MAYO CLINIC LABORATORIES

Test Definition: HIVDR

HIV-1 Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma

Overview

Useful For

Identifying HIV-1 genotypic mutations associated with resistance to nucleotide and non-nucleoside reverse-transcriptase inhibitors, protease inhibitors, and integrase strain transfer inhibitors

Guiding initiation or change of combination antiretroviral therapy in individuals, including children, with HIV-1 infection

Testing Algorithm

For information see HIV Treatment Monitoring Algorithm

Special Instructions

- HIV Treatment Monitoring Algorithm

Highlights

This assay uses next-generation sequencing to identify HIV-1 antiviral drug resistance-associated codon mutations in patients prior to or while receiving combination antiretroviral therapy. This test can be used to predict the likelihood of a favorable response to current US Food and Drug Administration-approved antiviral drug combinations used for treatment of HIV-1 infection.

Method Name

Reverse Transcription Polymerase Chain Reaction (RT-PCR) followed by Targeted Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Plasma EDTA

Ordering Guidance

This test is intended for detection and identification of drug resistance-associated HIV-1 genotypic mutations in plasma specimens of individuals prior to or while receiving combination antiretroviral therapy.

Prior to requesting this test, patients must have a confirmed plasma HIV-1 RNA level (ie, viral load) of 1000 copies/mL or higher within the preceding 30 days. HIVQN / HIV-1 RNA Detection and Quantification, Plasma is available to provide this prerequisite test result. Alternately, if the patient's viral load is unknown, order HIQDR / HIV-1 RNA Quantification with Reflex to Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma, which will

perform viral load followed by genotype, if appropriate.

For initial diagnosis of HIV, order HIVDX / HIV-1 and HIV-2 Antigen and Antibody Diagnostic Evaluation, Plasma.

Shipping Instructions

If shipment will be delayed for more than 24 hours, freeze plasma specimen at -70 degrees C (up to 60 days) until shipment on dry ice.

Necessary Information

The following ask-at-order entry question must be answered at the time of test ordering (mark answer on the test request form if not ordering electronically):

HIV-1 RNA level copies/mL in last 30 days = *(select answer option)*
<1000
1000 to 1,000,000
1,000,001 to 10,000,000
>10,000,000

Note: Test requests for submitted specimens with less than 1000 copies/mL (not sufficient amount for testing), "No," or no response entered will be canceled.

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Lavender top (EDTA)
Submission Container/Tube: Plastic vial
Specimen Volume: 2.2 mL
Collection Instructions:
1. Centrifuge blood collection tube and aliquot plasma into plastic vial per collection tube manufacturer's instructions (eg, centrifuge and aliquot within 2 hours of collection for BD Vacutainer tubes).
2. Freeze aliquoted plasma for shipment.
Additional Information: Specimens submitted for HIV-1 genotyping must contain 1000 copies/mL or more of HIV-1 RNA.

Forms

If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

0.8 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK

Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Plasma EDTA	Frozen (preferred)	60 days	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Antiviral resistance may compromise the efficacy of antiretroviral therapy (ART) in patients receiving such therapy for HIV1 infection. When combination therapy fails, detection and analysis of antiviral drug resistance-associated viral genotypic mutations can guide necessary changes to ART to suppress viral replication (ie, reduce viral load), thereby improving patient outcome.

HIV-1 is an RNA virus that infects cells and is then converted to complementary DNA by the action of the viral reverse transcriptase (RT) gene product. RT has little proofreading capacity and therefore, incorporates errors in the proviral DNA. These errors are transcribed into infectious viral particles when the proviral DNA is transcribed into RNA. Similarly, the enzyme protease catalyzes a polyprotein to produce peptides necessary for active viral replication. Although ART (combination of nucleoside and nonnucleoside reverse-transcriptase inhibitors, protease inhibitors, and/or integrase strain transfer inhibitors) may be effective in reducing the viral load, genotypic mutations arising in the drug-targeted HIV-1 genome due to selective pressure from antiviral therapy will result in antiviral resistance that may compromise such therapy.

Amplification and analysis of drug-targeted HIV-gene sequence allows identification of changes in nucleotide bases and associated amino acid codons that may cause antiviral drug resistance. Such genotypic changes are deemed as variants by comparing the sequence data of the patient's HIV strain to those of a wild-type HIV strain. The significance of these genotypic mutations in relation to antiviral resistance is then determined by a set of interpretive rules developed by a consensus panel of leading experts in the field of HIV-1 resistance. Relevant data presented at a recognized scientific conference or published in peer-reviewed journals are considered by the consensus panel in developing these rules. When necessary, reliable unpublished drug resistance data known to consensus panel members may be considered in the process. The interpretive rules are updated by the consensus panel annually after reviewing newly published data on HIV-1 genotypic drug resistance mutations.

