Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV

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# Drug-Resistance Testing

**Panel’s Recommendations**

## 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 (AII).
- 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 (AII).
- 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:

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<td>A</td>
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### Rating of Evidence:

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<tr>
<td>I</td>
<td>Data from randomized controlled trials</td>
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<td>II</td>
<td>Data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes</td>
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## Genotypic and Phenotypic Resistance Assays

Genotypic and phenotypic resistance assays are used to assess viral strains and select treatment strategies. These assays provide information on resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and integrase strand transfer inhibitors (INSTIs). In some circumstances, INSTI-resistance tests may need to be ordered separately, and clinicians should check this with the testing laboratory. INSTI-resistance testing is particularly important in persons who experience virologic failure while taking an INSTI-containing regimen. Testing for fusion inhibitor resistance can also be ordered separately. **There is currently no commercially available resistance test for the CD4 T lymphocyte post-attachment inhibitor ibalizumab.** For a description of co-receptor tropism testing, see Co-receptor Tropism Assays.
Genotypic Assays

Genotypic assays detect drug-resistance mutations in relevant viral genes; in general, these assays require a plasma viral load of at least 500 to 1,000 copies/mL. Most genotypic assays involve conventional Sanger sequencing of the reverse transcriptase (RT), protease (PR), and integrase (IN) genes of circulating RNA in plasma to detect mutations that are known to confer drug resistance. A genotypic assay that assesses mutations in the gp41 (envelope) gene associated with resistance to the fusion inhibitor enfuvirtide is also commercially available. Genotypic assays can be performed rapidly and results are available within 1 to 2 weeks of sample collection. Interpreting these test results requires knowledge of the mutations selected by different antiretroviral (ARV) drugs and of the potential for cross resistance to other drugs conferred by certain mutations. The International AIDS Society-USA (IAS-USA) maintains an updated list of significant resistance-associated mutations in the RT, PR, IN, and envelope genes. The Stanford University HIV Drug Resistance Database also provides helpful guidance for interpreting genotypic resistance test results. Various additional tools are also available to assist providers in interpreting genotypic test results. Clinical trials have demonstrated that consulting with specialists in HIV drug resistance improves virologic outcomes. Clinicians are thus encouraged to consult a specialist to interpret genotypic test results and design optimal new regimens.

A next-generation sequencing genotypic resistance assay that analyzes HIV-1 proviral DNA in host cells is now commercially available. This test aims to detect archived resistance mutations in patients with HIV RNA below the limit of detection or with low-level viremia.

Phenotypic Assays

Phenotypic assays measure the ability of a virus to grow in different concentrations of ARV drugs. RT, PR, and, more recently, IN and envelope gene sequences derived from patient plasma HIV RNA are inserted into the backbone of a laboratory clone of HIV or used to generate pseudotyped viruses that express the patient-derived HIV genes of interest. Replication of these viruses at different drug concentrations is monitored by expression of a reporter gene and is compared with replication of a reference HIV strain. The drug concentration that inhibits viral replication by 50% (i.e., the median inhibitory concentration [IC\textsubscript{50}]) is calculated, and the ratio of the IC\textsubscript{50} of test and reference viruses is reported as the fold increase in IC\textsubscript{50} (i.e., fold resistance).

Automated phenotypic assays that can produce results in 2 to 3 weeks are commercially available, but they cost more to perform than genotypic assays. In addition, interpreting phenotypic assay results can be complicated by incomplete information regarding the specific resistance level (i.e., fold increase in IC\textsubscript{50}) associated with drug failure, although clinically significant fold increase cutoffs have been described for some drugs. Again, consulting with a specialist to interpret test results can be helpful.

