A TECHNICAL REVIEW

LabCorp

GenoSure Archive®

Cell-associated viral DNA assay for HIV-1 protease, reverse transcriptase, and integrase inhibitors

Introduction

Human immunodeficiency virus type 1 (HIV-1) was identified in 1983 as the cause of acquired immune deficiency syndrome (AIDS). During the decade between 1985 and 1995, more than 100,000 people in the United States lost their lives to HIV.[1] In March of 1987, the US Food and Drug Administration (FDA) approved the first antiretroviral (ARV) drug azidothymidine (AZT; zidovudine) for the treatment of HIV/AIDS, and more than 30 ARV drugs comprising six drug classes have been approved to date.[2]

By design, most ARV drugs target the activity of the viral enzymes required by HIV-1 to complete its lifecycle: nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) target the activity of the reverse transcriptase enzyme; integrase inhibitors (INIs) target the activity of the integrase enzyme; and protease inhibitors (PIs) target the activity of the protease enzyme. ARV drugs, however, do not target, disrupt, or delete integrated copies of viral DNA (provirus) that are archived within the host cell genome during replication. Thus HIV remains an incurable, yet manageable, disease.

The plurality of approved ARV drugs combined with therapeutic advances, such as improved efficacy, lower pill burden, less frequent dosing, and reduced side effects, have resulted in more patients with complete and sustained suppression of virus replication. This has redefined HIV/AIDS from a terminal illness to a chronic, manageable disease.[3] Advancements in ARV therapy have increased life expectancy for HIV-1-infected patients in the US and Canada from 36 years in 2000 to 51 years in 2006.[4]

The success of ARV therapy for each patient is predicated on the identification of a combination of ARV drugs that is active against HIV-1. During replication, the virus may select for mutations that confer reduced susceptibility/resistance to the current ARV regimen. The ability of the virus to develop ARV resistance was first documented and described in the early 1990s when the use of AZT as monotherapy was common.[5-7] Drug resistance has since been described for all ARV drugs approved for HIV-1 treatment.[8] Consequently, the Department of Health and Human Services recommends screening for drug resistant virus prior to treatment initiation and prior to retreatment after ARV failure.[9]

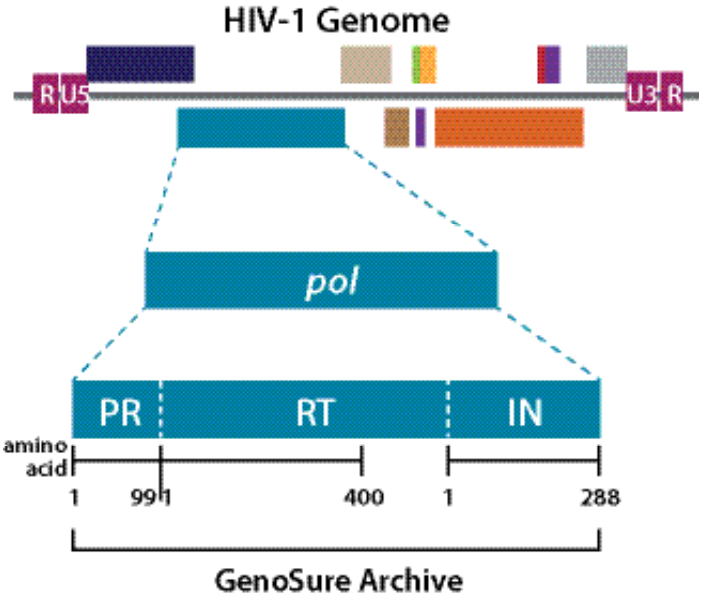
Resistance tests are routinely used to identify effective ARV drug options so that suppressive regimens can be administered to each individual patient. ARV drug regimens are typically switched or adjusted when the existing/current regimen fails to maintain complete viral suppression. Increasingly common today is the need for "fine tuning" regimens while the patient's virus remains suppressed. Reasons for these treatment adjustments most often include side effects, adverse events, regimen intolerance, and drug-drug interactions.

Standard resistance testing to guide ARV drug selection cannot be employed in the setting of viral suppression because the currently available assays sample plasma virus and require viral loads ≥500 copies/mL. Consequently, new diagnostic tools are needed to give treatment providers ARV drug susceptibility information in the setting of complete viral suppression (undetectable viral load) or low level viremia (<500 copies/mL).

