clinically indistinguishable from HIV-1 infection and typically occurs at higher CD4 counts. Many HIV-1 drugs are ineffective or less effective against HIV-2 and, as HIV-2 viral load testing is not widely available, infection may be hard to monitor, particularly as disease progression can occur with undetectable viral loads. HIV-2 should be considered in at risk individuals from or with a history of travel to West Africa or with discordant test results.

Subtypes



The strains (or clades) of HIV-1 can be classified into three groups: the "major" group M, the "outlier" group O and the "new" group N. Group O appears to be restricted to West-Central Africa and group N - discovered in Cameroon - is extremely rare. More than 90% of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K.

Occasionally, two viruses of different subtypes can meet in the cell of an infected individual and recombine together their genetic material to create a new hybrid virus (recombinant). Many of these new strains do not survive for long, but those that infect more than one person are known as Circular Recombinant Forms (CRFs).

Pathogenesis

Within 3-6 weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterised by flu-like symptoms and associated with high levels of viraemia in the peripheral blood, although HIV-2 viral loads rarely exceed 1600 copies/mL.

In most infected individuals this is followed by an HIV-specific immune response and a decline in plasma viraemia, usually within 4-6 weeks of the onset of the symptoms. At this point, antibody tests can detect the presence of infection (termed seroconversion). After seroconversion, infected individuals typically enter a clinically stable, asymptomatic phase that can last for years. This asymptomatic period is characterised by persistent low-level plasma viraemia and a gradual depletion of CD4+ T lymphocytes. This eventually leads to severe immunodeficiency (AIDS), multiple opportunistic infections, malignancies and death.

Although virus levels in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which the high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected cells and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viraemia and CD4+ cells.

Diagnostic tests

Serological evidence of HIV infection may be obtained by testing for HIV antigens and/or antibodies in the serum or plasma of individuals with suspected HIV infection. Antigens can only be detected during the acute phase and during the symptomatic phase of AIDS. Antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at or shortly after the acute phase and lasting until the end stages of AIDS. The use of highly sensitive 4th Generation or 5th Generation antigen/antibody assays is therefore an established approach in the serodiagnosis of HIV infection and in the screening of blood products.

Increased antibody test sensitivity has reduced the time between infection and the moment at which antibodies can be detected. On average, it takes 22 days for antibodies to reach detectable levels. However, the 2020 BHIVA guidelines recommend that the window period from infection to detection of serological markers by 4th generation tests is 45 days. Incorporating HIV p-24 antigen detection into assays can reduce this window period. The p-24 antigen is produced in excess early in infection and '4th generation' EIA tests, used for HIV diagnosis in the UK, include monoclonal antibodies that will detect this antigen. Generally, if the patient has not taken any treatment, the use of a 4th generation test will reduce the window period by about a week. The levels of p-24 antigen in the serum will decline and may become undetectable following seroconversion and as the infection progresses.

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- ***HIV 'ECLIA' (Electrochemiluminescence immunoassay)***

We use the Roche Cobas HIV combi assay, which is a sandwich-based assay performed on the Roche Cobas e411 analyser. This simultaneously detects HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2 in human serum and plasma. Recombinant antigens are used to determine HIV-specific antibodies, whilst specific monoclonal antibodies are used for the detection of HIV-1 p24 antigen. The 2020 British HIV Association guidelines state that initial laboratory diagnoses of HIV infection should be confirmed using an assay that discriminates between HIV-1 and HIV-2 and detects anti-HIV antibodies and p24 antigen simultaneously (at least 4th generation) and that emergency departments may offer testing in areas with high HIV seroprevalence.

- ***HIV LIA (Line immunoassay)***

We use the INNO-LIA™ HIV I/II Score assay as a confirmatory test for samples found to be positive in a screening test. This assay will distinguish between HIV 1 and 2 infections.

Five HIV-1 antigens are present on the LIA strip; sgp120 and gp41 detect specific antibodies to HIV-1 (HIV-1 group O peptides are present in the HIV-1 gp120 band); p31, p24 and p17 cross react with antibodies to HIV-2. In addition, the LIA strip contains antigens gp36 and sgp105 which are specific for antibodies to HIV-2 only.

- ***Molecular detection of HIV-1 proviral DNA***

Reverse transcription of HIV-1 RNA and its subsequent integration into the genome of the host results in the presence of proviral DNA (provirus) in lymphocytes. The molecular detection of this proviral DNA is particularly useful for the diagnosis of HIV-1 in babies born to infected mothers. These babies can carry maternal antibodies for up to 18 months and antibody detection is, therefore, not a reliable indicator of HIV infection.

It is important whilst testing neonates and young infants (less than 18 months old) for HIV infection that simultaneous testing of HIV proviral DNA in the mother is performed. In the event of HIV proviral DNA not being detected in the baby, its detection in the mother ensures that this is not due to the inability of the primers to detect the particular sub-type of the HIV virus infecting the mother. We use assays that detect HIV-1 proviral DNA genes encoding proteins gag, pol, env and the transcription regulator LTR (Long Terminal Repeat). The use of four markers of infection decreases the chances of missing a gene not detected by a particular set of primers.

Detection of proviral DNA can reduce the post-exposure window period to about 11 days and so may also be used as a diagnostic test to detect HIV-1 in individuals who have not yet mounted an immune response, or to resolve indeterminate serology

results. This assay is also used by laboratories seeking additional confirmation of HIV-1 negative status in donor tissues.

We also offer a two marker (pol and LTR) diagnostic assay for HIV-2 proviral DNA/RNA. This may be used in situations where an individual is at risk of HIV-2 infection or has discordant test results.

