# Test Specific Guidelines

# Human Immunodeficiency Virus Laboratory Testing

**MOL.CS.321.A**
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## Introduction

**In-vitro testing for Human Immunodeficiency Virus (HIV) is addressed by this guideline.**

## Procedures Addressed

**The inclusion of any procedure code in this table is provided for informational purposes and is not a guarantee of coverage nor an indication that prior authorization is required.**

| Procedure addressed by this guideline | Procedure code |
|---|---|
| Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, reverse transcriptase and protease regions | 87901 |
| Infectious agent genotype analysis by nucleic acid (DNA or RNA); HIV-1, other region (eg, integrase, fusion) | 87906 |
| Nucleic acid testing (NAT): HIV-1, direct probe technique | 87534 |
| Nucleic acid testing (NAT): HIV-1, amplified probe technique, includes reverse transcription when performed | 87535 |
| Nucleic acid testing (NAT): HIV-1, quantification, includes reverse transcription when performed | 87536 |
| Nucleic acid testing (NAT): HIV-2, direct probe technique | 87537 |
| Nucleic acid testing (NAT): HIV-2, amplified probe technique, includes reverse transcription when performed | 87538 |

| Procedure addressed by this guideline | Procedure code |
|---|---|
| Nucleic acid testing (NAT): HIV-2, quantification, includes reverse transcription when performed | 87539 |
| Sentosa® SQ HIV-1 genotyping | 0219U |

## What Is HIV?

### Definition

HIV is an RNA retrovirus that attacks the immune system, making individuals more susceptible to opportunistic infections.

HIV natural history, diagnosis and treatment is the subject of a number of government, organizational and academic reviews, free online video courses, as well as guidelines.[1-11] According to the CDC, HIV is transmitted from person to person in various ways, including unprotected anal or vaginal sex, sharing needles for injection drug use, being stuck with an HIV contaminated needle, and less commonly, perinatal transmission.[2]

There are two main virotypes of HIV, HIV-1 and HIV-2. HIV-1 is the predominant infection in the United States. Within each virotype, there are multiple subtypes.[5,9]

The CDC publishes prevalence statistics for HIV on its website.[2]

The natural history of untreated HIV is a chronic infection that slowly disables the immune system. Individuals are often symptom free for months or years as the virus multiples. During this period, individuals are infectious. Eventually, slow destruction of the immune system leads individuals to become symptomatic and succumb to a myriad of different opportunistic infections or cancers. Less commonly, patients exhibit an acute HIV syndrome 2-4 weeks after infection with flu-like signs and symptoms including swollen lymph nodes, headache, sore throat, arthralgia, and rash.[2,5]

The HIV mechanism of action is through the infection of CD4-positive (CD4+) T lymphocytes. HIV enters the cell after binding to an envelope protein. HIV then manufactures DNA via a reverse transcriptase, integrates the viral DNA into the host cell DNA through a viral integrase enzyme and eventually drives replication of the HIV virus with the integrated DNA.[2,5] The formation of the virion inside the host cell is enabled by a protease. Anti-retroviral therapy consists of a multidrug regimen with the drugs primarily targeting the reverse transcriptase and the protease. Alternative treatments, often employed if drug resistance develops, target the envelope protein and the integrase enzyme.[6,7,8]

CDC guidelines describe the progression of HIV from acute to chronic infection and the characteristic findings of in-vitro blood testing associated with this progression. This includes the “window period” after individuals have been

infected but before their tests become positive.[9,10] The CDC lists the effective timing of specific test's ability to identify HIV infection:[2] The window period varies between individuals, and is dependent upon the test used to detect HIV, with shorter window periods generally associated with nucleic acid tests.

During the window period, individuals are infectious and pose a public health risk if they engage in behavior that can transmit the virus. It is important to detect HIV infection as early as possible since people in the window period are more infectious and responsible for a disproportionate amount of disease transmission. The earlier it can be determined whether an individual is infected, the earlier they can be counseled regarding initiating prophylactic treatment, retesting, and the high risk of infecting others in order to reduce disease transmission. Early treatment is strongly associated with better outcomes.[2,7,8]

Quantitation of HIV RNA in the blood by HIV-NAT and the quantification of CD4+ T cells are the foundation of the chronic monitoring of HIV infection.[2,3,6-8] Quantitative HIV RNA assessment is also known as viral load testing. In adults, the natural history of infection is associated with rising quantities of HIV virus in the blood and falling CD4 cell counts. In general, rising viral load and falling CD4 counts are associated with worse clinical disease and prognosis. Successful treatment is indicated by stabilizing or increasing the CD4 count with a corresponding decrease in HIV RNA levels, eventually lowering them below the limits of detection of the quantitative HIV-NAT.