Limitations of Genotypic and Phenotypic Assays

Limitations of both genotypic and phenotypic assays include lack of uniform quality assurance testing for all available assays, relatively high cost, and insensitivity to minor viral species. Drug-resistant viruses that constitute <10% to 20% of the circulating virus population will probably not be detected by commercially available assays. This limitation is important to note because a wild-type virus often re-emerges as the predominant population in the plasma after discontinuation of drugs that exert selective pressure on drug-resistant populations. As a consequence, the proportion of virus with resistance mutations can decrease to below the 10% to 20% threshold. In the case of some drugs, this reversion to predominantly wild-type virus can occur in the first 4 to 6 weeks after the drugs are discontinued. Prospective clinical studies have shown that despite this plasma reversion, re-initiation of the same ARV agents (or those sharing similar resistance pathways) is usually associated with early drug failure, and that the virus present at failure is derived from previously archived resistant virus. Therefore, resistance testing is most valuable when performed while a person experiencing virologic failure is still taking ARV drugs or, if that is not possible,
then within 4 weeks after discontinuing therapy (AII). Because resistant viruses may persist longer in the plasma of some patients, resistance testing that is done 4 to 6 weeks after discontinuation of drugs or later may still detect mutations and provide useful information to guide therapy (CIII). However, the absence of detectable resistance in such patients must be interpreted with caution when designing subsequent ARV regimens. Importantly, in addition to considering prior antiretroviral therapy (ART) history, prior genotypic- or phenotypic-resistance test results should be obtained from old records when possible. Because the most current drug-resistance test may not be able to detect resistance mutations that were previously detected, these prior test results are clinically important and should be used when designing a new regimen (AIII).

A next-generation sequencing genotypic assay that analyzes HIV-1 proviral DNA may provide additional information on drug resistance in patients with low levels of plasma HIV RNA or in patients whose levels are below the limit of detection (CIII). However, these assays might miss some or all the previous drug-resistance mutations, and they should be interpreted with caution. The usefulness of these assays in the clinic is still under investigation and has yet to be fully determined.

Use of Resistance Assays in Clinical Practice (See Table 5)

Use of Resistance Assays in Determining Initial Treatment

Transmission of drug-resistant HIV strains is well documented and associated with suboptimal virologic response to initial ART.16-19 The risk of acquiring drug-resistant virus is related to the prevalence of drug resistance in people with HIV who engage in high-risk behaviors within a given community. In high-income countries, approximately 10% to 17% of ART-naive individuals have resistance mutations to at least one ARV drug.20 Up to 8%, but generally <5%, of transmitted viruses will exhibit resistance to drugs from more than one class.20-23 Transmitted resistant HIV is generally either NNRTI- or NRTI-resistant. Transmitted PI resistance is much less common, and to date, transmitted INSTI resistance is rare.24,25

Resistance testing can guide therapy selection to optimize virologic response in people 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. Therefore, resistance testing in these situations is recommended (AII). A genotypic assay is preferred for this purpose (AIII). In these settings, treatment initiation should not be delayed pending resistance testing results if the individual is willing and able to begin treatment. Once results are reported, the regimen can be modified if warranted (see also Acute and Recent HIV [Early] Infection). In the absence of ART, resistant viruses may decline over time to less than the detection limit of standard resistance tests. However, when ART is eventually initiated, even low levels of resistant viruses may still increase the risk of treatment failure.26-28 Therefore, if ART is deferred, resistance testing should still be performed during early HIV infection (AIII). In this situation, the genotypic resistance test result should be used for regimen selection when the person begins ART. Repeat resistance testing at the start of treatment may also be considered, because a patient may acquire drug-resistant virus (i.e., superinfection) between entry-into-care and the initiation of ART (CIII).

Interpretation of drug-resistance testing before ART initiation in persons with chronic HIV is less straightforward. The rate at which transmitted resistance-associated mutations revert to wild-type virus has not been completely delineated, but mutations present at the time of HIV transmission are more stable than those selected under drug pressure. It is often possible to detect resistance-associated mutations in viruses that were transmitted several years earlier.30-32 Though no prospective trial has directly addressed whether drug-resistance testing before initiation of therapy confers benefit in this population, data from several studies, including one prospective clinical trial, suggest that virologic responses in persons with baseline resistance mutations are suboptimal.16-19,33,34 In addition, an analysis of early RT and PR genotypic resistance testing in ARV-naive persons suggests that baseline testing in this population is cost effective and should be performed.35 Therefore, resistance testing in people with chronic infections is recommended at the time of entry into HIV care (AII).
Although no definitive prospective data exist to support the choice of one type of resistance testing over another, genotypic testing is generally preferred over phenotypic testing because of lower cost, more rapid turnaround time, greater sensitivity for detecting mixtures of wild-type and resistant virus, and easier interpretation of test results (AIII). If therapy is deferred, repeat testing shortly before initiating ART may be considered, because the patient may have acquired drug-resistant virus (i.e., superinfection) (CIII).29

