CLINICAL GUIDELINES PROGRAM
NEW YORK STATE DEPARTMENT OF HEALTH AIDS INSTITUTE | HIV · HCV · SUBSTANCE USE · LGBT HEALTH

HIV Resistance Assays

Guideline Information

Intended users	Clinicians providing ambulatory care for patients with HIV
Last reviewed and updated	February 2020
Original publication	June 2016
Committee	Medical Care Criteria Committee
Developer and funding	New York State Department of Health AIDS Institute (NYSDOH AI)
Development	See Supplement: Guideline Development and Recommendation Ratings

HIV Resistance Assays

Contents

Determining HIV Drug Resistance

☑ RECOMMENDATIONS

Determining HIV Drug Resistance

- Clinicians should consult with an expert to interpret the results of resistance assays because such results are often complex. (A3)
  - The NYSDOH AI Clinical Education Initiative line is available for phone consultation: 866-637-2342.
- When determining the optimal regimen for achieving viral suppression, clinicians should perform genotypic resistance testing that includes the protease (A2), RT (A2), and integrase genes (B2) at baseline, whether ART is being initiated or not.
  - 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 wild-type 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)

Abbreviations: ART, antiretroviral therapy; RT, reverse transcriptase.

Notes:

a. Virologic failure is defined as >200 copies/mL. See the NYSDOH AI guideline Virologic and Immunologic Monitoring in HIV Care.

The interpretation of HIV resistance assays is one of the most challenging tasks clinicians encounter when caring for patients with HIV and is crucial for tailoring an effective therapeutic ART regimen. The replicative mechanisms of HIV lack proof-reading capacity, making them error-prone and subject to cumulative mutations (i.e., changes in its genetic sequence). This lack of replicative fidelity, coupled with the selective pressure of sub-therapeutic drug levels, can lead to the development of clinically significant (i.e., resistance-bearing) mutations.

→ KEY POINT

- Resistance testing is recommended when patients are interrupting incompletely suppressive ART. Because of the rapid return of wild-type virus without selective pressure from ART [Devereux, et al. 1999], testing is preferred before cessation of treatment. In cases where the patient has already stopped therapy, testing should be performed as soon as practical and no more than 4 weeks after cessation, before the return of wild-type virus. Mutations detected in this setting may provide useful information, but the absence of mutations does not rule out their presence in minor variants.

The most commonly used ART drugs are targeted to inhibit the activity of three specific viral enzymes: the protease, RT, and integrase. Mutations have been identified that interfere with the ability of one or more ART agents to inhibit viral protein activity, thus rendering the virus resistant to the drug(s). HIV resistance mutations and mechanisms for less commonly used ART drugs that target fusion and viral entry have also been identified.

New resistance mutations and the emerging clinical significance of these mutations frequently change. Several resources are available for more information on drug resistance mutations and resistance testing, including:

- Stanford University HIV Drug Resistance Database
- IAS-USA 2019 Update of the Drug Resistance Mutations in HIV-1
- HIV Resistance Response Database Initiative
- Los Alamos National Laboratory HIV Databases
- HIV French Resistance Database

Two methods are used to determine drug resistance for HIV: genotyping, which detects treatment-resistant genetic mutations; and phenotyping, which assesses the viral response to ART agents. Genotyping is the preferred test in most clinical situations.

In New York State, third-party reimbursement programs, including Medicaid, the New York State AIDS Drug Assistance Program (ADAP), and private insurers, often limit the number of resistance tests per year (within 12 months following date of first use). Medicaid Managed Care Plans (MMCPs) and private insurers may require prior authorization for these services and may limit the number of resistance tests performed annually, such as three tests per year, regardless of whether genotyping, phenotyping, or a combination of testing is obtained.

Providers should refer to their patient's specific plan regarding frequency, annual limits, and whether prior authorization is required for any genotypic and phenotypic HIV resistance tests. Detailed information regarding Medicaid managed care-covered benefits for resistance testing, including current procedural terminology (CPT), codes are available at www.health.ny.gov/health_care/medicaid/program/update/2014/2014-03.htm#exp.

Investigational technologies, such as "single-copy" assays or "deep sequencing," are under development; however, because they are not currently in use in clinical settings, these tests are not addressed here.

