Reimbursement Policy:

HIV Genotyping and Phenotyping - Lab Benefit Program (LBM)

POLICY NUMBER	EFFECTIVE DATE:	APPROVED BY
AHS-M2093	3/01/2023	RPC (Reimbursement Policy Committee)

Policy Description:

Human immunodeficiency virus (HIV) is an RNA retrovirus that infects human immune cells (specifically CD4 cells), causing progressive deterioration of the immune system ultimately leading to acquired immune deficiency syndrome (AIDS) characterized by susceptibility to opportunistic infections and HIV-related cancers (CDC, 2014).

Indications and/or Limitations of Coverage:

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

1) HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** in patients who have failed a course of antiviral therapy OR have suboptimal viral load reduction OR have been noncompliant with therapy.

2) HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for guiding treatment decisions in patients with acute or recent infection (within the last 6 months).

3) HIV genotyping or phenotyping in antiretroviral naive patients entering treatment **MEETS COVERAGE CRITERIA.**

4) HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** for all HIV-infected pregnant individuals in the following situations:

a) Before initiation of antiretroviral therapy

b) For those with detectable HIV RNA levels.

5) HIV genotyping or phenotyping **MEETS COVERAGE CRITERIA** and is required prior to beginning doravirine

6) HIV phenotyping **MEETS COVERAGE CRITERIA** in treatment-experienced individuals on failing regimens who are thought to have multidrug resistance

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

7) Routine use of combined genotyping and phenotyping **DOES NOT MEET COVERAGE CRITERIA.**

8) Drug susceptibility phenotype prediction using genotypic comparison to known genotypic/phenotypic database **DOES NOT MEET COVERAGE CRITERIA**.

Definitions:

Term	Definition
AI/A1	Strong panel support – Evidence from ≥1 RCTs published in the peer-reviewed literature or presented in abstract form at peer-reviewed scientific meetings
AIDS	Acquired immune deficiency syndrome
AII/A2	Strong panel support - Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes
AIIa	Strong panel support – Evidence from cohort or case-control studies published in the peer-reviewed literature
AIII	Strong panel support – Based on the panel's analysis of the available evidence
ART	Antiretroviral therapy
ARV	Antiretroviral
ASHM	The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine
ATCC	American Type Culture Collection
BII/B2	Moderate panel support - Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes
BIIa	Moderate panel support – Evidence from cohort or case-control studies published in the peer-reviewed literature
BIII	Moderate panel support – Based on the panel's analysis of the available evidence
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4

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Term	Definition
CDC	Centers for Disease Control and Prevention
CIII	Limited or weak panel support – Based on the panel's analysis of the available evidence
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DTG	Dolutegravir
EACS	European Acquired Immune Deficiency Syndrome Clinical Society
FDA	Food and Drug Administration
GIS	Genotypic interpretation systems
GT	Genotype
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus - Type 1
HIV-2	Human immunodeficiency virus - Type 2
HIVDR	HIV drug resistance
HIV-VL	HIV viral load
INSTI	Integrase strand transfer inhibitor
K103N	Lysine to aspartate polymorphism
LADRVs	Low abundant drug resistant variants
LDTs	Laboratory-developed tests
NGS	Next generation sequencing
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NYSDOH	New York State Department of Health
PEP	Postexposure prophylaxis
PIs	Protease inhibitors
PR	Protease
RAL	Raltegravir
RCT	Randomized controlled trial
RNA	Ribonucleic Acid
RT	Reverse transcriptase

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Term	Definition
rt-PCR	Real time- polymerase chain reaction
RVAs	Recombinant virus assays
SS	Sanger sequencing
TDR	Total drug resistance

Scientific Background:

Human immunodeficiency virus (HIV) targets the immune system, eventually hindering the body's ability to fight infections and diseases. If not treated, an HIV infection may lead to acquired immunodeficiency syndrome (AIDS) which is a condition caused by the virus. There are two main types of HIV: HIV-1 and HIV-2; both are genetically different. HIV-1 is more common and widespread than HIV-2.

HIV replicates rapidly; a replication cycle rate of approximately one to two days ensures that after a single year, the virus in an infected individual may be 200 to 300 generations removed from the initial infection-causing virus (Coffin & Swanstrom, 2013). This leads to great genetic diversity of each HIV infection in a single individual. As an RNA retrovirus, HIV requires the use of a reverse transcriptase for replication purposes. A reverse transcriptase is an enzyme which generates complimentary DNA from an RNA template. This enzyme is error-prone with the overall single-step point mutation rate reaching $\sim 3.4 \times 10^{-5}$ mutations per base per replication cycle (Mansky & Temin, 1995), leading to approximately one genome in three containing a mutation after each round of replication (some of which confer drug resistance). This rate is comparable to other RNA viruses. This pace of replication, duration of infection, and size of the replicating population allows the retrovirus to evolve rapidly in response to selective influences (Coffin & Swanstrom, 2013).

Due to the high rate of mutation in HIV viruses, drug resistance mutations are common. Some drugs may be resisted by a single mutation—these drugs have a "low genetic barrier" to resistance. Such mutations are common enough to be termed "signature mutations," which are frequently associated with a specific drug resistance. For example, the K103N mutation commonly leads to resistance for efavirenz. Efavirenz is a standard retroviral medication used to treat and prevent HIV and AIDs. To combat this, medical professionals can now assess drug-resistant HIV variants using phenotypic testing and genotypic testing (Kozal, 2019a).

