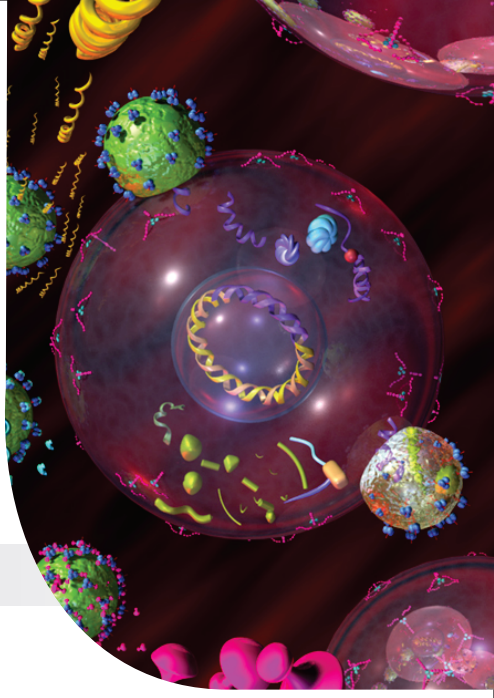
Unipath
SPECIALTY LABORATORY Ltd.



HIV-1 Proviral DNA Detection by Real Time PCR

Department of Molecular Biology

INTRODUCTION

HIV-1 Proviral DNA test detect HIV-infected lymphocytes. During HIV replication, the virus creates a double-stranded DNA copy of its genome and this provirus is integrated into the DNA of the host cell. When the host cell replicates, the daughter cell also receive a copy of the proviral DNA.

CLINICAL INFORMATION:

Human immunodeficiency virus (HIV)-1 infection in humans is usually confirmed by detection of HIV-1-specific antibodies in serum. However, serologic testing may not reliably identify HIV-1 infection in neonates with passively acquired maternal HIV-1 antibodies or with incompletely developed immune system, in individuals with early infection (<30 days from infection), or with "indeterminate" antibody profile by Western blot assays. In these situations, detection of HIV-1 proviral DNA by PCR can provide early evidence of HIV-1 infection (approximately 10-14 days after infection), when results of routine diagnostic assays are inconclusive.

Upon entry into human cells (including peripheral blood mononuclear cells), the HIV-1 RNA is converted into complementary DNA (cDNA) by reverse transcription. These linear cDNA strands are then integrated into the host genome, thus representing the proviral form of HIV-1. mRNA, transcribed from the proviral DNA, is used to synthesize the proteins required to make new viral particles. These proteins and viral RNA are packaged in the host's cytoplasm and released from the cell, completing the life cycle of the virus.

The presence of integrated HIV-1 proviral DNA can be detected by a PCR that targets a segment of the highly conserved HIV-1 gag gene. Clinical studies have indicated that detection of HIV-1 proviral DNA in whole blood specimens by PCR is highly sensitive (>95%) and specific (>98%) for the presence of early detection of HIV-1 infection in infants <2 years old.

HIV-1 proviral testing is recommended at birth, 1 to 2 months of age, and 6 months after birth, in infants born to HIV-1-infected mothers. Two serially positive HIV-1 virologic test results (HIV-1 proviral DNA & HIV-1 RNA) are necessary for the diagnosis of HIV-1 infection in infants <2 years of age.

CLINICAL UTILITY:

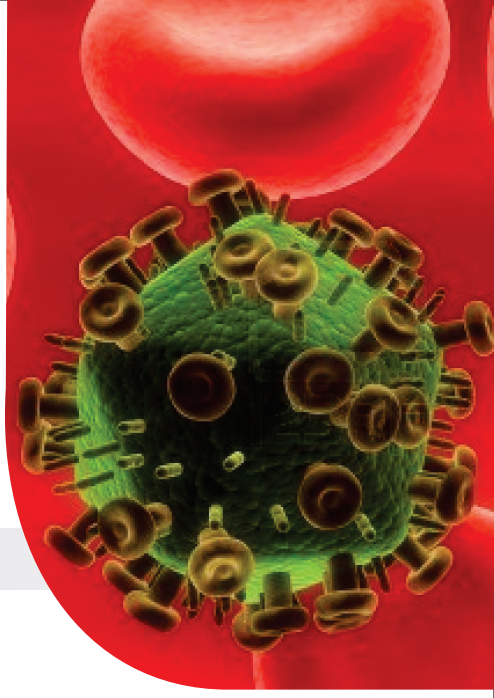
- Virologic detection of HIV-1 infection in infants of <2 years of age (an age group for which serologic tests are unreliable) born to HIV-1-infected mothers
- Early detection of HIV-1 infection in children and adults prior to appearance of HIV-1 RNA, HIV-1 p24 antigen, or HIV-1 antibodies in blood
- Individuals whose HIV screening test by EIA/MEIA is reactive, but whose status is indeterminate or inconclusive by Western blot.
- High-risk individuals with a negative serological screen and a history of recent exposure
- Determining eradication of HIV-1 in individuals receiving investigational highly active antiretroviral therapies

