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

HIV Resistance Assays

Updates, Authorship, and Related Guidelines

Date of current publication	June 20, 2023
Highlights of changes, additions, and updates in the June 20, 2023 edition	Comprehensive update
Intended users	Clinicians in New York State who provide care for patients with HIV
Lead author	Steven M. Fine, MD, PhD
Writing group	Rona M. Vail, MD; Joseph P. McGowan, MD, FACP, FIDSA; Samuel T. Merrick, MD; Asa E. Radix, MD, MPH, PhD; Jessica Rodrigues, MS; Charles J. Gonzalez, MD; Christopher J. Hoffmann, MD, MPH
Author and writing group conflict of interest disclosures	None
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Developer and funder	New York State Department of Health AIDS Institute (NYSDOH AI)
Development process	See Supplement: Guideline Development and Recommendation Ratings
Related NYSDOH AI guidelines	• Diagnosis and Management of Acute HIV Infection • Second-Line ART After Treatment Failure or for Regimen Simplification • Use of Injectable CAB/RPV LA as Replacement ART in Virally Suppressed Adults • Virologic and Immunologic Monitoring in HIV Care

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Contents

Purpose of This Guideline

Clinical trials as well as ample anecdotal evidence support better antiretroviral therapy (ART) outcomes when HIV resistance testing is performed [Palella, et al. 2009; Baxter, et al. 2000]. This guideline was developed by the New York State Department of Health AIDS Institute (NYSDOH AI) as a resource for clinicians in New York State who provide care for patients with HIV. The guideline includes evidence-based recommendations for using HIV drug resistance testing to help improve ART outcomes. Toward that end, the goals of this guideline are to:

- Assist clinicians in determining when to order HIV drug resistance testing
- Inform clinicians about the different types, benefits, and limitations of currently available resistance assays
- Assist clinicians in choosing resistance testing to improve treatment outcomes

Note on "experienced" and "expert" HIV care providers: Throughout this guideline, when reference is made to "experienced HIV care provider" or "expert HIV care provider," those terms are referring to the following 2017 NYSDOH AI definitions:

- Experienced HIV care provider: Practitioners who have been accorded HIV Experienced Provider status by the American Academy of HIV Medicine or have met the HIV Medicine Association's definition of an experienced provider are eligible for designation as an HIV Experienced Provider in New York State. Nurse practitioners and licensed midwives who provide clinical care to individuals with HIV in collaboration with a physician may be considered HIV Experienced Providers as long as all other practice agreements are met (8 NYCRR 79-5:1; 10 NYCRR 85.36; 8 NYCRR 139-6900). Physician assistants who provide clinical care to individuals with HIV under the supervision of an HIV Specialist physician may also be considered HIV Experienced Providers (10 NYCRR 94.2)
- Expert HIV care provider: A provider with extensive experience in the management of complex patients with HIV.

Determining HIV Drug Resistance

☑ RECOMMENDATIONS

Determining HIV Drug Resistance

- Clinicians should consult with an expert HIV care provider to interpret the results of resistance assays because such results can be complex [a]. (A3)
- Clinicians should perform genotypic resistance testing that includes the protease (A2), reverse transcriptase (A2), and integrase genes (B2) at baseline.
- For a patient experiencing treatment failure [b] or incomplete viral suppression while taking oral ART, the clinician should perform resistance testing while the patient is still on therapy but no later than 4 weeks after stopping ART, to minimize the rapid return of wild-type virus when the selective pressure from ART is removed. (A2)
- For patients receiving CAB/RPV LA, the clinician should obtain resistance testing while the patient is still on or as soon as possible after they have discontinued effective ART, although the time limit for obtaining useful resistance information after discontinuation of CAB/RPV LA is unknown. (A3)
- Clinicians should perform coreceptor tropism testing before initiating a CCR5 antagonist. (A1)
- For patients whose treatment with a fusion inhibitor has failed, the clinician should test for fusion inhibitor resistance as a supplement to other genotypic resistance testing. (A2)

Abbreviations: ART, antiretroviral therapy; CAB/RPV LA, injectable long-acting cabotegravir and rilpivirine.

Notes:

a. The NYSDOH AI Clinical Education Initiative line is available for phone consultation: 1-866-637-2342.
b. Virologic failure is defined as an HIV RNA level (viral load) >200 copies/mL.