Genotypic and Phenotypic Resistance Assays

Genotyping

Genotypic resistance assays detect mutations known to be associated with therapeutic failure by directly sequencing the genomic coding region of the protein inhibited by the ART drug. The genomic mutations, which may include substitutions, insertions, or deletions in the viral protein's coding region, are then compared with the known mutation(s) associated with the ART agent(s) clinical resistance profile.

Direct sequencing-based methods have been approved by the FDA, but the ViroSeq HIV-1 Genotyping System (Abbott Laboratories) is the only FDA-approved assay currently available. In addition, laboratory-developed ("in-house") genotyping assays are available through several commercial laboratories (e.g., GenoSure MG, Monogram/LabCorp). Advances in genotyping assays continue to evolve. Testing for resistance to integrase strand transfer inhibitors and fusion inhibitors is now available and should be considered when resistance to these classes of drugs is a concern, such as when transmission of resistant virus is suspected or when a patient fails a regimen that includes one of these drugs.

In the RNA-based genotyping assays, the HIV-1 RNA is isolated from a plasma specimen and reverse-transcribed to produce complementary DNA (cDNA). Specific regions of the HIV genome are amplified by PCR and sequenced. This sequence is then compared with that of a drug-sensitive ("wild-type") strain of HIV, and differences (mutations) present in the specimen sequence are noted. Computer software is generally used to perform this comparison and to predict whether resistance to specific drugs is likely to result from the particular combination of mutations detected in the virus. For most genotypic assays, this prediction is based on a set of rules derived from clinical observations, laboratory studies, and the advice of experts in the field. The actual prediction of resistance may vary from laboratory to laboratory for some combinations of mutations, depending on the interpretation algorithm used to define the rules.

Currently available RNA genotypic assays require a minimum viral load in the range of 500 to 2,000 copies/mL, depending on the assay, and generally require 2 weeks or less for results. DNA-based genotypic assays [White, et al. 2013] are becoming commercially available, such as the GenoSure Archive (Monogram/LabCorp). These assays use next-generation sequencing technology and are designed to overcome the limitations that commonly used RNA genotypic assays encounter in the presence of low-level viremia. In traditional genotypic assays, identification of resistance mutations is often not possible when viral load levels are below the lower limit of detection of a given assay; the lower limit may range from 500 to 1,000 copies/mL across available assays.

In DNA-based genotypic assays, integrated proviral DNA is extracted from HIV-infected cells, rather than from the circulating HIV in the plasma. Once the proviral HIV cell-associated DNA is extracted, the DNA is PCR-amplified, sequenced, and analyzed in analogous fashion to the older genotype RNA methodologies. The coding sequences for reverse transcriptase-, protease-, and integrase-targeted inhibitors are matched, as with the RNA-resistance genotype assays, with known resistance-associated mutations. The results are usually reported as "sensitive," "resistant," or "resistance possible" for a given ART agent. Although the clinical efficacy of the DNA-based genotype assays has not been fully validated, this technology can provide information on "archived," or noncirculating, viral resistance. It should not be assumed that all previous mutations will be detected. Although concordance across various studies using in-house, laboratory-developed tests was relatively high, the peripheral blood mononuclear cell (PBMC)-derived DNA assays often did not detect known previous mutations that had been documented with plasma-based RNA tests [Lubke, et al. 2015; Banks, et al. 2012; Delaugerre, et al. 2012]; the results could vary by class, with the manufacturer's own study showing lower concordance for protease mutations relative to those of reverse transcriptase in patients whose current viral load was undetectable [Toma, et al. 2015]. However, testing of archived proviral DNA may provide useful additional information when making decisions about switching ART regimens for those who are virologically suppressed or those with repeated low-level viremia, especially when historical data are unavailable [Booth, et al. 2014]. The commercial assay has not been validated for patients with viral loads >500 copies/mL, although some studies are investigating the assay's performance at higher viral loads, when wild-type virus may have replaced drug-resistant variants typically detected by RNA-based assays [Derache, et al. 2015]. The results obtained from archived proviral DNA testing should be used to supplement all other available information regarding treatment and resistance history.

Neither the RNA- nor DNA-based resistance assays can detect mutations associated with currently available HIV entry inhibitors (see below).