References:
1. DeSimone JA, Pomerantz RJ: New methods for the detection of HIV. Clin Lab Med 2002;22:573-592
2. Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection. Available from URL: http://www.aidsinfo.nih.gov/ContentFiles/PediatricGL_SuplPDA.pdf 2005, pp 4

Specimen: EDTA blood/plasma (Immediately send to lab within 24 hours)

TAT: 2 DAYS

Unipath
SPECIALTY LABORATORY Ltd.



HIV-1 Qualitative Detection by Real Time PCR

Department of Molecular Biology

INTRODUCTION

The HIV-1 qualitative assay detects the presence of HIV-1 viral RNA in plasma. A positive result indicates that viral replication is occurring. This procedure utilizes a real-time reverse-transcriptase PCR procedure to amplify a portion of the 5' Long Terminal Repeat region of the HIV-1 genome. This is highly conserved.

CLINICAL INFORMATION:

This test may be used to resolve indeterminate HIV western blot results and to detect early HIV infection. The Centers for Disease Control and Prevention recommends the use of plasma RNA testing in conjunction with an HIV antibody test in cases where acute retroviral syndrome is suspected. The HIV-1 Qualitative Assay can be used for this purpose.
The HIV-1 Qualitative assay is not a replacement for the HIV-1 Proviral DNA test and both tests are available from Unipath Specialty Laboratory.

CLINICAL UTILITY:

The HIV-1 Qualitative testing may be advantageous in the following circumstances:
- Detection of acute retroviral syndrome
- Detection of early HIV-1 infection
- Resolving indeterminate western blot results
- To evaluate low-level viremia in patients who are HIV-1 antibody positive.

METHODOLOGY: Taqman Probe based Real Time PCR

Comparison of the HIV-1 Proviral DNA test and the HIV-1 Qualitative Assay

	HIV – 1 QUALITATIVE	HIV – 1 PROVIRAL DNA
Specimen	4ML EDTA BLOOD/1ML EDTA PLASMA	4ML EDTA BLOOD
Assay Target	Pol/Integrase region of HIV-1 RNA genome	Integrated HIV-1 proviral DNA
Positive Results Indicates	**HIV-1 Replication**	**HIV-1 Infection**
Evaluation of Infants born to HIV-infected women	No	**Yes**
Resolving Intermediate western Blots	**Yes**	**Yes**
Detecting early infections	**Yes**	**Yes**
Evaluating Low Level Viremia	**Yes**	**No**
Evaluating Acute Retroviral Syndrome	**Yes**	**No**

References:
1. Bøgh M, Machuca R, Gerstoft J, Pedersen C, Obel N, Kvinesdal B, Nielsen H, Nielsen C. Subtype-specific problems with qualitative Amplicor HIV-1 DNA PCR test. J Clin Virol. 2001 Feb;20(3):149-53.
2. Centers for Disease Control and Prevention. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. MMWR 2006;55(No. RR-14):1-17.

Specimen: EDTA blood/plasma (Immediately send to lab within 24 hours)

TAT: 2 DAYS

ISO 9001 International Standard Certification LIC No QAC/R91/3585 NABL M-0520

Unipath
SPECIALTY LABORATORY Ltd.

Unipath Specialty Laboratories Ltd.
102, Sanoma Plaza, B/S JMC House, Opp. Parimal Garden, Ellis Bridge, Ahmedabad - 380006
Phone No. 079-49006800/31
Mail to: unipathmdx@gmail.com