MAYO CLINIC LABORATORIES

Test Definition: HIQDR

HIV-1 RNA Quantification with Reflex to Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma

Overview

Useful For

Quantifying plasma HIV-1 RNA levels (viral load) in individuals (including children) with known HIV-1 infection, followed by identification of 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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
HIVDR	HIV-1 Genotypic Drug Resistance, P	Yes	No

Testing Algorithm

If HIV-1 RNA level is 1000 copies/mL or greater, then HIV-1 genotypic drug resistance mutation testing will be performed at an additional charge.

The following algorithms are available:
-HIV Prenatal Testing Algorithm, Including Follow-up of Reactive Rapid Serologic Test Results
-HIV Testing Algorithm (Fourth Generation Screening Assay), Including Follow-up of Reactive Rapid Serologic Test Results
-HIV Treatment Monitoring Algorithm

Special Instructions

- HIV Testing Algorithm (Fourth-Generation Screening Assay), Including Follow-up of Reactive Rapid Serologic Test Results
- HIV Prenatal Testing Algorithm, Including Follow-up of Reactive Rapid Serologic Test Results
- HIV Treatment Monitoring Algorithm

Highlights

This test starts with a US Food and Drug Administration (FDA)-approved reverse-transcription polymerase chain reaction assay to quantify HIV-1 RNA in plasma of individuals with known HIV-1 infection. For specimens that yield HIV-1 RNA levels of 1000 copies/mL or greater, reflex testing is performed using a next-generation sequencing assay to identify HIV-1 antiviral drug resistance-associated codon mutations in patients prior to or while receiving combination antiretroviral therapy to predict the likelihood of a favorable response to current FDA-approved antiretroviral combination therapy.

Method Name

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Test Definition: HIQDR

HIV-1 RNA Quantification with Reflex to Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma

NY State Available
Yes

Specimen

Specimen Type
Plasma EDTA

Ordering Guidance
This test is intended for quantification of HIV-1 RNA level in plasma specimens of individuals with known HIV-1 infection and for identification of drug resistance-associated HIV-1 genotypic mutations prior to or while receiving combination antiretroviral therapy.

Prior to requesting this test, the patient must have a known HIV-1 infection. The following tests are available to screen and confirm the HIV-1 infection status:
-HVCOP / HIV-1 and HIV-2 Antigen and Antibody Routine Screen, Plasma
-HIVDX / HIV-1 and HIV-2 Antigen and Antibody Diagnostic Evaluation, Plasma

If only HIV-1 genotypic mutation testing is needed, order HIVDR / HIV-1 Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma.

Shipping Instructions
1. Ship specimen frozen on dry ice.
2. If shipment will be delayed for greater than 24 hours, freeze plasma specimen at -20 to -80 degrees C until shipment on dry ice.

Specimen Required
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube: Lavender top (EDTA)
Submission Container/Tube: Plastic vial
Specimen Volume: 3.6 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.

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

Specimen Minimum Volume
2 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)	55 days	
	Refrigerated	5 days	

Clinical & Interpretive

Clinical Information
Human immunodeficiency virus-1 (HIV-1) is an RNA virus that infects human host cells and is then converted to complementary DNA by the action of viral reverse transcriptase. HIV-1 is the causative agent of AIDS, a severe, life-threatening condition, and the virus has been isolated from asymptomatic, infected individuals at high-risk for AIDS. Accounting for over 99% of HIV infections in the world, HIV-1 is transmitted by sexual contact, by exposure to infected blood or blood products, from an infected pregnant woman to fetus in utero or during birth, or from an infected mother to infant via breast feeding.

Multiple clinical studies of plasma HIV-1 viral load (expressed as HIV-1 RNA copies/mL of plasma) have shown a clear relationship of HIV-1 RNA copy number to stage of HIV-1 disease and efficacy of anti-HIV-1 therapy. Quantitative HIV-1 RNA level in plasma (ie, HIV-1 viral load) is an important surrogate marker in assessing the risk of disease progression and monitoring response to anti-HIV-1 drug therapy in the routine medical care of HIV-1-infected patients.

Studies have identified a number of mutations associated with antiviral resistance. Genotypic analysis allows identification of nucleotide changes associated with HIV drug resistance. When combination therapy fails, genotyping for drug resistance mutations may help direct appropriate changes in antiretroviral therapy and may result in at least a short-term benefit, as evidenced by viral load reduction.

Reference Values
Undetected

Interpretation
HIV-1 RNA Quantification:

This assay has a HIV-1 RNA quantification result range of 20 to 10,000,000 copies/mL (1.30-7.00 log copies/mL) in plasma.

An "Undetected" result indicates that the assay was unable to detect HIV-1 RNA within the plasma specimen.

A result of "<20 copies/mL" indicates that HIV-1 RNA is detected, but the level present is less than the lower quantification limit of this assay. Due to the increased sensitivity of this assay, patients with previously low or undetectable HIV-1 viral load may show increased or detectable viral load with this assay. However, the clinical implications of a viral load below 20 copies/mL remain unclear. Possible causes of such a result include very low plasma HIV-1 viral load present (eg, in the range of 1-19 copies/mL), very early HIV-1 infection (ie, <3 weeks from time of infection), or absence of HIV-1 infection (ie, false-positive result).

A result of ">10,000,000" with the result comment of "HIV-1 RNA level is >10,000,000 copies/mL (>7.00 log copies/mL). This assay cannot accurately quantify HIV-1 RNA above this level" indicates that HIV-1 RNA is detected, but the level present is above the upper quantification limit of this assay.