Interpreting HIV resistance assays can be challenging but is necessary to craft a reliably suppressive ART regimen. HIV replicates via reverse transcriptase, which lacks proofreading capacity and is susceptible to mutations during viral replication (i.e., changes in its genetic sequence). Random mutations coupled with the selective pressure of subtherapeutic drug levels can lead to selection of drug-resistant HIV strains. Once established, drug-resistant HIV strains may continue to replicate even in patients who are adherent to their ART regimens. Thus, if a patient's HIV viral load remains detectable and they are known to be adherent to their ART regimen, resistance testing should be performed [Kantor and Gupta 2023; Wood and Stekler 2022].

→ KEY POINT

- Resistance testing is recommended when incompletely suppressive ART is interrupted. Because of the rapid return of wild-type virus without selective pressure from ART [Devereux, et al. 1999], testing is preferred before treatment is stopped. If the patient has already stopped ART, testing should be performed as soon as is practical and, if possible, no more than 4 weeks after cessation, before the return of wild-type virus. If resistance testing is performed more than 4 weeks after ART cessation, some mutations may no longer be detected by the assay and clinically relevant mutations may not be recognized. For patients who were receiving CAB/RPV LA, resistance testing should be done as soon as possible but may be useful any time after cessation of ART.

The most commonly used ART drugs are targeted to inhibit the activity of 3 specific viral enzymes: protease, reverse transcriptase, and integrase. Mutations have been identified that interfere with the ability of 1 or more ART agents to inhibit viral replication, thus rendering the virus resistant to the drug(s). HIV resistance mutations for less commonly used ART drugs that target fusion, viral entry, and capsid formation have also been identified.

As new ART drugs are developed and knowledge about the clinical significance of resistance mutations evolves, consulting the following resources for information on drug resistance mutations and resistance testing may be useful:

- Stanford University HIV Drug Resistance Database
- International Antiviral Society-USA 2022 Update of the Drug Resistance Mutations in HIV-1
- HIV Resistance Response Database Initiative
- Los Alamos National Laboratory HIV Databases
- HIV French Resistance Database

HIV resistance testing is generally accomplished through genotyping, which directly sequences the HIV genome to detect mutations. Phenotyping, which measures HIV replication in the presence of drug, may also be available, but genotyping is generally preferred. Single-copy and deep sequencing technologies that analyze proviral DNA are also available in special circumstances to detect mutations present at very low levels or in virally suppressed patients. Each of these options for HIV drug resistance testing is discussed in the guideline section Genotypic and Phenotypic Resistance Assays.

Insurance coverage of drug resistance testing: In New York State, health plans may impose annual limits on the number of resistance tests that can be performed for a given patient, and some plans may require prior authorization. Refer to the patient's specific insurance plan regarding frequency, annual limits, and whether prior authorization is required for any genotypic and phenotypic HIV resistance tests.

Genotypic and Phenotypic Resistance Assays

Genotyping

Sanger and next-generation sequencing: Genotypic resistance assays directly sequence the protein coding regions of genes for viral reverse transcriptase, protease, and integrase. The mutations identified via genotyping are compared with a list of mutations that are known to confer resistance to current antiretroviral medications (ARVs), generating a list of ARVs that are likely to remain active against virus versus those to which resistance is likely.

When interpreting resistance test results, it is important to include mutations from all prior resistance tests whenever available because these mutations are thought to be cumulative in most cases. The resources noted in the guideline section Determining HIV Drug Resistance may be helpful when interpreting genotypic resistance testing results, but consultation with an expert HIV care provider is also advised.

Currently, there are 2 methods for sequencing for genotypic drug resistance:

- Standard genotype sequencing (Sanger or population sequencing) derives a consensus sequence for each gene in the sample and is useful for detecting mutations that are dominant or are present at high levels in the sample. Sanger sequencing is still used at most sites.
- Next-generation sequencing (NGS) [FDA 2019], which is beginning to replace standard genotyping technology at some sites, allows for simultaneous sequencing of thousands of individual genomes and can detect mutations at far lower copy numbers than Sanger sequencing. NGS may detect low copy number mutants in patients who have stopped antiretroviral therapy (ART) for some time and in whom the majority of replicating HIV has been replaced by wild-type sequences.