The natural history of untreated HIV in children is more variable than what is observed in adults. Whereas, in adults, clinical progression correlates with falling CD4 counts and rising HIV RNA levels, the relationship in children is not as direct with more exceptions than in adults. Some children with minimal disease burden have high HIV-RNA levels while some with low RNA levels have significant clinical findings. In general, infants have higher viral loads than adolescents or adults.[5,8] In children <18 months old, the basis of diagnosis is nucleic acid testing rather than combined antibody/antigen testing.[4]

HIV transmission from mother to child has been dramatically reduced because of 1) early recognition of HIV infection during pregnancy because of more aggressive and widespread testing; 2) initiation of ART during pregnancy, and 3) Prophylactic antiretroviral treatment of infants at risk of infection.[8]

Over time, HIV has been transformed from a fatal illness to a chronic disease through the development of multidrug, anti-retroviral therapy (ART) aimed at disabling various parts of the HIV infection and replication cycle.[6-8,10] HIV can become resistant to these therapies. Resistance can be predicted and managed with the help of in-vitro blood testing for HIV genotype or phenotype. HIV genotypic testing is generally favored over phenotype testing. Resistance testing is performed at the beginning of therapy, and then used again if there is evidence of treatment failure.

# Test Information

## Introduction

The laboratory tests discussed in this guideline play a foundational role in the diagnosis, treatment, and monitoring of HIV infection. Population screening for HIV disease by laboratory methods is important for reducing the spread of the disease and improving outcomes through the use of antiretroviral therapy (ART). HIV tests are offered by most of the major in-vitro diagnostic manufacturers and the instrument platforms vary from large high-throughput instruments for the commercial lab setting to small point-of-care instruments that can be used in ambulatory clinics and public health settings. The primary methods incorporated into these technologies are a variety of immunoassay formats (e.g. bead-based, immunochromatography, microplate enzyme immunoassay, and others) or nucleic acid detection, also by a variety of formats.

### Combined HIV Assays That Detect HIV-1 Antigen and Antibodies to HIV-1 and HIV-2

These are the most common tests for diagnosing HIV infection and are the first tests recommended in the CDC guideline and algorithm, which have been widely adopted.[9,10] The most advanced of these are known as 4th or 5th generation antigen/antibody tests. In most individuals, if a combined antigen/antibody test is negative, HIV has been ruled and no further testing is necessary. The one exception is individuals with a recent exposure who are in the window period. These individuals require HIV-NAT (see below) for maximal sensitivity or they may need to be tested again if they are within the window period for HIV-NAT. Individuals who test positive by the combined antigen/antibody assay then have confirmatory testing to establish the diagnosis.[9,10]

### Nucleic Acid Testing

Nucleic acid testing (NAT) for HIV is referred to in guidelines as “virologic” testing because it directly detects virus.[7,8] For diagnosing HIV infection, the main uses of NAT are: 1) diagnosing HIV in the window period before the combined antigen-antibody test turn positive,[9,10] 2) diagnosing HIV in infants and children < 24 months old (due to potential residual maternal HIV antibodies)[8], and 3) resolving cases that are positive by the combined test but then have an indeterminate confirmatory test by another antibody-based method.[9,10] In addition, nucleic acid methods are available on some point of care devices. A CDC guideline recommended that individuals testing positive by point of care devices then undergo testing based on the current algorithm, which starts with the combined HIV-1/HIV-2 antibody plus HIV-1 antigen assay.[9,10]