Genotypic assays detect the presence of specific drug-resistance mutations in several different genes (protease, reverse transcriptase, and integrase genes). For example, assays may test for resistance in nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs). The definition of a resistance conferring mutation is blurred, but generally includes one or more of the following conditions:

- The mutation confers phenotypic resistance when introduced into a drug-sensitive laboratory strain of HIV.
- The mutation is selected for during serial in vitro passage of the virus in the presence of a drug.
- The mutation is selected for during clinical therapy with that drug.
- The presence of the mutation in clinical isolates is associated with phenotypic resistance and virologic failure (Kozal, 2019b).

Interpretation of genotypic data may be done either by clinical expertise or through a database (in which the genotype is correlated with the phenotype) (Kozal, 2019b).

Several HIV genotypic assays are available. The ViroSeq HIV-1 Genotyping system by Abbott helps to detect HIV-1 genomic mutations that may lead to resistance to certain types of antiretroviral drugs (Abbott, 2018). Further, the ATCC® HIV-1 Drug Resistance Genotyping Kit has been developed by the American Type Culture Collection (ATCC), the Centers for Disease Control and Prevention (CDC) and Thermo Fischer Scientific; this is a real time- polymerase chain reaction (rt-PCR) assay which may help to identify and monitor HIV-1 drug resistance (ATCC, 2014).

Phenotypic resistance assays measure the extent to which an antiretroviral drug inhibits viral replication. Phenotypic testing typically assesses the fold-change in susceptibility of a patient's virus and the treatment response, while also correlating the mutations present with the fold-change in susceptibility. Recombinant virus assays (RVAs) are used; protease, reverse transcriptase, or integrase gene sequences from circulating viruses are inserted into a reference strain of HIV, and this new HIV strain is measured by the phenotypic assay. The primary phenotypic assay is "PhenoSense" from LabCorp although "Antivirogram" was used in the past (Kozal, 2019b). The Human Immunodeficiency Virus 1 (HIV-1) PhenoSense GT® Plus Integrase (Monogram® Phenotype + Genotype) test by LabCorp measures HIV genotypic and phenotypic resistance from plasma samples (LabCorp, 2020).

Advantages of the genotype assays include lower cost and shorter turnaround time. However, interpretation of these assays is complicated by combinations of individual mutations that may have a differential effect on resistance that differs from the individual mutation alone (Kozal, 2019b). Mutation combinations are known to cause resistance to certain drugs, but increase susceptibility to others, impact viral fitness, and contribute to major pathways of resistance; additionally, the interactions of mutations affecting various mechanisms can be difficult to predict. Over 20 rules-based genotypic interpretation systems (GIS) have been proposed (Fox et al., 2007; Kozal, 2019b).

Advantages of phenotypic assays include an ability to measure resistance more directly and examine the relative effect of multiple mutations on drug resistance. Limitations of the phenotypic assays include a longer turnaround time, greater expense, and biologic cut-offs above achievable drug levels. Phenotypic resistance assays may be helpful when evaluating HIV strains with known or suspected complex drug resistance mutation patterns as their actual resistance may not be accurately predicted by simply detecting the presence of multiple mutations (Kozal, 2019b). Both assays are limited by decreased sensitivity for low-level minority variants that comprise less than 5 to 20 percent of the virus population (Kozal, 2019b).

Analytical Validity

Rosemary et al. (2018) performed a comparison of two genotyping assays, ViroSeq and ATCC kit. A total of 183 samples with a viral load ≥1000 copies/mL were sequenced by ViroSeq and randomly selected (85 successfully genotyped, 98 unsuccessfully genotyped). The ATCC kit also genotyped 115 of the 183 samples, and out of the 98 unsuccessfully genotyped samples, the ATCC kit was able to genotype 42. Overall, 127 of the 183 samples were genotyped. The authors noted that the sequences of the genotyped samples were 98% identical and had "similar HIVDR profiles at individual patient level" (Rosemary et al., 2018).

Clinical Utility and Validity

Zhang et al. (2005) compared two phenotyping assays, Antivirogram and PhenoSense. Reverse transcriptase inhibitor susceptibility results were evaluated for 202 isolates from Antivirogram and 126 from PhenoSense. The authors found the median deviance for wild-type and mutant isolates to be lower for PhenoSense compared to Antivirogram, and PhenoSense was more likely to detect resistance to abacavir, didanosine, and stavudine when common drug resistance mutations were present (Zhang et al., 2005).

Shen et al. (2016) assessed the ability to predict phenotypic drug resistance from genotypic data. The authors used two machine learning algorithms to predict drug resistance to HIV protease inhibitors and reverse transcriptase inhibitors as well as the severity of that resistance from a query sequence. The accuracy of these classifications was found to be >0.973 for eight PR inhibitors and 0.986 for ten RT inhibitors and the r^2 was 0.772–0.953 for the PR cohort and 0.773–0.995 for the RT cohort. The algorithms' results were verified by "five-fold cross validation" on the genotype-phenotype datasets (Shen et al., 2016).