Genotypic resistance assays generally require that an individual have an HIV RNA level (viral load) of >500 copies/mL, but individual laboratories may have their own minimum viral load requirements.

Proviral DNA-based (archive) genotypic assays: NGS technology can also be used to sequence HIV proviral DNA, which is nonreplicating, in patients who may wish to change their suppressive ART regimen. With these assays, integrated proviral DNA is extracted from within the cell and the coding sequences for reverse transcriptase, protease, and integrase are sequenced as they are in the standard genotypic assays, to predict whether a virus derived from these proviral sequences would be resistant to ARVs [Chu, et al. 2022].

For patients whose previous ART regimens failed and whose viral load is currently undetectable, and for whom past genotype test results are not available, proviral DNA-based genotypic assays may detect "archived" mutations; these assays are often referred to as "archive genotypes." Archive genotypes may be useful for patients with a complex ART history or when previous resistance test results are unavailable, but the clinical significance of archived mutations is not yet fully understood.

One potential use of archive genotypes is for patients who are currently on a suppressive oral ART regimen but are considering switching to CAB/RPV LA. In this situation, the archive genotype might identify non-nucleoside reverse transcriptase inhibitor resistance-associated mutations, which are known to increase the risk of treatment failure.

Because the availability of HIV genotypic resistance testing is evolving, it is best to check with local laboratories to see which tests are currently available and for specific sample requirements.

Phenotyping

Although still available, phenotypic assays generally do not add to the information provided by currently used genotypic assays. If a patient has experienced multiple regimen failures and has a large number of ARV resistance mutations, which is rare, a phenotypic assay may be used to help devise an effective ART regimen. A phenotypic assay provides a direct measure of drug resistance and is analogous to antibiotic-susceptibility testing of bacteria. Currently available phenotypic assays use recombinant DNA methods to measure the ability of a patient's virus to replicate in the presence of a drug. Therefore, results from a phenotypic test include the net effect of multiple resistance mutations.

In the phenotypic assay, HIV RNA is isolated from plasma and converted into cDNA, and the relevant region is amplified by polymerase chain reaction (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 an individual's virus is greater than that of the reference virus for a particular drug, then the individual'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. A phenotypic test for resistance to enfuvirtide that may help predict the drug's activity is available through Labcorp-Monogram Biosciences. Phenotypic assays have a minimum viral load requirement of 500 to 1,000 copies/mL and results are generally available in 3 to 5 weeks.

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 standard genotypic or phenotypic RNA-based resistance assays, all resistance assays, including the DNA-based genotype, are limited by sampling bias. Acute HIV 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. 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 virus containing resistance-associated mutations (i.e., nonreplicating virus present within cells as proviral DNA). If ART is interrupted, the selective pressure from the ART is removed and generally favors replication of wild-type HIV strains, which eventually will predominate. When this occurs, the RNA-based genotypic and phenotypic resistance assays may fail to detect low-level ART-resistant virus. A DNA-based proviral assay may have utility in these circumstances; see the NYSDOH AI guideline Second-Line ART After Treatment Failure or for Regimen Simplification > Identifying and Managing Virologic Failure for more information. For these reasons, all previous genotype and phenotype resistance testing results, along with the patient's ART medication history, should be retained, and this 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.

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 in a patient is calculated as the ratio of patient-derived sequences to wild-type sequences. A ratio of less than 1 reflects a reduced replicative capacity 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.

Coreceptor Tropism Assays

CCR5 and CXCR4 tropism: HIV-1 binds to a chemokine receptor, either CCR5 or CXCR4, as a coreceptor to enter CD4 cells. Viruses that use CCR5 are referred to as CCR5 (or R5) tropic and those that use CXCR4 as CXCR4 (or X4) tropic. The antiretroviral drug maraviroc blocks HIV from binding to CCR5. Therefore, if use of maraviroc is being considered, it is essential to determine that no CXCR4-tropic virus is present. Both genotypic and phenotypic assays measure CCR5 and CXCR4

tropism. Generally, the choice of which assay to use will be determined by which assay is locally available (see discussion below). CCR5-tropic virus predominates early in HIV infection, whereas CXCR4-tropic virus is often present in late-stage disease, and virus that uses CCR5 may be preferentially transmitted compared with CXCR4 variants. The majority of individuals with acute or recent HIV infection, including perinatally infected children, have CCR5-tropic virus.