These new diagnostic tools exploit the archived HIV-1 proviral DNA that is embedded in host cells during virus replication. Access to this genetic information is enabled by the lysis of HIV-1-infected cells in whole blood samples. The first of these diagnostic tools offered by Monogram Biosciences®, Trofile® DNA, was launched in 2010. Trofile® DNA evaluates the envelope (env) region of the HIV-1 genome and is used to determine tropism in the setting of complete virologic suppression to guide CCR5 antagonist administration.[10] An additional tool is now available: **GenoSure Archive®,** which interrogates the HIV-1 polymerase (pol) region to identify archived mutations. This new assay provides susceptibility assessments for PIs, NRTIs, NNRTIs, and INIs, which enable providers to obtain comprehensive ARV drug susceptibility information in the setting of virologic suppression and low-level viremia. This assay facilitates adjustments for a majority of ARV drug regimens.

Laboratory Method

GenoSure Archive is performed by amplifying cell-associated HIV-1 DNA from infected cells in whole-blood samples and then analyzing the pol region using next-generation sequencing (NGS) methods. The assay is analytically validated to identify resistance-associated mutations in the HIV-1 pol gene. **GenoSure Archive** evaluates the full-length protease and integrase coding regions and amino acids 1 to 400 of the HIV-1 reverse transcriptase region. (Figure 1).



Briefly, the **GenoSure Archive** requires one 4 mL tube of whole blood. Total DNA is extracted from whole blood, and nested PCR is performed to amplify specifically the HIV-1 region of interest. The resulting amplification products are purified and prepared for nucleic acid sequencing using next generation sequencing methods. After sequencing, the data are analyzed for the presence of resistance-associated mutations using Monogram's proprietary bioinformatics pipeline. Susceptibility calls of "resistant," "sensitive," or "resistance possible" are assigned. These assignments employ the same genotypic interpretive database used in Monogram's GenoSure PRIme® assay, relying on >100,000 paired genotypic and phenotypic susceptibility determinations.

Clinical Application

Studies evaluating the concordance between the HIV-1 plasma RNA and cellular DNA compartments have demonstrated variability. This discordance is likely due to differences that exist between the viral population currently circulating in the plasma and the proviral DNA archived in infected cells. Of note, ARV drug-resistant HIV-1 variants in the context of emerging failure are identified earlier in the plasma relative to peripheral blood mononuclear cells (PBMCs).[11] In contrast, resistant variants may persist longer in the latter compartment,[12] especially in the absence of ongoing drug pressure.

A recent study demonstrated favorable concordance, however, between Monogram's HIV-1 DNA genotyping assay and historical plasma genotypes among enrolled patients.[13] In this study, patients who were fully suppressed on their ARV drug regimens, consisting of a boosted protease inhibitor and two NRTIs, were switched to a single-tablet regimen consisting of rilpivirine, tenofovir, and emtricitabine. The authors concluded that cellular DNA genotyping may have clinical utility in detecting archived mutations in aviremic patients prior to switching ARV regimens.

GenoSure Archive is designed to provide HIV-1 ARV drug resistance data when standard resistance testing cannot be performed due to inadequate plasma viral load. The assay provides a useful tool in suppression

management, specifically to aid in regimen switches or simplification.[14] Often, ARV regimen changes are needed to address side effects, adverse events, and concerns regarding long-term toxicities. There also may be a desire to streamline an overly complex regimen, implemented during an earlier ARV treatment era, when less ideal options existed. Additionally, in the absence of historical drug resistance data for patients maintaining viral suppression, cell-associated viral DNA resistance testing may provide useful information.[15]

The descriptive information above also applies to **HIV-1 DNA Sequencing Protease – Reverse Transcriptase** and **HIV-1 DNA Sequencing Integrase.** HIV-1 DNA Sequencing Protease – Reverse Transcriptase provides susceptibility data for nucleosides, nucleotides, nonnucleosides, and protease inhibitors. HIV-1 DNA Sequencing Integrase provides susceptibility data for integrase inhibitors.

Relevant Assays*

Test Name	Test Nº
Human Immunodeficiency Virus 1 (HIV-1) GenoSure Archive®	551776
Human Immunodeficiency Virus 1 (HIV-1) DNA Sequencing Protease – Reverse Transcriptase	551730
Human Immunodeficiency Virus 1 (HIV-1) DNA Sequencing Integrase	551965
Human Immunodeficiency Virus 1 (HIV-1) GenoSure Archive® Plus Trofile® DNA	552020

*For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.LabCorp.com.

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