An older, algorithmic resistance profile based on genomic sequencing "virtual phenotype" (VIRCO, vircoTYPE) ceased to be clinically available in the United States as of December 2013. It compared the results of a patient's genotype and predicted potential drug sensitivities by comparing a patient's genotypic mutational profile with a database of laboratory and genotypic (sequence) and phenotypic (drug sensitivity) data and samples.

Phenotyping

Although still available, phenotypic assays generally do not add to the information provided by currently used genotypic assays. A phenotypic assay provides a direct measure of drug resistance and is analogous to antibiotic-susceptibility testing of bacteria. The currently available phenotypic assays use recombinant DNA methods to measure the ability of a patient's virus to grow in the presence of a drug. Therefore, results from a phenotypic test include the net effect of any and all resistance mutations.

In the phenotypic assay, HIV RNA is isolated from plasma and converted into cDNA, and the relevant region is amplified by PCR. This amplified material is inserted into a recombinant virus system whereby the susceptibility to different drugs can be tested. The result from the phenotypic assay is a value that defines the concentration of the drug required to reduce growth of the virus by 50% (IC50). The IC50 of the patient's virus is compared with the IC50 of a drug-sensitive (wild-type) reference virus, and the fold change is defined. If the IC50 of a person's virus is greater than that of the reference virus for a particular drug, then the person's virus has decreased sensitivity to the drug. The relative fold change helps determine whether the drug should still be included in the ART regimen or whether it should be removed entirely. Monogram Biosciences offers phenotypic resistance testing through clinical laboratories with the PhenoSense assay. Phenotypic assays have a minimum viral load requirement of 500 to 1,000 copies/mL and generally require 3 to 5 weeks for results.

Phenotypic assays are more technically complex, labor-intensive, and expensive than genotypic assays.

Technical Limitations of Genotypic and Phenotypic Assays

In addition to the minimum viral load requirements needed for amplification (generally at least 500 to 1,000 copies/mL) in genotypic or phenotypic RNA-based resistance assays, all resistance assays, including the DNA-based genotype, are limited by sampling bias. Acute infection is often established by a single progenitor virion [Cohen, et al. 2011], whereas in established HIV infection, HIV exists as a virus population comprising multiple genomic variants (see the NYSDOH AI guideline Diagnosis and Management of Acute HIV). Genotypic and phenotypic resistance assays are each more likely to detect the common viral variants and fail to identify the minor variants. Similarly, standard genotypic and phenotypic resistance testing performed on plasma specimens will not detect noncirculating, or archived, resistant virus (i.e., virus resistant to ART agents from previous regimens). If therapy is stopped altogether, the selective pressure from the ART agents suppressing the noncirculating virus is removed and a pan-sensitive or wild-type HIV population over time will begin to resurface and dominate the circulating virus population. When this occurs, the RNA-based genotypic and phenotypic resistance assays may fail to detect the ART-resistant virus, despite being present either as archived virus or at low levels. Although a DNA-based assay may have utility in these circumstances, clinical data are insufficient to recommend for or against its use in the patient care setting. For these reasons, all copies of the patient's previous genotype and/or phenotype resistance testing, along with the ART medication history, should be retained, and the information should be combined and used in constructing a subsequent ART regimen. Once resistance develops, it can be expected to persist indefinitely to that specific drug in archived form.

Another, more subtle, limitation is related to the level at which a virus is sensitive to a given ART agent. This "cutoff" may vary across assays, even when the same viral sample is used. Consultation with an experienced provider for interpretation of results is crucial.

Replicative Capacity

Replicative capacity information may be provided as an adjunct to phenotypic or combination genotypic-phenotypic resistance assays. The relative replicative capacity of the virus from the patient is calculated as the ratio of the patient-derived sequences to wild-type sequences. A ratio of less than 1 reflects a reduced replicative capacity as compared with that of the wild-type control. The full clinical value of this adjunctive information remains under investigation, and it has no clear clinical value at this time.

Co-Receptor Tropism Assay

Co-receptor tropism analysis determines which cellular co-receptor (CCR5 or CXCR4) is used by the HIV-infected individual's dominant viral population to gain access to host cells. The majority of acutely or recently infected individuals, including perinatally infected children, have a CCR5-tropic virus.