Reference Values

An interpretive report will be provided.

Interpretation

Detectable HIV-1 genotypic mutations conferring resistance to an antiviral drug are reported as amino acid codon changes (eg, M184V) resulting from the nucleic acid base alterations, according to the interpretative algorithm of the Stanford HIV Database program. The codon mutations are categorized and interpreted in relation to previously

published data of phenotypic antiviral susceptibility tests on virus that harbor such mutations. Each codon mutation is assigned a drug penalty score. The total score generated from all mutations relevant to the specific antiviral drug is used to estimate the level of resistance to that drug. These interpretive rules may be updated periodically by the Stanford HIV Database Team after reviewing newly published data on HIV-1 genotypic drug resistance-associated codon mutations.

Susceptible (SUSC) indicates that the codon mutations present in patient's HIV-1 strain have not been associated with resistance to the specific drug (Stanford HIVdb total score 0 to 9).

Potential Low-Level Resistance (PLR) indicates that codon mutations detected have been associated with possible reduction in susceptibility to the specific drug (Stanford HIVdb score 10 to 14).

Low-Level Resistance (LR) indicates that codon mutations detected have been associated with reduction in susceptibility to the specific drug (Stanford HIVdb score 15 to 29).

Intermediate Resistance (IR) indicates that codon mutations detected have been associated with reduction in susceptibility to the specific drug (Stanford HIVdb score 30 to 59).

High-level Resistant (HR) indicates that codon mutations detected have been associated with maximum reduction in susceptibility to the specific drug (Stanford HIVdb > or = 60).

Unable to genotype indicates that the sequence data obtained are of poor quality to determine the presence or absence of resistance-associated codon mutations in the patient's HIV-1 strain. Probable causes of such poor sequence data include polymorphism in the region of the sequencing primers interfering with primer binding and subsequent sequencing reaction, or low viral load (ie, <1000 copies/mL).

Inconclusive indicates inability of the assay to reliably determine antiviral resistance because of the presence of polymerase chain reaction inhibitors or ambiguous or incomplete viral target sequences generated from the assay.

Cautions

Due to the complexity of the results generated, the International AIDS Society-USA Panel recommends expert interpretation of genotyping and phenotype test results for patient care management. A patient's response to antiviral therapy depends on multiple factors, including the percentage of patient's viral populations that is drug resistant, patient compliance with the prescribed drug therapy, patient access to adequate care, drug pharmacokinetics, and drug interactions. Drug resistance test results should be interpreted only in conjunction with clinical presentation and other laboratory markers when making therapeutic decisions.

Absence of resistance to a drug does not rule out the presence of reservoirs of drug-resistant virus in the infected individual.

The HIV-1 genotypic test is not a direct measure of drug resistance. Although genotypic testing can detect variants in the relevant HIV-1 genome, the significance of these variants requires careful interpretation to predict drug susceptibility. This assay's ability to amplify the target and detect genotypic mutation is poor and unreliable when the plasma HIV-1 viral load (VL) is less than 1000 copies/mL. Specimens submitted for this test should contain greater than or equal to

1000 copies/mL of HIV-1 RNA. Per assay manufacturer claims, the assay's ability to detect minor drug-resistant HIV-1 variants among 90% or more of HIV-1 group M strains varies depending on the VL in the tested plasma specimen; 20% or higher at VL of 1000 copies/mL, 10% or higher at VL of 5000 copies/mL, and 5% or higher at VL of 15,000 copies/mL.

The list of drug resistance-associated codon mutations and interpretive rules used by the Stanford HIV database are updated periodically by the Stanford HIV Database team. Therefore, the test results do not necessarily include all resistance-associated codon mutations described in the current medical literature.

Possible causes of treatment failure other than the development of drug resistance are poor adherence to medication regimen, drug potency, and individual variation in pharmacokinetics (eg, inadequate phosphorylation of nucleosides).