Quantitative HIV-NAT, which is often referred to as an HIV RNA viral load or HIV “quant” is used for monitoring HIV treatment.[6-8] The diagnostic uses of NAT described above involve setting a threshold for a positive test and then resulting

the test as positive or negative. In contrast, quantitative HIV-NAT uses the same technology, but the assay is designed to give a quantitative result, in copies of the virus per volume (such as copies/mL) of blood. The goal of ART treatment is to drive the quantitative HIV RNA to undetectable levels. Individuals who achieve undetectable levels of virus have a very good prognosis but are still monitored by quantitative HIV-NAT to monitor for changes in viral load.[6-8] In general, rising HIV viral loads are a poor prognostic sign and indicative of disease progression, ART treatment failure, or non-compliance. Falling levels are indicative of a treatment effect and slowing of disease progression.[1-3,6-8]

HIV-1 Genotyping

Genotyping for HIV-1 drug resistant mutations may be performed by several methods, including Sanger sequencing and next generation sequencing (NGS). Commercially available test kits utilizing Sanger sequencing or allele specific polymerase chain reaction technology and automated sequence detection allow for the detection of HIV-1 resistance to protease inhibitors, nucleoside reverse-transcriptase inhibitors, and non-nucleoside reverse-transcriptase inhibitors, with limits of detection as low as 1000 copies/ml for plasma and 2000 copies/ml for dried blood spot samples. NGS allows for the detection of low-abundance drug resistant variants, which may have a prevalence of as high as 33% in some studies.[13]

The Sentosa® SQ HIV-1 Genotyping Assay is an NGS assay for the detection of HIV-1 genomic mutations in patients with diagnosed HIV-1 Group M infection. It is intended for use in detecting HIV-1 genomic mutations (in the protease, reverse transcriptase and integrase regions of the pol gene) as an aid in monitoring and treating HIV-1 infection. This test is used in adjunct to the therapeutic management of patients diagnosed with HIV-1 Group M infection with viral loads of at least 1,000 RNA copies per mL in EDTA plasma specimens.

Results should be used in conjunction with other available laboratory and clinical information and are not intended for use as an aid in the diagnosis of infection with HIV or to confirm the presence of HIV infection, or for screening donors of blood, plasma or human cells, tissues and cellular and tissue-based products (HCT/Ps).

HIV Confirmatory Test by Immunofluorescence Assay (IFA) or Western Blot

This older test was in frequent use when HIV western blot was the predominant confirmatory test in the HIV guidelines with IFA being an alternative, less frequently used confirmatory method.[14] This test has been largely replaced by automated, less subjective methods of antibody and antigen detection. It is still occasionally used and, although it is no longer in the current CDC guideline, it can still be appropriate in some settings as a backup test. Moreover, although the newer guideline is more effective than the older guideline, which was based on an

enzyme, immunoassay (EIA) followed by confirmation with Western Blot or IFA, the older guideline produced excellent results with very few false results.[9,10]

HIV-1 Antibody, HIV-2 Antibody, Combined HIV-1 and HIV-2 Antibody in a Single Test, HIV-1 Antigen, HIV-2 Antigen

Before the advent of the new CDC guidelines which emphasize the later 4th generation combined antibody/antigen assays described above, single antibody tests or single antigen tests were used alone or in combinations as a mainstay of HIV screening.[9,10,14] Under the old algorithm, the antibody tests for HIV-1 were the foundation, and antigen testing for HIV-1 was used to improve detection in the window period for antibodies. HIV-2 testing, which was less frequent under the old guidelines and limited to cases where it was suspected, was accomplished using HIV-2 antibody testing primarily, with HIV-2 antigen testing performed in rare cases where individuals were both HIV-2 infected and potentially in the window period for HIV-2 antibodies. Although they are not included in current guidelines, these single antigen and antibody tests may be useful in selected settings. These settings include point of care testing in ambulatory clinics or in public health settings. The current CDC guideline recommended that individuals testing positive by point of care devices subsequently undergo the current algorithm, which starts with the combined HIV-1/HIV 2 antibody plus HIV-1 antigen assay.[9,10] Similarly, the older combined HIV-1/HIV-2 antibody without antigen can still be useful in that it tests for two antibodies in one assay.

## Guidelines and Evidence

### Introduction

This section includes guidelines and evidence pertaining to in-vitro testing for HIV.