Because CCR5-tropic virus predominates early in HIV infection, whereas CXCR4-tropic virus is often present in late-stage disease, the CCR5 variant may be preferentially transmitted compared with CXCR4 variants. In patients with chronic HIV infection, a population of mixed CCR5- and CXCR4-tropic viruses, as well as dual-tropic viruses, may also be detected. The tropism of these viral populations is often referred to as dual/mixed or D/M HIV.

In the United States, most co-receptor tropism testing involves phenotypic assays. However, genotypic assays, which predict tropism based on algorithmic analysis of viral V3 sequencing binding site [McGovern, et al. 2010; Vandekerckhove, et al. 2009], are also available.

Although phenotypic testing can determine a viral population containing both tropisms, it is not sufficiently sensitive to differentiate between mixed and dual tropism. The Trofile (Monogram Biosciences) co-receptor tropism assay is an RNA-based test that permits phenotypic identification of CCR5, CXCR4 co-receptor, or dual/mixed-tropic (CXCR4/CCR5-utilizing) HIV-1 and should be used prior to the initiation of a receptor antagonist.

Another commercially available recombinant phenotypic assay for assessing HIV chemokine co-receptor tropism is the Phenoscript assay (Eurofins VIRalliance). In this assay, a 900-bp portion containing the patient's V1-V3 envelope virus is amplified and inserted into a HIV-1 vector lacking the corresponding V1-V3 section. The fully complemented HIV-1 is then able to produce virus that can be used to infect cell lines with either CCR5 or CXCR4 on their surfaces with a colorimetric readout. The results are reported in a similar manner as the Trofile (i.e., CCR5-trophic, CXCR4-trophic, or dual/mixed tropic). This assay has not been validated in a clinical trial setting or against the Trofile assay.

Two DNA-based tropism assays are also available. The HIV-1 Coreceptor Tropism, Proviral DNA (Quest Diagnostics) uses population sequencing of the HIV envelope V3 loop to detect the presence of CXCR4-tropic HIV-1 [Baumann, et al. 2009]. The Trofile DNA (Monogram Biosciences) uses the complete gp160 coding region to distinguish whether the HIV-1 population uses CCR5, CXCR4, or both (i.e., dual/mixed tropism) to gain entry into the cell. Unlike HIV-1 RNA-based assays, both the Trofile DNA and HIV-1 Coreceptor Tropism can detect virus in the setting of undetectable HIV-1 viral load levels and should be used when HIV RNA is beneath the lower limit recommended for RNA-based tropism assays (<1,000 copies/mL).

Resistance to the class of CCR5 co-receptor antagonists develops by two unrelated mechanisms. First, the patient's viral population shifts its co-receptor usage (i.e., uses CXCR4 exclusively or uses both CCR5 and CXCR4 receptors to gain entry into the cell). The current assays are not sufficiently sensitive to discriminate between mixed- or dual-tropic populations. The second method by which resistance to a CCR5 receptor antagonist may develop is by the virus mutating and binding to the CCR5 receptor with the drug antagonist still in place. This second method can be discerned by a flattening of the IC90curves in a phenotypic assay or potentially by genotypic analysis. Analysis by phenotypic assay is the preferred method for this purpose because genotypic data are more complex.

All Recommendations

☑ ALL RECOMMENDATIONS: HIV RESISTANCE ASSAYS

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- Clinicians should consult with an expert to interpret the results of resistance assays because such results are often complex. (A3)
  - The NYSDOH AI Clinical Education Initiative line is available for phone consultation: 866-637-2342.
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Notes:

a. Virologic failure is defined as >200 copies/mL. See the NYSDOH AI guideline Virologic and Immunologic Monitoring in HIV Care.

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Supplement: Guideline Development and Recommendation Ratings