For the purpose of monitoring patient's response to antiretroviral therapy, the US Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents defines virologic failure as a confirmed viral load of greater than 200 copies/mL, which eliminates most cases of viremia resulting from isolated blips or assay variability. Confirmed viral load rebound (ie, >200 copies/mL) on 2 separate tests obtained at least 2 to 4 weeks apart should prompt a careful evaluation of patient's tolerance of current drug therapy, drug-to-drug interactions, and patient adherence.

If the viral load is greater than or equal to 1000 copies/mL, genotypic antiviral drug resistance mutation analysis is performed automatically at an additional charge.

Genotypic Drug Resistance:
Codon sequences of the reverse transcriptase, protease and integrase coding region of the HIV-1 genome are compared with those in the Stanford HIV database of known antiretroviral resistance-associated mutations determined with the assay software application. Results are provided on the interpretation of those codon changes associated with resistance to individual antiretroviral drugs.

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

The HIV-1 RNA detection and quantification assay is not approved by the US Food and Drug Administration (FDA) as a screening test for HIV-1 infection in donors of blood, human cells, tissues, or tissue products.

Although this quantitative HIV-1 RNA test is not FDA approved for diagnostic purposes, the US Department of Health and Human Services Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children recommends the use of molecular-based assays to detect HIV-1 RNA or proviral DNA for the diagnosis of HIV infection in infants younger than 18 months of age and born to HIV-infected mothers.

A single HIV-1 viral load test result should not be used as the sole criterion in guiding therapeutic decisions and intervention in the clinical care of HIV-1-infected patients. Viral load results should be correlated with patient symptoms, clinical presentation, and CD4 cell count. Due to the inherent variability in the assay, physiologic variation and concurrent illnesses in the infected patients, changes of less than 100-fold (<2 log) in plasma HIV-1 viral load should not be considered significant changes.

Viral load results below 20 copies/mL do not necessarily indicate absence of HIV-1 viral replication. Inhibitory substances may be present in the plasma specimen, leading to negative or falsely low HIV-1 RNA results. Improper specimen collection or storage may lead to negative or falsely lower plasma viral load results.

Although this commercial HIV-1 viral load assay is optimized for quantification of plasma viral load in HIV-1 infection due to HIV-1 groups M (subtypes A to H) and O strains, results generated from HIV-1 group O strains may be discordant (> or =0.5 log copies/mL) with those obtained from other commercially available HIV-1 viral load assays. The assay is not reliable for quantifying plasma viral loads in infection caused by HIV-1 group N and HIV-2 strains.

ACD plasma specimens are not optimal for HIV-1 viral load testing because such plasma specimens show HIV-1 RNA levels approximately 15% lower than those collected in tubes containing EDTA. Plasma specimens stored frozen in plasma preparation tubes (PPT) are not suitable for HIV-1 viral load testing due to falsely high viral load results from release of intracellular HIV-1 nucleic acids (DNA and RNA) during the freezing process.

Clinical Reference

1. Branson BM, Owen SM, Wesolowski LG, et al. Laboratory testing for the diagnosis of HIV infection: Updated recommendations. Centers for Disease Control and Prevention; June 27, 2014. Accessed January 29, 2025. Available at http://stacks.cdc.gov/view/cdc/23447
2. 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 1, 2022. Accessed January 29, 2025. Available at https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-arv/guidelines-adult-adolescent-arv.pdf
3. Gunthard HF, Calvez V, Paredes R, at al. Human immunodeficiency virus drug resistance: 2018 recommendations of the International Antiviral Society–USA Panel. Clin Infect Dis. 2019; 68(2):177-187. doi:10.1093/cid/ciy463

Performance

Method Description

HIV-1 RNA quantification:
The cobas HIV-1 assay is a US Food and Drug Administration (FDA)-approved, in vitro nucleic acid amplification test for the quantification of HIV-1 RNA in human plasma using the cobas 5800/6800/8800 systems for fully automated viral nucleic acid extraction (generic silica-based capture technique) and automated amplification and detection of the viral nucleic acid sequence. This polymerase chain reaction (PCR) assay amplifies sequences within the gag gene and long terminal repeat (LTR) region of the HIV-1 genome and generates amplification products that are detected and quantified in real-time with 2 sequence-specific TaqMan probes. A non-HIV armored RNA quantitation standard (RNA-QS) is introduced into each specimen during sample preparation to serve as internal control for nucleic acid extraction and PCR amplification/detection processes. Fluorescent reporter dye-labeled TaqMan probes hybridized to the complementary HIV-1 target sequences and RNA-QS sequence undergo hydrolysis during PCR amplification step to generate fluorescent signal detected in 2 different dye channels. Concentration of the HIV-1 RNA in a patient's plasma sample is determined by a ratio of the intensity of the fluorescent dye from the cleaved HIV-1 target sequence probes and that from the RNA-QS target probe detected throughout the PCR process.(Package insert: HIV-1-Quantitative nucleic acid test for use on the cobas 5800/6800/8800 Systems. Roche Molecular Systems Inc; Doc rev. 4.0, 11/2022)

HIV-1 genotypic drug resistance:
This test utilizes the FDA-approved, commercially available Sentosa SQ HIV-1 Genotyping Assay, 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 (RT)-PCR 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 1.0, 10/2019)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
1 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 modified from the manufacturer's instructions. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87536
0219U (if appropriate)

LOINC® Information

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Test Definition: HIQDR

HIV-1 RNA Quantification with Reflex to Genotypic Drug Resistance to Reverse Transcriptase, Protease, and Integrase Inhibitors, Plasma

Test ID	Test Order Name	Order LOINC® Value
HIQDR	HIV-1 RNA Quant Reflex to Resist, P	70241-5

Result ID	Test Result Name	Result LOINC® Value
616917	HIV-1 RNA Detect/Quant, P	70241-5