Taylor et al. (2019) have developed a MiSeq-HyDRA platform for enhanced HIV drug resistance genotyping and surveillance; this platform uses next generation sequencing (NGS) as opposed to Sanger sequencing (SS) methods which are limited due to low data throughput and limited detection of low abundant drug resistant variants (LADRVs). NGS and SS are both DNA sequencing techniques. The authors tested this novel platform with HIV-1 samples amplified at viral loads of ≥1,000 copies/ml. "The gross error rate of this platform was determined at 0.21%, and minor variations were reliably detected down to 0.50% in plasmid mixtures" (Taylor et al., 2019). The authors conclude by stating that this genotypic platform using NGS has many advantages including an increased sensitivity for LADRV detection, reduced costs and labor, and the potential to routinely monitor for HIV drug resistance.

Raymond et al. (2020) evaluated the performance of the Vela Dx Sentosa next-generation sequencing (NGS) system for HIV-1 DNA genotypic resistance. 40 DNA samples were analyzed with Vela Dx Sentosa assay and the results were compared with Sanger sequencing. The Vela Dx Sentosa assay was 100% successful in amplifying and sequencing the protease and reverse transcriptase, and 86% successful in amplifying integrase sequences when the HIV DNA load was greater than 2.5 log copies/million cells. The Sentosa and Sanger sequencing were concordant for predicting protease-reverse transcriptase resistance in 20% of the 14/18 samples which were successfully sequenced. Sentosa was able to predict a higher level of resistance in three of the samples. The Vela Dx Sentosa predicted the prevalence of drug resistance to protease inhibitors (7%), nucleoside reverse transcriptase inhibitor (59%), nonnucleoside reverse transcriptase inhibitor (31%), and integrase inhibitors (20%). Overall, the authors conclude that the Vela Dx Sentosa assay can accurately predict HIV DNA drug resistance (Raymond et al., 2020).

Fogel et al. (2020) also analyzed the ability of next-generation sequencing methods to analyze HIV drug resistance. In this case, 145 plasma samples were analyzed using the ViroSeq HIV-1 Genotyping System and the veSEQ-HIV assay. Results were compared with the Abbott RealTime Viral Load assay. 142 HIV protease and reverse transcriptase sequences and 138 integrase sequences were obtained with ViroSeq. On the other hand, veSEQ-HIV detected 70.4% of the samples with protease, reverse transcriptase, and integrase sequences. Drug resistance mutations were detected in 33 ViroSeq samples and 42 veSEQ-HIV samples. Overall, veSEQ-HIV predicted more drug resistance mutations and worked better for larger viral loads. Results from veSEQ-HIV strongly correlated with the results from Abbott RealTime Viral Load assay. The authors conclude that the veSEQ-HIV assay provided results for most samples with higher viral loads, was accurate for predicting drug resistance mutations, but detected mutations at lower levels compared with the ViroSeq assay (Fogel et al., 2020).

Pröll et al. (2022) investigated whether NGS from proviral DNA and RNA could be an alternative to using plasma viral RNA as the material of choice for genotypic resistance testing at the start of ART and virologic failure for patients with low viremia. When taking samples from 36 patients, with varying viral loads of 96 to 390,000 copies/mL, the researchers found 2476 variants/drug resistance mutations by SS, while 2892 variants were found by NGS. Researchers stated, "An average of 822/1008 variants were identified in plasma viral RNA by Sanger or NGS sequencing, 834/956 in cellular viral RNA, and 820/928 in cellular viral DNA." This demonstrates that cellular RNA and cellular viral DNA could serve as viable substitutes when testing for variant detection and genotypic resistance among patients with HIV and low viremia (Pröll et al., 2022).

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Guidelines and Recommendations:

Department of Health and Human Services (DHHS)

The Department of Health and Human Services (DHHS, 2022a, 2022b, 2022c) updated their guidelines for using drug resistance assays in HIV infections. The guidelines recommend HIV genotyping or phenotyping in the following situations among pregnant individuals and reducing perinatal HIV transmission in the US:

- "General Principles Regarding Use of Antiretroviral Drugs During Pregnancy
  - Antiretroviral (ARV) drug-resistance genotype evaluations or assays should be performed before starting ARV drug regimens in people who are ARV-naive (AII) or ARV-experienced (AIII) and before modifying ARV drug regimens (AII) in people whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL).
  - In pregnant people who are not already receiving ART, ART should be initiated before results of drug resistance testing are available because earlier viral suppression has been associated with lower risk of transmission. When ART is initiated before results are available, the regimen should be modified, if necessary, based on resistance assay results (AII)."
- "Pregnant People with HIV Who Have Never Received Antiretroviral Drugs (Antiretroviral Naive)
  - The results of ARV drug-resistance studies should guide the selection of ARV regimens in people whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL) (see Antiretroviral Drug Resistance and Resistance Testing in Pregnancy) (AII). However, ART initiation should not be delayed while awaiting results of resistance testing. When ART is initiated before the results of the drug resistance assays are available, the ARV regimen should be modified, if necessary, based on the resistance assay results (AII)."
- "Pregnant People with HIV Who Are Currently Receiving Antiretroviral Therapy
  - ARV drug-resistance testing should be performed to assist the selection of active drugs when changing ARV regimens in pregnant people who are experiencing virologic failure on ART and who have HIV RNA levels >500 copies/mL to 1,000 copies/mL (AII). In individuals who have HIV RNA levels >500 copies/mL but <1,000 copies/mL, testing may be unsuccessful but still should be considered (BII)."
- "Pregnant People with HIV Who Have Previously Received Antiretroviral Medications but Are Not Currently Receiving Any Antiretroviral Medications
  - If HIV RNA is above the threshold for standard genotypic drug resistance testing (i.e., >500 to 1,000 copies/mL), ARV drug-resistance testing should be performed prior to starting an ARV drug regiment (AIII)
  - ART should be initiated prior to receiving results of current ARV resistance assays. ART should be modified based on the results of the resistance assay, if necessary (AII)."
- "Monitoring during Pregnancy
  - HIV drug-resistance testing (genotypic testing and, if indicated, phenotypic testing) should be performed during pregnancy in those whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 copies/mL to 1,000 copies/mL) before –
    - Initiating ART in antiretroviral (ARV)-naive pregnant people who have not been previously tested for ARV drug resistance (AII);
    - Initiating ART in ARV-experienced pregnant people (including those who have received preexposure prophylaxis) (AIII); or
    - Modifying ARV regimens for people with HIV who become pregnant while receiving ARV drugs or people who have suboptimal virologic response to ARV drugs that were started during pregnancy (AII).
  - ART should be initiated in pregnant patients prior to receiving the results of ARV-resistance tests. ART should be modified, if necessary, based on the results of resistance testing (AII)."

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- "Antiretroviral Drug Resistance and Resistance Testing in Pregnancy
  - HIV drug-resistance testing (genotypic and, if indicated, phenotypic) should be performed in persons living with HIV whose HIV RNA levels are above the threshold for resistance testing (i.e., >500 to 1,000 copies/mL) before
    - Initiating ART in ARV-naive pregnant women who have not been previously tested for ARV resistance (AII),
    - initiating ART in ARV-experienced pregnant women (including those who have received pre-exposure prophylaxis) (AIII), *or*
    - modifying ART regimens for those who are newly pregnant and receiving ARV drugs or who have suboptimal virologic response to the ARV drugs during pregnancy (AII).
  - Phenotypic resistance testing is indicated for treatment-experienced persons on failing regimens who are thought to have multidrug resistance (BIII).
  - ART should be initiated in pregnant persons before receiving results of ARV-resistance testing; ART should be modified, if necessary, based on the results of resistance assays (AII).
  - If the use of an integrase strand transfer inhibitor (INSTI) is being considered and INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay (AIII). INSTI resistance may be a concern if-
    - a patient received prior treatment that included an INSTI, *or*
    - a patient has a history with a sexual partner on INSTI therapy who was not virologically suppressed or with unknown viral load
    - documented zidovudine (ZDV) resistance does not affect the indications for use of intrapartum intravenous ZDV (BIII)." (DHHS, 2022c).

Among adults and adolescents living with HIV, the DHHS recommends the following for drug resistance testing:

- "For antiretroviral therapy-naïve persons:
  - HIV drug-resistance testing is recommended at entry into care for persons with HIV to guide selection of the initial antiretroviral therapy (ART) regimen (AII). If therapy is deferred, repeat testing may be considered at the time of ART initiation (CIII)
  - Genotypic, rather than phenotypic, testing is the preferred resistance testing to guide therapy in antiretroviral (ARV)-naive patients (AIII)
  - In persons with acute or recent (early) HIV infection, in pregnant people with HIV, or in people who will initiate ART on the day of or soon after HIV diagnosis, ART initiation should not be delayed while awaiting resistance testing results; the regimen can be modified once results are reported (AIII)
  - Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the reverse transcriptase (RT) and protease (PR) genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is a concern, providers should ensure that genotypic resistance testing also includes the integrase gene (AIII).
- For Antiretroviral Therapy-Experienced Persons:
  - HIV drug-resistance testing should be performed to assist the selection of active drugs when changing ART regimens in the following patients:
    - Persons with virologic failure and HIV RNA levels >1,000 copies/mL (AI)
    - Persons with HIV RNA levels >500 copies/mL but <1,000 copies/mL, drug-resistance testing may be unsuccessful but should still be considered (BII)
    - Persons with suboptimal viral load reduction (AII)
  - When a person with HIV experiences virologic failure while receiving an INSTI-based regimen, genotypic testing for INSTI resistance (which may need to be ordered separately) should be performed to determine whether to include a drug from this class in subsequent regimens (AII).
  - Drug-resistance testing in the setting of virologic failure should be performed while the person is taking prescribed ARV drugs or, if that is not possible, within 4 weeks after discontinuing therapy (AII). If more than 4 weeks have elapsed since the ARVs were discontinued, resistance testing may

still provide useful information to guide therapy; however, it is important to recognize that previously selected resistance mutations can be missed due to lack of drug-selective pressure (CIII).
    - Genotypic testing is preferred over phenotypic resistance testing to guide therapy in persons with suboptimal virologic response or virologic failure while on first- or second-line regimens and in individuals in whom resistance mutation patterns are known or not expected to be complex (AII).
    - The addition of phenotypic to genotypic resistance testing is recommended for persons with known or suspected complex drug resistance mutation patterns (BIII).
    - All prior and current drug-resistance test results, if available, should be considered when constructing a new regimen for a patient (AIII)."