Screening for HIV is broadly recommended. The United States Preventive Services Taskforce recommendation stated:[12]

“The USPSTF recommends that clinicians screen for HIV infection in adolescents and adults aged 15 to 65 years. Younger adolescents and older adults who are at increased risk should also be screened.”

“The USPSTF recommends that clinicians screen all pregnant women for HIV, including those who present in labor who are untested and whose HIV status is unknown."

This recommendation has become widely accepted and promulgated in the United States. For example, an HIV guideline from the International Antiviral Society USA (IAS-USA) Panel stated:[6]

“HIV testing is recommended at least once for anyone who has ever been sexually active and more often for individuals at ongoing risk for infection.”

**The recommendation from the CDC stated:[2]**

**"CDC recommends that everyone between the ages of 13 and 64 get tested for HIV at least once as part of routine health care."**

**Screening intervals for HIV testing are based on risk assessment and there is no "one size fits all" approach. The USPSTF summarized the approach to screening intervals as follows:[12]**

**The evidence is insufficient to determine optimum time intervals for HIV screening. One reasonable approach would be one-time screening of adolescent and adult patients to identify persons who are already HIV-positive, with repeated screening of those who are known to be at risk for HIV infection, those who are actively engaged in risky behaviors, and those who live or receive medical care in a high-prevalence setting. According to the CDC, a high-prevalence setting is a geographic location or community with an HIV seroprevalence of at least 1%. … Given the paucity of available evidence for specific screening intervals, a reasonable approach may be to rescreen groups at very high risk …for new HIV infection at least annually and individuals at increased risk at somewhat longer intervals (for example, 3 to 5 years)….Women screened during a previous pregnancy should be rescreened in subsequent pregnancies."**

**The CDC published a guideline and a brief update regarding the best approach to screening and diagnosis of HIV.[9,10] The algorithm starts with screening for HIV-1 and HIV-2 with a 3rd, 4th, or 5th generation FDA-approved, combined antigen-antibody immunoassay. If the testing is negative, there is no additional testing necessary unless there is evidence of recent exposure suggesting acute infection in the window period before combined antigen antibody testing is positive. This is when HIV-NAT is recommended. The updated guideline stated:[10]**

**"Laboratories should conduct initial testing for HIV with an FDA-approved antigen/antibody immunoassay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to test for established HIV-1 and HIV-2 infection and for acute HIV-1 infection, respectively. No further testing is required for specimens that are non-reactive on the initial immunoassay. However, if there is a possibility of very early infection leading to a non-reactive initial antigen/antibody immunoassay, such as when recent HIV exposure is suspected or reported, then conduct an HIV-1 nucleic acid test (NAT)…"**

**Due to its lower sensitivity during the acute phase of HIV, rapid HIV-1 and/or HIV-2 testing is not preferred; instrumented assays are the preferred method.[10,11]**

**"The FDA-approved single-use rapid HIV-1/HIV-2 antigen/antibody immunoassay can be used as the initial assay in the laboratory HIV testing algorithm for serum or plasma. If any instrumented antigen/antibody test is available, it is preferred due to its superior sensitivity for detecting HIV during acute infection."**

**When initial testing is positive, an assay is run to distinguish HIV-1 from HIV-2 since HIV-1 and HIV-2 may have different treatments. The CPT coding for this will include some combination of immunoassay codes in the table above, but it will**

vary based on the exact methods used to distinguish the types of HIV. The guideline stated:[10]

"…accurate diagnosis of HIV-2 is clinically important because some antiretroviral agents effective against HIV-1 (including nonnucleoside reverse transcriptase inhibitors and some protease inhibitors) are not effective against HIV-2."

"Specimens with a reactive antigen/antibody immunoassay result (or repeatedly reactive, if repeat testing is recommended by the manufacturer or required by regulatory authorities) should be tested with an FDA-approved supplemental antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies."

If the assay to distinguish HIV-1 from HIV-2 is indeterminate, then the guideline recommended that an HIV-NAT be used to resolve the indeterminate result.[10]

In infants and children up to 24 months of age, HIV-NAT is the test of choice for diagnosing or ruling out HIV infection, rather than HIV antibody and antigen testing. This is because persistence of maternal HIV antibodies in the child's blood makes it difficult to interpret results. Moreover, in children < 6 months old, the immune system is not mature enough to develop a detectable immune response by HIV antibody testing. According to the NIH pediatric guideline:[8]

"Virologic assays (i.e., HIV RNA and HIV DNA nucleic acid tests) that directly detect HIV must be used to diagnose HIV infection in infants and children younger than 18 months with perinatal and postnatal HIV exposure; HIV antibody tests should not be used."