Standard genotypic drug-resistance testing in ARV-naive persons involves testing for mutations in the RT and PR genes. Although reports of transmission of INSTI-resistant virus are rare, as use of INSTIs increases, the potential for transmission of INSTI-resistant virus may also increase. Therefore, when INSTI resistance is suspected, providers should supplement standard baseline genotypic resistance testing with genotypic testing for resistance to this class of drugs, which may need to be ordered separately (AIII).

The next-generation sequencing genotypic resistance assay that analyzes proviral DNA in host cells can be considered when conventional HIV RNA drug resistance testing is unsuccessful or unavailable for patients initiating therapy (CIII). As outlined above, the results should be interpreted with caution, as this assay might miss some or all previously existing drug-resistance mutations.

**Use of Resistance Assays in the Event of Virologic Failure**

Resistance assays are important tools to inform treatment decisions for patients who experience virologic failure while on ART. Several prospective studies have assessed the utility of resistance testing to guide ARV drug selection in patients who experience virologic failure. These studies involved genotypic assays, phenotypic assays, or both.6,39-45 In general, these studies found that changes in therapy based on resistance test results produced better early virologic response to salvage regimens than regimen changes guided only by clinical judgment.

In addition, one observational cohort study found that the use of genotypic drug-resistance testing in ART-experienced patients with detectable plasma HIV RNA was independently associated with improved survival.46 Thus, resistance testing is recommended as a tool for selecting active drugs when changing ARV regimens because of virologic failure in persons with HIV RNA >1,000 copies/mL (AI) (see also Virologic Failure). In persons with HIV RNA >500 copies/mL but <1,000 copies/mL, testing may be unsuccessful but should still be considered (BII). Conventional drug-resistance testing in persons with plasma viral loads <500 copies/mL is not usually recommended, because resistance assays cannot be consistently performed at low HIV RNA levels (AIII).

Resistance testing can also help guide treatment decisions for patients with suboptimal viral load reduction (AII). Virologic failure in the setting of ART is, for certain patients, associated with resistance to only one component of the regimen.47-49 In this situation, substituting individual drugs in a failing regimen may be an option, but this concept will require clinical validation (see Virologic Failure).

Genotyping is preferred for resistance testing in patients who experience virologic failure or suboptimal viral load reduction while on a first or second ARV drug regimen and in individuals in whom resistance mutation patterns are known or not expected to be complex (i.e., mutations that are straightforward, usually limited in number, and/or those that have clear significance) (AII). Often in these situations, the mutation patterns detected can be interpreted by algorithms used to predict the impact of subsequent regimens on virologic response. For patients with extensive treatment history, complex mutational patterns may occur. In such situations, the interpretation of complex genotypes and the impact of the mutation pattern on subsequent treatment regimens can be challenging. For these individuals, phenotypic resistance testing may provide additional helpful information (BIII). Rather than only predicting the impact of the detected mutations, these assays can measure in vitro the actual fold change in drug susceptibility, as well as the actual impact of mutation combinations and interactions on each drug under consideration.

When compared with phenotypic testing, genotypic testing costs less to perform and has a faster turnaround time.
and greater sensitivity for detecting mixtures of wild-type and resistant virus. In addition, observations show that genotypic and phenotypic assays are comparable predictors of virologic response to subsequent ART regimens.\textsuperscript{50}

In patients who experience virologic failure while on INSTI-based regimens, testing for INSTI resistance should be performed to determine whether to include drugs from this class in subsequent regimens (AII). In this circumstance, clinicians should confirm that, when they order a resistance test, their laboratory is testing for INSTI resistance in addition to NNRTI, NRTI, and PI resistance. If INSTI-resistance testing needs to be ordered separately (as is the case in some laboratories), clinicians should request this assay in addition to standard drug-resistance testing. Addition of phenotypic to genotypic testing is generally indicated for persons with known or suspected complex drug-resistance mutation patterns (BIII).