In terms of the usage of drug-resistance assays among adolescents and adults with HIV, the DHHS recommends the following:

- "In acute or recent (early) HIV infection: Drug-resistance testing is recommended (AII). A genotypic assay is generally preferred (AIII). Treatment should not be delayed while awaiting results of resistance testing (AIII).
    - If ART is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In ART-naive patients with chronic HIV: Drug-resistance testing is recommended at entry into HIV care to guide selection of initial ART (AII). A genotypic assay is generally preferred."
    - For pregnant persons, or if ART will be initiated on the day of or soon after HIV diagnosis, treatment can be initiated prior to receiving resistance testing results.
    - If an INSTI is considered for an ART-naïve patient and/or transmitted INSTI resistance is a concern, providers should supplement standard resistance testing with a specific INSTI genotypic resistance assay, which may need to be ordered separately (AIII).
    - If therapy is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AIII)."
- "In patients with virologic failure: Drug-resistance testing is recommended in patients on combination ART with HIV RNA levels >1,000 copies/mL (AI). In patients with HIV RNA levels >500 copies/mL but <1000 copies/mL, testing may not be successful but should still be considered (BII).
    - Resistance testing should be done while the patient is taking ART or, if that is not possible, within 4 weeks after ART discontinuation (AII). If >4 weeks have elapsed, resistance testing may still be useful to guide therapy; however, previously-selected mutations can be missed due to lack of drug selective pressure (CIII).
    - A standard genotypic resistance assay is generally preferred for patients experiencing virologic failure on their first or second regimens and for those with noncomplex resistance patterns (AII).
    - All prior and current drug-resistance testing results should be reviewed and considered when designing a new regimen for a patient experiencing virologic failure (AIII).
    - When virologic failure occurs while a patient is on an INSTI based regimen, genotypic testing for INSTI resistance should be performed to determine whether to include drugs from this class in subsequent regimens (AII).
    - Adding phenotypic testing to genotypic testing is generally preferred in patients with known or suspected complex drug-resistance patterns. (BIII)"
- "In patients with suboptimal suppression of viral load: Drug resistance testing is recommended in patients with suboptimal viral load suppression after initiation of ART. (AII)"
- "In HIV-infected pregnant women: Genotypic resistance testing is recommended for all pregnant women before initiation of ART (AIII) and for those entering pregnancy with detectable HIV RNA levels while on therapy. (AI)"
- "In patients with undetectable viral load or low-level viremia: HIV-1 proviral DNA resistance assays may be useful in patients with HIV RNA below the limit of detection or with low-level viremia, where a HIV RNA genotypic assay is unlikely to be successful (CIII)" (DHHS, 2022a).

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The DHHS also added guidelines on genotypic and phenotypic testing for pediatric HIV infection:

- "Antiretroviral (ARV) drug-resistance testing is recommended at the time of HIV diagnosis, before initiation of therapy, in all ART naive patients, and before switching regimens in patients with treatment failure (AII). Genotypic resistance testing is preferred for this purpose (AIII)."
- "Phenotypic resistance testing should be considered (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after a patient has experienced virologic failure on multiple ARV regimens (CIII)" (DHHS, 2022b).

The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine (ASHM) Sub-Committee for Guidance on HIV Management in Australia

The Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine (ASHM) Sub-Committee for Guidance on HIV Management in Australia has released commentary to the US DHHS Guidelines for the use of Antiretroviral Agents in HIV-1 Infected Adults and Adolescents. The Panel's recommendations are below:

"For Antiretroviral Therapy-Naive Persons:

- HIV drug-resistance testing is recommended at entry into care for persons with HIV to guide selection of the initial antiretroviral therapy (ART) regimen (AII). If therapy is deferred, repeat testing may be considered at the time of ART initiation (CIII).
- Genotypic, rather than phenotypic, testing is the preferred resistance testing to guide therapy in antiretroviral (ARV)-naive patients (AIII).
- In persons with acute or recent (early) HIV infection, in pregnant people with HIV, or in people who will initiate ART on the day of or soon after HIV diagnosis, ART initiation should not be delayed while awaiting resistance testing results; the regimen can be modified once results are reported (AIII).
- Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the reverse transcriptase (RT) and protease (PR) genes. If transmitted integrase strand transfer inhibitor (INSTI) resistance is a concern, providers should ensure that genotypic resistance testing also includes the integrase gene (AIII).