"Positive virologic tests (i.e., nucleic acid tests [NAT]—a class of tests that includes HIV RNA and DNA polymerase chain reaction [PCR] assays, and related RNA qualitative or quantitative assays) indicate likely HIV infection. The first test result should be confirmed as soon as possible by a repeat virologic test on a second specimen…"

For ruling out HIV infection, HIV-NAT tests are relied upon before age 6 months, and then antibody tests or combined antigen-antibody tests (see table for list of possible CPT codes) can be used after 6 months to rule out disease as these children can mount a sufficient immune response to the virus. Nevertheless, HIV-NAT is still needed to rule in disease in individuals of age > 6 months. The guideline stated:[8]

"Definitive exclusion of HIV infection in non-breastfed infants is based on 2 or more negative virologic tests, with 1 obtained at age ≥1 month and 1 at age ≥4 months, or 2 negative HIV antibody tests from separate specimens obtained at age ≥6 months …Some experts confirm the absence of HIV infection at 12 to 18 months of age in children with prior negative virologic tests by performing an HIV antibody test to document loss of maternal HIV antibodies."

Oral fluid antibody testing detects fewer HIV infections than other methods.[15] In a retrospective observational analysis of 287 patients that seroconverted, oral fluid

antibody testing in 80 patients yielded false negative results.[16] The CDC's updated HIV testing algorithm does not include oral fluid antibody testing as an applicable method.[9]

**Monitoring Antiretroviral Treatment for HIV Infection Using Quantitative HIV NAT**

The 2018 NIH guideline on the use of antiretroviral treatment (ART) in adolescents and adults gives an overview of treatment criteria and describes the goal of treatment.[7] ART is universally recommended to patients diagnosed with HIV.

The 2020 guideline on ART from the International Antiviral Society USA (IAS-USA) Panel summarized the widely accepted role that laboratory testing plays throughout treatment for monitoring the success of ART.[6]

During treatment, ART is serially monitored with laboratory testing. Monitoring for treatment failure is important because changing treatment may re-establish control of the infection and produce good clinical outcomes. The NIH guideline summarized the widely accepted, evidence-based approach regarding monitoring ART with laboratory testing using HIV RNA level and CD4 counts:[8]

"Evaluation of virologic failure should include an assessment of adherence, drug-drug and drug-food interactions, drug tolerability, HIV RNA level and CD4 T lymphocyte (CD4) cell count trends over time, ART history, and prior and current drug-resistance test results."

For patients who become drug resistant, the goal is to change therapy and drive down the quantitative HIV RNA level. The NIH guideline stated:[7]

"The goal of treatment for ART-experienced patients with drug resistance who are experiencing virologic failure is to establish virologic suppression (i.e., HIV RNA levels below the lower limits of detection of currently used assays)."

Monitoring of an early HIV infection is carried out in a similar fashion. The guideline stated:[7]

"Once initiated, the goal of ART is to suppress plasma HIV-1 RNA to undetectable levels…Testing for plasma HIV-1 RNA levels, CD4 T lymphocyte cell counts, and toxicity monitoring should be performed as recommended for patients with chronic HIV-1 infection."

The NIH guidelines for adolescents, adults, and children provided recommended intervals for testing for quantitative HIV viral load.[7,8] In adults, after initiation of ART or change in ART due to treatment failure, RNA viral load testing occurs at 2-8 weeks and again in 4 to 8 week intervals until viral load is sufficiently suppressed. After suppression is achieved, then repeat testing is every 3-6 months. The adolescent and adult guideline stated:[7]

"If HIV RNA is detectable at 2 to 8 weeks, repeat testing every 4 to 8 weeks until viral load is suppressed to <200 copies/mL. Thereafter, repeat testing every 3 to 6 months."