The next-generation sequencing genotypic resistance assay that analyzes proviral DNA can be considered for patients who are experiencing treatment failure and for whom conventional HIV RNA genotypic drug-resistance testing is unavailable or unsuccessful (CIII). As outlined above, results should be interpreted with caution, as these assays might miss some or all previously existing drug-resistance mutations.

When the use of a CCR5 antagonist is being considered, a co-receptor tropism assay should be performed (AI) (see Co-receptor Tropism Assays).

**Use of Resistance Assays for Optimizing Antiretroviral Regimen in Persons with Viral Suppression**

In the past decade, simpler, more potent, and better-tolerated ARV medications have become available and new ARV drugs will likely continue to emerge. Switching individual ARV drugs in a regimen is sometimes considered for patients with a suppressed viral load in order to simplify a regimen, avoid drug interactions or toxicity, or for other reasons. Because the patient’s viral load is suppressed, standard drug-resistance testing will not be successful.

The next-generation sequencing genotypic resistance assay that analyzes proviral DNA can be considered for these individuals, particularly if complex or semi-complex pre-existing resistance is suspected. In individuals who have experienced no prior virologic failures and who are on their first or second regimen, or who have genotypic testing results from when they had prior virologic failures, the use of the proviral DNA genotypic test is unlikely to provide additional useful information. However, in individuals who have experienced multiple prior failures, a prolonged history of prior ARV regimens, and/or for whom prior genotypic resistance test results are not available, it may be appropriate to utilize proviral DNA genotypic testing (CIII). When such testing is obtained, results should be combined with all prior genotypic and phenotypic test results to construct a cumulative genotype, which incorporates all current and previously detected drug-resistance mutations. Results from this test should be interpreted with caution, as these assays might miss some or all previously existing drug-resistance mutations. The usefulness of these assays in the clinic is still under investigation and has yet to be fully determined.

**Use of Resistance Assays in Pregnancy**

In pregnancy, the goal of ART is to rapidly and maximally reduce plasma HIV RNA to provide optimal maternal therapy and to prevent perinatal transmission of HIV. Genotypic resistance testing is recommended for all pregnant persons with HIV before initiation of therapy (AIII) and for those entering pregnancy with detectable HIV RNA levels while on therapy (AI). Phenotypic testing in those found to have complex drug-resistance mutation patterns may provide additional information (BIII). Optimal prevention of perinatal transmission requires prompt initiation of ART pending resistance testing results. Once the results are available, the ARV regimen can be changed as needed.
### Table 5. Recommendations for Using Drug-Resistance Assays (page 1 of 2)