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
Developer	New York State Department of Health AIDS Institute (NYSDOH AI) Clinical Guidelines Program
Funding Source	NYSDOH AI
Program Manager	Clinical Guidelines Program, Johns Hopkins University School of Medicine, Division of Infectious Diseases. See Program Leadership and Staff.
Mission	To produce and disseminate evidence-based, state-of-the-art clinical practice guidelines that establish uniform standards of care for practitioners who provide prevention or treatment of HIV, viral hepatitis, other sexually transmitted infections, and substance use disorders for adults throughout New York State in the wide array of settings in which those services are delivered.
Expert Committees	The NYSDOH AI Medical Director invites and appoints committees of clinical and public health experts from throughout NYS to ensure that the guidelines are practical, immediately applicable, and meet the needs of care providers and stakeholders in all major regions of NYS, all relevant clinical practice settings, key NYS agencies, and community service organizations. See Expert Committees.
Committee Structure	• Leadership: AI-appointed chair, vice chair(s), chair emeritus, clinical specialist(s), JHU Guidelines Program Director, AI Medical Director, AI Clinical Consultant, AVAC community advisor • Contributing members • Guideline writing groups: Lead author, coauthors if applicable, and all committee leaders
Conflicts of Interest Disclosure and Management	• Annual disclosure of financial relationships with commercial entities for the 12 months prior and upcoming is required of all individuals who work with the guidelines program, and includes disclosure for partners or spouses and primary professional affiliation. • The NYSDOH AI assesses all reported financial relationships to determine the potential for undue influence on guideline recommendations and, when indicated, denies participation in the program or formulates a plan to manage potential conflicts. Disclosures are listed for each committee member.
Evidence Collection and Review	• Literature search and review strategy is defined by the guideline lead author based on the defined scope of a new guideline or update. • A comprehensive literature search and review is conducted for a new guideline or an extensive update using PubMed, other pertinent databases of peer-reviewed literature, and relevant conference abstracts to establish the evidence base for guideline recommendations. • A targeted search and review to identify recently published evidence is conducted for guidelines published within the previous 3 years. • Title, abstract, and article reviews are performed by the lead author. The JHU editorial team collates evidence and creates and maintains an evidence table for each guideline.
Recommendation Development	• The lead author drafts recommendations to address the defined scope of the guideline based on available published data. • Writing group members review the draft recommendations and evidence and deliberate to revise, refine, and reach consensus on all recommendations. • When published data are not available, support for a recommendation may be based on the committee's expert opinion. • The writing group assigns a 2-part rating to each recommendation to indicate the strength of the recommendation and quality of the supporting evidence. The group reviews the evidence, deliberates, and may revise recommendations when required to reach consensus.

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
Review and Approval Process	• Following writing group approval, draft guidelines are reviewed by all contributors, program liaisons, and a volunteer reviewer from the AI Community Advisory Committee. • Recommendations must be approved by two-thirds of the full committee. If necessary to achieve consensus, the full committee is invited to deliberate, review the evidence, and revise recommendations when required. • Final approval by the committee chair and the NYSDOH AI Medical Director is required for publication.
External Reviewers	• External peer reviewers recognized for their experience and expertise review guidelines for accuracy, balance, clarity, and practicality and provide feedback. • Peer reviewers may include nationally known experts from outside of New York State.
Update Process	• JHU editorial staff ensure that each guideline is reviewed and determined to be current upon the 3-year anniversary of publication; guidelines that provide clinical recommendations in rapidly changing areas of practice may be reviewed annually. Published literature is surveilled to identify new evidence that may prompt changes to existing recommendations or development of new recommendations. • If changes in the standard of care, newly published studies, new drug approval, new drug-related warning, or a public health emergency indicate the need for immediate change to published guidelines, committee leadership will make recommendations and immediate updates. • All contributing committee members review and approve substantive changes to, additions to, or deletions of recommendations; JHU editorial staff track, summarize, and publish ongoing guideline changes.

Table S2: Recommendation Ratings and Definitions		
Strength	**Quality of Evidence**	
A: Strong B: Moderate C: Optional	1	Based on published results of at least 1 randomized clinical trial with clinical outcomes or validated laboratory endpoints.
	*	Based on either a self-evident conclusion; conclusive, published, in vitro data; or well-established practice that cannot be tested because ethics would preclude a clinical trial.
	2	Based on published results of at least 1 well-designed, nonrandomized clinical trial or observational cohort study with long-term clinical outcomes.
	2†	Extrapolated from published results of well-designed studies (including nonrandomized clinical trials) conducted in populations other than those specifically addressed by a recommendation. The source(s) of the extrapolated evidence and the rationale for the extrapolation are provided in the guideline text. One example would be results of studies conducted predominantly in a subpopulation (e.g., one gender) that the committee determines to be generalizable to the population under consideration in the guideline.
	3	Based on committee expert opinion, with rationale provided in the guideline text.