Disease state monitoring

- ***HIV-1 RNA quantitation***

Quantitative measurements of HIV-1 viraemia in the peripheral blood have shown that higher virus levels may correlate with increased risk of clinical progression of HIV disease and that reduction in plasma virus levels may be associated with decreased risk of clinical progression. Virus levels may be measured in the peripheral blood by direct measurement of viral RNA in plasma using nucleic acid amplification technologies. We use the IVD/CE marked Roche COBAS® 5800 HIV-1 quantitative test to monitor HIV-1 viral load in HIV-1 group M and group O infected patients.

The test can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy (ART) by measuring changes in the viral load over time. The levels of detection of HIV-1 RNA in this assay range from 20-10,000,000 copies/ml.

BHIVA guidelines no longer state that this test should NOT be used as a diagnostic test to confirm the presence of HIV-1 infection (screening). Molecular assays should not be used for routine diagnosis currently, as detailed in the current BHIVA testing guidelines (2020), where only serological testing should be used for this purpose. HIV-1 viral load monitoring should ONLY be used on samples from people living with HIV (PLWH), but the assay may be used for confirmatory testing if no other means are available, or serological assays yield equivocal/conflicting results. It should be noted that the use of this assay instead of proviral DNA testing in individuals on cART may yield false negative results.

- ***HIV-1 drug resistance***

HIV-1 treatment failures arise due to poor adherence to treatment regimens and/or the emergence of virus strains that are resistant to the drugs. These HIV-1 mutant strains may be resistant to one or more drugs in each class, including nucleoside reverse transcriptase (RT) inhibitors (NRTIs), non-nucleoside RT inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, entry inhibitors and HIV integrase strand transfer inhibitors (INIs). Additionally, resistance to a given drug may generate resistance to different drugs of the same class. However, treatment failure does not always result in resistance to all drugs within the regime.

A key factor in identifying new treatment regimens is knowledge of the viral resistance genotype within the viral population in the individual's plasma. Studies have provided

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evidence supporting the clinical utility of resistance testing and they indicate that the presence of drug resistance is an independent risk factor for treatment failure.

Currently HIV-1 resistance testing is recommended for:

- New diagnoses of PLWH who enter into care, regardless of whether therapy is initiated. It should be repeated when ART is initiated
- PLWH changing antiretroviral treatment regimens following failure
- pregnant women prior to commencing ART
- women who become pregnant whilst on therapy

We use an in-house RT-PCR/Sanger Sequencing based assay to determine the nucleotide sequence of the complete HIV-1 protease gene, codons 1 to ~350 of the reverse transcriptase gene and codons 6-273 of the integrase gene. This sequence data is analysed using the online HIV Drug Resistance Database maintained and regularly updated by Stanford University (HIVdb).

The minimum viral load for these assays is 500 HIV-1 RNA copies/mL for subtype B viruses. Any viral loads less than this are significantly more likely to not produce genotypic resistance results, as theoretical detection of <10 individual HIV-1 genomes could miss low level resistance mutations in the population (i.e. a wild-type result, if the RT-PCR is successful, does not necessarily mean that no resistance mutations are present). All samples with a higher viral load are tested, but due to variability in the HIV-1 genome, it is not possible to guarantee successful amplification and/or sequencing on viral loads <7,000 copies/mL.

An example of one of our antiretroviral drug resistance reports and a downloadable drug resistance request form are available from our website: www.micropathology.com.

- ***CD4 testing***

HIV infects CD4 expressing T-lymphocytes bringing about lysis of these, and bystander cells, by various mechanisms, resulting in the destruction of billions of CD4 T-lymphocytes every day. This eventually overwhelms the immune system's regenerative capacity resulting in an inability to mount a desirable immune response to any pathogen and vulnerability to opportunistic pathogens characteristic of AIDS.

Since CD4 cells are usually destroyed more rapidly than other types of lymphocytes and because absolute counts can vary from day to day, it is sometimes useful to look at the number of CD4 cells compared to other types of lymphocytes with the result expressed as a percentage. Also a CD4 cell count may be compared to a CD8 cell count and the result expressed as a ratio.

Patients with AIDS exhibit T-cell lymphopenia, a loss of CD4+ lymphocytes and a relative increase in the CD8+ and CD3+CD4-CD8- subtypes. Therefore, a precise enumeration of CD4+ T-cells is necessary for reliable and controlled ART and patient monitoring.

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Until recently, there was a generally used threshold of 200 CD4+ T-lymphocytes/µl blood to start ART for adult patients may not be appropriate for children since the CD4 counts in infants are significantly higher than in adults. However, current guidelines recommend starting ART as soon after diagnosis as possible to increase the chance of reducing the reservoir of virus integrated into the genome and improving prognosis. In paediatric patients a CD4% measurement should be performed for controlled and reliable ART and patient monitoring.

Sample requirements

Please refer to our user handbook (available for download from our website www.micropathology.com) for details of which samples are acceptable for each test and any requirements regarding transport, storage, and separation of plasma from cells.

Summary of HIV test methods and their applications

Method	Marker	Application
EIA	Antibody/antigen	Screening assay
LIA	Antibody/antigen	Confirmatory assay for serological diagnosis
(RT)-PCR (in-house)	HIV-1 proviral DNA/RNA (gag, pol, env, LTR) HIV-2 proviral DNA/RNA (pol, LTR)	Diagnostic assays. Vertical transmission, window period, donor screening, equivocal or discordant serological assays
Roche Cobas 5800	Viral RNA (LTR)	Viral load monitoring
PCR and sequencing (in-house)	Genotypic Antiviral resistance	Monitoring for antiviral resistance

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