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<tr>
<th>Clinical Setting and Recommendation</th>
<th>Rationale</th>
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<tr>
<td><strong>In Acute or Recent (Early) HIV Infection:</strong> Drug-resistance testing is recommended (AII). A genotypic assay is generally preferred (AIII). Treatment should not be delayed while awaiting results of resistance testing (AII).</td>
<td>Drug-resistance testing can determine whether drug-resistant virus was transmitted. The initial regimen can be modified, if necessary, once resistance test results are available. Genotypic testing is preferred to phenotypic testing because of lower cost, faster turnaround time, and greater sensitivity for detecting mixtures of wild-type and resistant virus.</td>
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<td>If ART is deferred, repeat resistance testing may be considered when therapy is initiated (CIII). A genotypic assay is generally preferred (AII).</td>
<td>Repeat testing when ART is initiated may be considered because the patient may have acquired a drug-resistant virus (i.e., superinfection).</td>
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<td><strong>In ART-Naive Patients with Chronic HIV:</strong> Drug-resistance testing is recommended at entry into HIV care to guide selection of initial ART (AII). A genotypic assay is generally preferred (AII).</td>
<td>Transmitted HIV with baseline resistance to at least 1 drug is seen in 10% to 17% of patients, and suboptimal virologic responses may be seen in patients with baseline resistant mutations to ARVs. If necessary, the ART regimen can be modified once resistance test results are available.</td>
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<td>If an INSTI is considered for an ART-naive 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).</td>
<td>Genotypic assays provide information on resistance to NRTIs, NNRTIs, PIs, and INSTIs. In some circumstances, INSTI resistance tests need to be ordered separately (clinicians should check with the testing laboratory). Currently, transmitted INSTI resistance is infrequent, but the risk of a patient acquiring INSTI-resistant strains may be greater in certain known exposure settings.</td>
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<tr>
<td>If therapy is deferred, repeat resistance testing may be considered before initiation of ART (CIII). A genotypic assay is generally preferred (AIII).</td>
<td>Repeat testing before initiation of ART may be considered because the patient may have acquired a drug-resistant virus (i.e., a superinfection).</td>
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<td>If use of a CCR5 antagonist is being considered, a co-receptor tropism assay should be performed (AII).</td>
<td>Genotypic testing is preferred to phenotypic testing because of lower cost, faster turnaround time, and greater sensitivity for detecting mixtures of wild-type and resistant virus.</td>
</tr>
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<td><strong>In Patients with Virologic Failure:</strong> Drug-resistance testing is recommended in patients on combination ART with HIV RNA levels &gt;1,000 copies/mL (AII). In patients with HIV RNA levels &gt;500 copies/mL but &lt;1,000 copies/mL, testing may not be successful but should still be considered (BII).</td>
<td>Drug-resistance testing can help determine the role of resistance in drug failure and maximize the clinician’s ability to select active drugs for the new regimen.</td>
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<td>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 &gt;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).</td>
<td>The absence of detectable resistance in such patients must be interpreted with caution when designing subsequent ARV regimens, as mutations may decay with time.</td>
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<td>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).</td>
<td>Genotypic testing is preferred to phenotypic testing because of lower cost, faster turnaround time, and greater sensitivity for detecting mixtures of wild-type and resistant HIV.</td>
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<td>All prior and current drug-resistance testing results should be reviewed and considered when designing a new regimen for a patient experiencing virologic failure (AII).</td>
<td>Drug resistance mutations may decay with time, and mutations detected in prior resistance tests may not be detected in current tests, though they remain clinically relevant.</td>
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<td>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).</td>
<td>Genotypic assays provide information on resistance to NRTI-, NNRTI-, PI-, and INSTI-associated mutations. In some circumstances, INSTI resistance tests need to be ordered separately (clinicians should check with the testing laboratory).</td>
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Table 5. Recommendations for Using Drug-Resistance Assays (page 2 of 2)

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<tr>
<td>Adding phenotypic testing to genotypic testing is generally preferred in patients with known or suspected complex drug-resistance patterns (BIII).</td>
<td>Phenotypic testing can provide additional useful information in patients with complex drug resistance mutation patterns.</td>
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<td>In Patients with Suboptimal Suppression of Viral Load:</td>
<td>Testing can determine the role of resistance in suboptimal viral suppression, and it can help the clinician identify the number of active drugs available in the current regimen and assess the need for a new regimen.</td>
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<td>Drug-resistance testing is recommended in patients with suboptimal viral load suppression after initiation of ART (AII).</td>
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<td>In Pregnant Persons with HIV:</td>
<td>The goals of ART in pregnant persons with HIV are to achieve maximal viral suppression for treatment of maternal HIV and to prevent perinatal transmission of HIV. Genotypic resistance testing will assist the clinician in selecting the optimal regimen for the patient. However, treatment should not be delayed while awaiting results of resistance testing. The initial regimen can be modified once resistance test results are available, if needed.</td>
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<td>Genotypic resistance testing is recommended for all pregnant persons before initiation of ART (AII) and for those entering pregnancy with detectable HIV RNA levels while on therapy (AII).</td>
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<tr>
<td>In Patients with Undetectable Viral Load or Low-Level Viremia:</td>
<td>This test may provide information about previously circulating resistant viral variants that are archived within proviral DNA. These assays may miss some or all prior resistance mutations that have occurred within the viral quasi-species, and therefore they should be interpreted with caution. The clinical utility of HIV-1 proviral DNA assays has not been fully determined.</td>
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<tr>
<td>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).</td>
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Key to Acronyms: ART = antiretroviral therapy; ARV = antiretroviral; INSTI = integrase strand transfer inhibitors; NNRTI = non-nucleoside reverse-transcriptase inhibitors; NRTI = nucleoside reverse-transcriptase inhibitors; PI = protease inhibitor

References


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