The pediatric guideline is similar with slightly more frequent monitoring of viral load. The guideline stated:[8]

"After initiation of ART, or after a change in ART regimen, children should be evaluated for clinical adverse effects and to support treatment adherence within 1 to 2 weeks, with laboratory testing for toxicity and viral load response recommended at 2 to 4 weeks after treatment initiation."

"Viral load measurement every 3 to 4 months is generally recommended to monitor ART adherence and disease progression."

## HIV Genotyping

The HIV genome has a high mutation rate, which leads to the possibility for each HIV positive patient to eventually carry and transmit an HIV variant with a drug resistant mutation.[17] The level of detection of drug resistant mutations varies with the method employed; conventional Sanger sequencing has a detection threshold of approximately 20%, whereas for next-generation sequencing (NGS) it is much lower.[18] However, the clinical impact of this testing on therapeutic decisions in antiretroviral therapy naïve patients has not been well established, and large prospective studies addressing the impact of drug resistant variants on different anti-viral therapy regimens are needed.[13] Virologic failure may not be experienced by all individuals harboring drug resistant mutant(s), and those detected in low abundance at the time of diagnosis may not be the culprit responsible for virologic failure.[13]

In some studies, drug resistant variants have been associated with an increased risk of treatment failure, however results of other studies demonstrate no effect.[19] In a study limited to 12 patients, a novel HIV-1 genotypic assay using deep sequencing revealed the presence of minority variants associated with virologic failure.[17] In a systematic review of 10 studies, patients with minority non-nucleoside reverse-transcriptase inhibitor (NNRTI) resistant variants had a 37% virologic failure rate compared to a 15% failure rate in those without NNRTI-resistant variants.[19] However, the assay methods used in the analyzed studies were heterogeneous, and whether the test results were used to tailor therapy was not examined.

The International Antiviral Society –USA (IAS-USA) Panel has stated the following:[20]

Regarding NGS:

"These newer technologies most likely will replace Sanger sequencing–based resistance testing within the next few years in research and commercial labs. Next-generation sequencing refers to high-throughput DNA sequencing technologies. Millions of DNA strands can be sequenced in parallel, yielding substantially more throughput and minimizing the need for the fragment-cloning methods that are often used in Sanger sequencing of genomes."

Regarding drug resistant variants:

"… testing for HIV drug resistance, and the appreciation of its role, is crucial to the prevention and management of failure of ART."

"Testing for minority variants harboring drug resistance may only be considered if treatments depend on a first-generation nonnucleoside analogue reverse transcriptase inhibitor. Different HIV-1 subtypes do not need special considerations regarding resistance testing."

The IAS –USA Panel has recommended HIV drug resistance testing for:

the newly diagnosed and presumably ART-naive

those receiving ART and demonstrating a rising HIV RNA copy number >200 copies/ml

those who have not achieved viral suppression within 6 months of therapy initiation

those who have interrupted ART containing an NNRTI with a long half-life

those who are drug-naïve and have an increase in plasma -viremia

In its recommendations for treatment of HIV infection, the panel states:[21]

"Before starting ART, recommended laboratory monitoring includes HIV RNA level, CD4 cell count, and reverse transcriptase and protease genotype (InSTI genotyping generally is not recommended because it is not cost-effective) …"

The Sentosa® SQ HIV-1 Genotyping Assay is an NGS assay for the detection of HIV-1 genomic mutations in patients with diagnosed HIV-1 Group M infection (Vela Diagnostics package insert Version 1.0). In a comparison study, the Sentosa® SQ HIV-1 Genotyping assay detected twice as many minority variants than Sanger sequencing.[22] In another study, the Vela Diagnostics assay was less sensitive for detecting minority variants compared to two other NGS platforms, but results were similar for all three platforms when variants exceeded 20% of the quasi-species.[23] In a later study, the Sentosa® assay had only an 82% success rate in identifying mutations in the protease-reverse transcriptase region.[24] In a prospective study comparing the Sentosa® assay to Sanger sequencing, detection of mutations was similar between the two methods, but whether results impacted clinical management or outcome was not examined.[25] Another study found Sanger sequencing and the Sentosa® assay to perform similarly at the 20% variant threshold, but again did not evaluate the impact of variant detection on clinical management or outcome.[26]

The preponderance of studies comparing NGS to Sanger sequencing for the detection of Group M HIV-1 resistance mutations have demonstrated that the methods have similar resistance mutation detection rates at the 20% threshold, however NGS lacks standardization.[27,28] Standardization of NGS methods and results reporting among laboratories will likely lead to greater acceptance of the method, and translate into lower testing costs. With that comes the increasing

importance of determining whether the detection of minor resistance variants by NGS will impact patient care or improve outcomes.