In patients with chronic HIV infection, a population of mixed CCR5- and CXCR4-tropic viruses, as well as dual-tropic (able to use either CCR5 or CXCR4 as coreceptor) viruses, may be detected. The tropism of these viral populations is often referred to as dual/mixed or D/M HIV.

Resistance to CCR5 coreceptor antagonists develops by 2 unrelated mechanisms. The first, and most common, occurs when a patient's viral population shifts its coreceptor usage from CCR5 to CXCR4. In this scenario, both CXCR4- and dual-tropic virus will be resistant to CCR5 antagonists. The second, and more rare, occurs when a virus develops the ability to continue to use CCR5 as its coreceptor despite the binding of the CCR5 antagonist. This latter type of resistance will be detected using phenotypic resistance assays.

Coreceptor tropism testing: Coreceptor tropism should be tested whenever the use of a CCR5 entry inhibitor (e.g., maraviroc) is being considered or when a patient's CCR5 antagonist-containing regimen is failing despite excellent adherence. Coreceptor testing can either be phenotypic or genotypic, but phenotypic tropism testing is most commonly used.

The phenotypic coreceptor tropism assay (Trofile) measures inhibition of viral replication by CCR5 antagonists in vitro. If the HIV viral load is undetectable, a DNA tropism assay (Trofile DNA) can be used instead. In the DNA tropism assay, proviral DNA is first extracted from peripheral blood mononuclear cells (PMBCs) and then used in the phenotypic assay to predict whether CXCR4- or dual-tropic sequences are present.

Genotypic coreceptor tropism assays predict tropism based on sequencing of the V3 region of the HIV *env* gene [Vandekerckhove, et al. 2011; McGovern, et al. 2010]. A standard genotyping assay can be performed if the HIV viral load is >1,000 copies/mL, and a proviral DNA genotype is used if the viral load is undetectable. These assays are currently available through Quest Diagnostics. Studies of genotypic assays suggest a lower sensitivity than phenotypic assays for finding CXCR4- or dual-tropic virus, but clinical trials suggest these assays are good at predicting response to maraviroc. No trial has compared phenotypic and genotypic coreceptor tropism assays head to head, so the best use of genotypic assays is still unclear. At this time, most tropism testing in the United States is performed using phenotypic assays.

When use of a CCR5 antagonist (i.e., maraviroc) is being considered or a CCR5 antagonist-containing ART regimen is failing, either the standard phenotypic (if viral load is ≥1,000 copies/mL) or genotypic (if viral load is <1,000 copies/mL) coreceptor tropism assay should be used.

All Recommendations

☑ ALL RECOMMENDATIONS: HIV RESISTANCE ASSAYS

Determining HIV Drug Resistance

- Clinicians should consult with an expert HIV care provider to interpret the results of resistance assays because such results can be complex [a]. (A3)
- Clinicians should perform genotypic resistance testing that includes the protease (A2), reverse transcriptase (A2), and integrase genes (B2) at baseline.
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- For patients receiving CAB/RPV LA, the clinician should obtain resistance testing while the patient is still on or as soon as possible after they have discontinued effective ART, although the time limit for obtaining useful resistance information after discontinuation of CAB/RPV LA is unknown. (A3)
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Abbreviations: ART, antiretroviral therapy; CAB/RPV LA, injectable long-acting cabotegravir and rilpivirine.

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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 New York State to ensure that the guidelines are practical, immediately applicable, and meet the needs of care providers and stakeholders in all major regions of New York State, all relevant clinical practice settings, key New York State agencies, and community service organizations.
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
Disclosure and management of conflicts of interest	• 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.

Table S1: Guideline Development: New York State Department of Health AIDS Institute Clinical Guidelines Program	
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.
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. • Final approval by the committee chair and the NYSDOH AI Medical Director is required for publication.
External reviews	• External review of each guideline is invited at the developer's discretion. • External reviewers recognized for their experience and expertise review guidelines for accuracy, balance, clarity, and practicality and provide feedback.
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 and will invite full committee review as indicated.

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.