For Antiretroviral Therapy-Experienced Persons:

- HIV drug-resistance testing should be performed to assist the selection of active drugs when changing ART regimens in the following patients:
  - Persons with virologic failure and HIV RNA levels >1,000 copies/mL (AI)
  - Persons with HIV RNA levels >500 copies/mL but <1,000 copies/mL, drug-resistance testing may be unsuccessful but should still be considered (BII)
  - Persons with suboptimal viral load reduction (AII)
- When a person with HIV experiences virologic failure while receiving an INSTI-based regimen, genotypic testing for INSTI resistance (which may need to be ordered separately) should be performed to determine whether to include a drug from this class in subsequent regimens (AII).
- Drug-resistance testing in the setting of virologic failure should be performed while the person is taking prescribed ARV drugs or, if that is not possible, within 4 weeks after discontinuing therapy (AII). If more than 4 weeks have elapsed since the ARVs were discontinued, resistance testing may still provide useful information to guide therapy; however, it is important to recognize that previously selected resistance mutations can be missed due to lack of drug-selective pressure (CIII).
- Genotypic testing is preferred over phenotypic resistance testing to guide therapy in persons with suboptimal virologic response or virologic failure while on first- or second-line regimens and in individuals in whom resistance mutation patterns are known or not expected to be complex (AII).
- The addition of phenotypic to genotypic resistance testing is recommended for persons with known or suspected complex drug-resistance mutation patterns (BIII).

- All prior and current drug-resistance test results, if available, should be considered when constructing a new regimen for a patient (AIII).

  Rating of Recommendations: A = Strong; B = Moderate; C = Optional
  Rating of Evidence: I = Data from randomized controlled trials; II = Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = Expert opinion" (ASHM, 2018).

European AIDS Clinical Society (EACS)

The EACS recommends a genotypic resistance test to be ideally done at the time of HIV diagnosis; testing "should not delay ART initiation (it may be re-adjusted after genotypic test results). Resistance testing is also recommended to be performed in the setting of virological failure, "preferably on failing therapy (usually routinely available for HIV-VL levels >200-500 copies/mL and in specialized laboratories for lower levels of viremia) and obtain historical resistance testing for archived mutations." For pregnant women, the EACS recommends performing resistance testing on women whose HIV-VL is not undetectable at third trimester, and "consider changing to or adding INSTI (RAL or DTG) if not on this class to obtain rapid HIV-VL decline." When considering PEP, the EACS recommends resistance testing if the HIV-VL is detectable in an HIV-positive source person on ART. They also recommend baseline resistance testing when considering a combination regiment for ART-naïve children and adolescents living with HIV. Resistance testing should also be used to help guide the choice of treatment.

Additional genotypic recommendations include if the patient was not previously tested or if the patient is at risk of a super-infection. Genotypic resistance testing is also required prior to beginning treatment with doravirine. When switching strategies for "virologically suppressed persons," Proviral DNA genotyping may be useful in persons with multiple virological failures, unavailable resistance history or low-level viremia at the time of switch. Results ought to be taken cautiously as proviral DNA genotype may not detect previous resistance mutations and can also detect clinically irrelevant mutations. Therefore, routine proviral DNA genotyping is currently not recommended." The EACS recommends a genotypic test over a phenotypic test as genotype tests are more available and more sensitive (EACS, 2021).

International Antiviral Society – USA Panel

The International Antiviral (formerly AIDS) Society-USA expert panel has provided the following recommendations:
"Recommendations for Resistance Testing in Clinical Practice: Who and When to Test"

- HIV resistance testing is recommended for all individuals with HIV infection
  - Who are newly diagnosed and presumably ART-naïve;
    - As soon as an individual is diagnosed with HIV-1 infection
    - In any case, before ART is started (AIIa)
  - Who are on antiretroviral treatment and have plasma HIV RNA that is rising to above 200 copies/mL by confirmed measurements after they have been suppressed to below 50 copies/mL;
    - Preferably while on failing ART (AIIa)
  - Who have not achieved full virus suppression after initiating ART
    - ≥6 months after ART initiation (AIIa)
  - Who have interrupted ART containing an NNRTI with a long half-life (eg, efavirenz); or
    - As soon as virus rebounds above 500 HIV-RNA copies/mL, respectively, before re-initiation of ART (AIIa)
  - Who have a significant increase in viral load in a drug-naïve individual not on treatment
    - After confirmation of increase in plasma viremia

- Increase of plasma viremia of >0.5$\log_{10}$ within approximately 3-6 months that is confirmed by a second HIV-1 RNA measurement (AIII)."

"Recommendations for Methods for HIV-1 Resistance Testing

- As a first choice, genotypic resistance testing is recommended (evidence rating AIIa).
- Phenotypic resistance testing is recommended, in certain situations:
  - 1. to evaluate HIV susceptibility to new and investigational drugs when drug-resistant mutation patterns have not been fully established (evidence rating AIIa);
  - 2. when genotypic test results are too complex to interpret (evidence rating CIII); or
  - 3. when ART options are highly limited and, as a result, salvage ART must rely on residual susceptibilities to different drugs that are difficult to predict from genotypic data (evidence rating CIII).
- The recommended compartment for drug resistance testing is plasma (evidence rating AII).
- Inclusion of the protease and first half of the reverse transcriptase (up to at least nucleotide 215) is recommended for all genotypic testing (evidence rating BIII).
- Routine InSTI resistance testing in drug-naive individuals is currently not recommended (BIII).
- Baseline InSTI resistance testing is recommended in select patients with evidence of TDR, such as those with nRTI- or multi-class resistance (evidence rating AIII).
- Monitoring of TDR/pretreatment drug resistance to InSTI in selected sites in resource-rich settings and low- and middle-income countries is recommended (evidence rating AIII).
- Sequencing of other regions (C-terminus of reverse transcriptase, gag) or even a near full-length of HIV-1 is not recommended for routine clinical management (evidence rating AIIa).
- Genotypic tropism testing is recommended if a CCR5 antagonist is considered for treatment (evidence rating BIIa).
- Peripheral blood mononuclear cell genotypic resistance testing is recommended in patients with low-level viremia or in patients who are virologically suppressed (evidence rating AIII) (Gunthard et al., 2019)"