In a prevalence study of 134 patients, ultra-deep sequencing was more sensitive than Sanger sequencing, and detected INSTI resistance variants in 57.5% of patients, but there was no association with therapeutic response.[29] A comparison study of test and follow-up cohorts reported similar mutation detection rates between NGS and Sanger sequencing, however virologic failure was not associated with low frequency mutations, but rather poor medication adherence.[30] Similarly, a retrospective multi-center study found that minority variants with resistance to rilpivirine were not associated with virologic failure, but baseline resistance at a 20% threshold was associated with viral response.[31] Further analysis of the study data determined that viral mutational load had a low positive predictive value for virologic failure.[32] One study found that mutation thresholds ranging from 1% to 20% were not significantly different in predicting virologic failure to NNRTI; however, the study defined viral suppression as less than 400 copies/ml, compared to the 50 copies/ml threshold used in other studies, and hence increases resulting still in values under 400 copies/ml would not be classified as virologic failure.[33]

A proof of concept study demonstrated that the detection of lamivudine resistance mutations by NGS was unrelated to the maintenance of virologic suppression in patients with a median duration of virus suppression of 6 years.[34]

A recent Cochrane Review found that resistance testing made little or no difference in mortality, change in CD4 cell count, adverse events, or progression to AIDS in patients failing ART. There was low certainty of evidence that resistance testing impacted virologic failure. The review could not address the possible value of resistance testing in patients naïve to treatment, those being considered or receiving INSTI containing regimens, or its long-term effects because of short median follow-up.[35]

Using the Cost-effectiveness of Preventing AIDS Complications model, a study demonstrated that baseline genotype was not cost-effective, and for newly diagnosed HIV positive patients, resulted in no more than 5 quality adjusted life days. The authors concluded that given first-line INSTI based regimens, baseline genotyping offered minimal clinical benefit.[36]

## Criteria

### Introduction

This guideline outlines coverage criteria for molecular testing for HIV. It does not address drug resistance testing as this is addressed by the guideline HIV Tropism Testing for Maraviroc Response.

## HIV Nucleic Acid Testing (NAT)

### CPT code(s): 87534, 87535, 87536, 87537, 87538, 87539

#### Medical necessity requirements

HIV nucleic acid testing (NAT) is considered medically necessary in the following circumstances:

Screening for a diagnosis of HIV in any individual 13 years or older

Screening for a diagnosis of HIV in any individual with a potential HIV exposure or engaging in behavior associated with increased risk of HIV infection who is within the window period when standard combined antibody/antigen screening may not be effective.

Screening for a diagnosis of HIV in pregnancy

Monitoring treatment of HIV

#### Billing and reimbursement

HIV screening by qualitative NAT is reimbursable up to 4 times per year for screening for new cases.

Monitoring HIV treatment with quantitative NAT is reimbursable up to 9 times per year.

## HIV Genotyping

### CPT code(s): 87901, 87906, 0219U

#### Medical necessity requirements

HIV-1 genotyping for drug resistance mutations is considered medically necessary in the following circumstances:

Upon diagnosis of HIV infection when an anti-retroviral therapy (ART) regimen not containing an INSTI is being considered

In treatment naïve patients where an increase in viral load has been demonstrated

In patients receiving an ART not containing INSTI who have demonstrated after 8 or more weeks of therapy a failure to reduce viral copy number below 50 copies/ml, or that have experienced an increase in viral load after having achieved viral suppression

#### Billing and reimbursement

The clinical utility of HIV genotyping for the identification of low level (“minority”) drug resistant mutations or INSTI mutations has not been demonstrated. HIV genotyping for low-level drug resistant mutations or INSTI mutations is considered investigational and experimental and is therefore not eligible for reimbursement for any clinical indication.

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## Introduction

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