Clinical Reference

1. Weber J, Volkova I, Sahoo MK, Tzou PL, Shafer RW, Pinsky BA. Prospective evaluation of the Vela Diagnostics next-generation sequencing platform for HIV-1 genotypic resistance testing. J Mol Diagn. 2019;21(6): 961-970. doi:10.1016/j.jmoldx.2019.06.003
2. Avila-Rios S, Parkin N, Swanstrom R, et al. Next-generation sequencing for HIV drug resistance testing: laboratory, clinical, and implementation considerations. Viruses. 2020;12(6):617.doi: 10.3390/v12060617
3. Raymond S, Nicot F, Abravanel F, et al: Performance evaluation of the Vela Dx Sentosa next-generation sequencing system for HIV-1 DNA genotypic resistance. J Clin Virol. 2020;122:104229. doi:10.1016/j.jcv.2019.104229
4. Panel on Antiretroviral Guidelines for Adults and Adolescents: Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. US Department of Health and Human Services. Updated September 12, 2024. Accessed March 27, 2025. Available at https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-arv/guidelines-adult-adolescent-arv.pdf

Performance

Method Description

This test utilizes the US Food and Drug Administration-approved, commercially available Sentosa SQ HIV-1 Genotyping Assay, which is a next-generation sequencing assay based on a "sequencing by synthesis" method. The assay is designed to generate 2 amplicons (approximately 1500 base pairs [bp] and approximately 1000 bp in length) spanning the PR / RT- and INT-coding regions, respectively, of the HIV-1 genome for sequencing. Codon positions 1 to 99, 1 to 387, and 1 to 288 in the PR-, RT-, and INT-coding regions, respectively, are subsequently analyzed by the assay software for clinically relevant codon substitutions.

Clinical plasma specimens are subjected to automated HIV-1 RNA extraction and purification, followed by reverse-transcription polymerase chain reaction of HIV-1 target sequences, with both a system control and a positive control included in each assay run for quality control purposes. Automated DNA library preparation is performed using the amplified products, including enzymatic shearing, adapter ligation, purification, and normalization, prior to DNA template preparation and sequencing. Sequencing reactions are conducted with the Sentosa SQ301 sequencer, and the assembled sequence data are analyzed using proprietary analysis and interpretive software applications. HIV-1 antiviral

drug-resistance interpretations are based on algorithms implemented in the most current version of the Stanford University HIV Drug Resistance Database (HIVdb; Stanford University) using a 5% variant detection cutoff threshold set by the assay manufacturer.(Instruction manual: Sentosa SQ HIV-1 Genotyping Assay User Manual. Vela Diagnostics; Version 1.3, 05/2023)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 10 days

Specimen Retention Time
60 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information
0219U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
HIVDR	HIV-1 Genotypic Drug Resistance, P	90901-0

Result ID	Test Result Name	Result LOINC® Value
616052	HIV-1 Genotypic Drug Resistance, P	80689-3

616729	HIV-1 group M subtype	100984-4
616737	Nucleos(t)ide RT mutations	45175-7
616918	Reverse Transcriptase failed codons	100983-6
616738	Abacavir	30287-7
616739	Didanosine	30284-4
616740	Emtricitabine	41402-9
616741	Lamivudine	30283-6
616742	Stavudine	30286-9
616743	Tenofovir	41396-3
616744	Zidovudine	30282-8
616745	Nonnucleoside RT mutations	45176-5
616746	Doravirine	91897-9
616747	Efavirenz	30291-9
616748	Etravirine	52749-9
616749	Nevirapine	30289-3
616750	Rilpivirine	68463-9
616751	Protease Mutations	33630-5
616919	Protease failed codons	100985-1
616752	Atazanavir + Ritonavir	49618-2
616753	Darunavir + Ritonavir	49630-7
616754	Fosamprenavir + Ritonavir	51409-1
616755	Indinavir + Ritonavir	49619-0
616756	Lopinavir + Ritonavir	42000-0
616757	Nelfinavir	30294-3
616758	Saquinavir + Ritonavir	49621-6
616759	Tipranavir + Ritonavir	49622-4
616760	Integrase mutations	61199-6
616920	Integrase failed codons	100986-9
616761	Bictegravir	90080-3
616762	Cabotegravir	96566-5
616763	Dolutegravir	72857-6
616764	Elvitegravir	72526-7
616765	Raltegravir	72525-9
HIRVL	HIV RNA level copies/mL <30 days =	89543-3
618206	HIVDR_PR-RT_SEQ:	No LOINC Needed
618207	HIVDR_INT_SEQ:	No LOINC Needed