New York State Department of Health AIDS Institute

Determining HIV Drug Resistance

- "When determining the optimal regimen for achieving viral suppression, clinicians should perform genotypic resistance testing that includes the protease (A2), reverse transcriptase (A2), and integrase genes (B2) at baseline, whether or not ART is being initiated.
  - In patients experiencing treatment failure [a] or incomplete viral suppression; such testing should be performed while patients are still on therapy, but no later than 4 weeks after stopping ART, given the rapid return of wildtype virus. (A2)
  - Perform co-receptor tropism testing prior to initiation of a CCR5 antagonist. (A1)
  - If fusion inhibitor resistance is suspected, that test should be obtained as a supplement to the other genotypic resistance tests. (A2)" (NYSDOH, 2020).

European HIV Drug Resistance Guidelines Panel

Guidelines from the European HIV Drug Resistance Guidelines Panel include the following:
"Postexposure prophylaxis

- Use genotypic information from the index case to guide PEP. If this genotype is not known, do not delay PEP, but if a sample from the index case is available, genotype index case to change or simplify PEP if needed.

Reimbursement Policy:

HIV Genotyping and Phenotyping - Lab Benefit Program (LBM)

Which assay to use

- The panel recommends the use of genotyping in most routine clinical situations. Current genotyping can be performed below a viral load of 1,000 copies/ml.

  Consider additional phenotyping for new drugs, in heavily pretreated patients and for HIV-2 where genotyping is not easily interpretable" (Vandamme et al., 2011).

Applicable State and Federal Regulations:

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes:

CPT	Code Description
87900	Infectious agent drug susceptibility phenotype prediction using regularly updated genotypic bioinformatics
87901	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, reverse transcriptase and protease regions
87903	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; first through 10 drugs tested
87904	Infectious agent phenotype analysis by nucleic acid (DNA or RNA) with drug resistance tissue culture analysis, HIV 1; each additional drug tested (List separately in addition to code for primary procedure)
87906	Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, other region (eg, integrase, fusion)
0219U	Infectious agent (human immunodeficiency virus), targeted viral next-generation sequence analysis (ie, protease [PR], reverse transcriptase [RT], integrase [INT]), algorithm reported as prediction of antiviral drug susceptibility Proprietary test: Sentosa® SQ HIV-1 Genotyping Assay Lab/Manufacturer: Vela Diagnostics USA, Inc

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Reimbursement Policy:

HIV Genotyping and Phenotyping - Lab Benefit Program (LBM)

Evidence-based Scientific References:

Abbott. (2018). *VIROSEQ HIV-1 GENOTYPING SYSTEM*. https://www.molecular.abbott/us/en/products/infectious-disease/viroseq-hiv-1-genotyping-system

ASHM. (2018). *Antiretroviral Guidelines*. https://arv.ashm.org.au/drug-resistance-testing/

ATCC. (2014). *ATCC Teams with CDC and Thermo Fisher Scientific on Public Health RT-PCR Assay*. https://www.labbulletin.com/articles/ATCC-Teams-CDC-Thermo-Fisher-Scientific-Public-Health-RT-PCR-Assay/categories/20130120_11

CDC. (2014). Revised surveillance case definition for HIV infection--United States, 2014. *MMWR Recomm Rep*, *63*(Rr-03), 1-10. https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm

Coffin, J., & Swanstrom, R. (2013). HIV Pathogenesis: Dynamics and Genetics of Viral Populations and Infected Cells. In *Cold Spring Harb Perspect Med* (Vol. 3). https://doi.org/10.1101/cshperspect.a012526

DHHS. (2022a, January 20, 2022). *Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV*. Department of Health and Human Services. Retrieved March 2 from https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv/whats-new-guidelines

DHHS. (2022b, April 11, 2022). *Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection*. https://clinicalinfo.hiv.gov/en/guidelines/pediatric-arv/whats-new-guidelines

DHHS. (2022c, March 17, 2022). *Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States*. https://clinicalinfo.hiv.gov/en/guidelines/perinatal/whats-new-guidelines

EACS. (2021). European AIDS Clinical Society Guidelines Version 11.0 October 2021. https://www.eacsociety.org/media/final2021eacsguidelinesv11.0_oct2021.pdf

Fogel, J. M., Bonsall, D., Cummings, V., Bowden, R., Golubchik, T., de Cesare, M., Wilson, E. A., Gamble, T., del Rio, C., Batey, D. S., Mayer, K. H., Farley, J. E., Hughes, J. P., Remien, R. H., Beyrer, C., Fraser, C., & Eshleman, S. H. (2020). Performance of a high-throughput next-generation sequencing method for analysis of HIV drug resistance and viral load. *Journal of Antimicrobial Chemotherapy*, *75*(12), 3510-3516. https://doi.org/10.1093/jac/dkaa352

Fox, Z. V., Geretti, A. M., Kjaer, J., Dragsted, U. B., Phillips, A. N., Gerstoft, J., Staszewski, S., Clotet, B., von Wyl, V., & Lundgren, J. D. (2007). The ability of four genotypic interpretation systems to predict virological response to ritonavir-boosted protease inhibitors. *Aids*, *21*(15), 2033-2042. https://doi.org/10.1097/QAD.0b013e32825a69e4

Gunthard, H. F., Calvez, V., Paredes, R., Pillay, D., Shafer, R. W., Wensing, A. M., Jacobsen, D. M., & Richman, D. D. (2019). Human Immunodeficiency Virus Drug Resistance: 2018 Recommendations of the International Antiviral Society-USA Panel. *Clin Infect Dis*, *68*(2), 177-187. https://doi.org/10.1093/cid/ciy463

Kozal, M. (2019a, September 17th, 2019). *Interpretation of HIV drug resistance testing*. https://www.uptodate.com/contents/interpretation-of-hiv-drug-resistance-testing

Kozal, M. (2019b, September 17th, 2019). *Overview of HIV drug resistance testing assays*. https://www.uptodate.com/contents/overview-of-hiv-drug-resistance-testing-assays

LabCorp. (2020). *Human Immunodeficiency Virus 1 (HIV-1) PhenoSense GT® Plus Integrase (Monogram® Phenotype + Genotype)*. https://www.labcorp.com/tests/551920/human-immunodeficiency-virus-1-hiv-1-phenosense-gt-plus-integrase-monogram-phenotype-genotype

Mansky, L. M., & Temin, H. M. (1995). Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *J Virol*, *69*(8), 5087-5094. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/7541846/

NYSDOH. (2020). *Clinical Guidelines Program*. https://cdn.hivguidelines.org/wp-content/uploads/20200506090926/NYSDOH-AI-HIV-Resistance-Assays_updated_5-5-2020_mbh.pdf

Pröll, J., Paar, C., Taylor, N., Skocic, M., Freystetter, A., Blaimschein, A., Mayr, R., Niklas, N., Atzmüller, S., Raml, E., & Wechselberger, C. (2022). New aspects of the Virus Life Cycle and Clinical Utility of Next Generation Sequencing based HIV-1 Resistance Testing in the Genomic, the Proviral and the Viral

Reservoir of Peripheral Blood Mononuclear Cells. *Curr HIV Res*. https://doi.org/10.2174/1570162x20666220324111418

Raymond, S., Nicot, F., Abravanel, F., Minier, L., Carcenac, R., Lefebvre, C., Harter, A., Martin-Blondel, G., Delobel, P., & Izopet, J. (2020). Performance evaluation of the Vela Dx Sentosa next-generation sequencing system for HIV-1 DNA genotypic resistance. *Journal of Clinical Virology*, *122*, 104229. https://doi.org/https://doi.org/10.1016/j.jcv.2019.104229

Rosemary, A., Chika, O., Jonathan, O., Godwin, I., Georgina, O., Azuka, O., Zaidat, M., Philippe, C., Oliver, E., Oche, A., David, O., Jay, S., Ibrahim, D., Mukhtar, A., Joshua, D., Chunfu, Y., Elliot, R., Beth, C., Phyllis, K., & Emmanuel, I. (2018). Genotyping performance evaluation of commercially available HIV-1 drug resistance test. *PLoS One*, *13*(6), e0198246. https://doi.org/10.1371/journal.pone.0198246

Shen, C., Yu, X., Harrison, R. W., & Weber, I. T. (2016). Automated prediction of HIV drug resistance from genotype data. *BMC Bioinformatics*, *17 Suppl 8*, 278. https://doi.org/10.1186/s12859-016-1114-6

Taylor, T., Lee, E. R., Nykoluk, M., Enns, E., Liang, B., Capina, R., Gauthier, M. K., Domselaar, G. V., Sandstrom, P., Brooks, J., & Ji, H. (2019). A MiSeq-HyDRA platform for enhanced HIV drug resistance genotyping and surveillance. *Sci Rep*, *9*(1), 8970. https://doi.org/10.1038/s41598-019-45328-3

Vandamme, A. M., Camacho, R. J., Ceccherini-Silberstein, F., de Luca, A., Palmisano, L., Paraskevis, D., Paredes, R., Poljak, M., Schmit, J. C., Soriano, V., Walter, H., & Sonnerborg, A. (2011). European recommendations for the clinical use of HIV drug resistance testing: 2011 update. *AIDS Rev*, *13*(2), 77-108. http://gruposdetrabajo.sefh.es/afvih/images/stories/Documentos/04resistan2011.pdf

Zhang, J., Rhee, S. Y., Taylor, J., & Shafer, R. W. (2005). Comparison of the precision and sensitivity of the Antivirogram and PhenoSense HIV drug susceptibility assays. *J Acquir Immune Defic Syndr*, *38*(4), 439-444.

Revision History

Company(ies)	DATE	REVISION
EmblemHealth ConnectiCare	7/6/2023	• Policy Archived **effective 11/13/2023;** replaced with policy AHS-M2116 Human Immunodeficiency Virus (HIV)
EmblemHealth ConnectiCare	11/2022	• Reformatted and reorganized policy, transferred content to new template with new Reimbursement Policy Number