

A NO-NONSENSE GUIDE TO HIV DRUG RESISTANCE TESTING

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CONTENTS

Introduction	1
What is drug resistance?	2
What's with all the strange numbers?	4
How is drug resistance measured?	4
Can you explain more about genotypic tests?	5
What are the advantages and limitations of genotypic resistance testing?	7
What about phenotypic testing? How does it work?	9
What are the advantages and limitations of phenotypic resistance testing?	11
Can phenotypic and genotypic testing be used together?	12
How can these tests help decide on an initial treatment regimen?	13
How can these tests help in choosing a new treatment regimen when an old one fails?	13
Would it be necessary to switch every drug?	14
Do most experts recommend these tests?	15
Where can I get these tests?	15
Will insurance cover the cost of resistance testing?	16
Glossary	17
Resource Guide	23

INTRODUCTION

Never before have so many treatment options been available to people living with HIV. At least 20 anti-HIV drugs are available now, which can be combined in a number of different ways. For HIV-positive people, the use of these drugs has translated into health benefits that, years ago, many experts never thought possible — the possibility of living a healthy life with HIV for many years.

Of course, the availability of new, powerful anti-HIV drugs is just one example of how things have improved in recent years. There are also laboratory tests that have been designed specifically to help HIV-positive people and their healthcare providers figure out when — and how best — to use these anti-HIV drugs. The latest tests to become available are HIV drug resistance tests, which have become standard medical procedures for people living with the virus.

HIV drug resistance tests are proving to be much like viral load tests when those first appeared. In 1996 there was a lot of hope — and confusion — regarding viral load technology and how it could be used to help people living with HIV and to enable their healthcare providers to make better treatment decisions. Much of the same optimism and confusion can be found today with respect to HIV drug resistance tests.

For people infected with the virus, drug resistance can render drugs less effective or even completely ineffective, thus significantly reducing treatment options.

This handbook explains how HIV drug resistance tests work and what role they can play in optimizing treatment decisions. The question-and-answer (Q&A) format is designed to answer some of the most frequently asked questions about the tests. Also included is a glossary of terms used in this publication and elsewhere.

What is drug resistance?

HIV drug resistance refers to a reduction in the ability of a particular drug or combination of drugs to block reproduction or “replication” of HIV. For people infected with the virus, drug resistance can render drugs less effective or even completely ineffective, thus significantly reducing treatment options.

Resistance typically occurs as a result of changes — called mutations — in HIV’s genetic structure (RNA). Mutations of RNA lead to alterations in certain proteins, most commonly enzymes, that regulate the production of infectious virus. Mutations are especially common in HIV, as this virus reproduces at an extraordinary rate and does not contain the proteins needed to correct mistakes made during copying of the genetic material. HIV relies on many enzymes — such as reverse transcriptase,

Once the wild-type virus is destroyed by the offending drug, the drug-resistant form can reproduce and eventually become the dominant strain.

integrase, and protease — to replicate inside a human cell. If a mutation of a single site in the reverse transcriptase gene occurs, the change will remain with the virus as long as it replicates or until another copying error alters its form yet again. Some mutations cause the virus to become so weak that it cannot replicate effectively; other mutations may cause the virus to become even more virulent.

Antiretroviral drugs, generally speaking, disrupt the HIV enzyme's ability for genetic copying, or making virus that can infect other cells. In a person who takes antiretroviral drugs, most of the HIV are killed or prevented from multiplying further. As a result of random mutations that occur on a daily basis, however, some strains of HIV are naturally resistant to the presence of such drugs. That is why treatment with monotherapy (a single antiretroviral drug) is destined to fail.

In essence, drug-resistant mutations are an example of Charles Darwin's principles of evolution. At first, these particular strains of HIV are fewer than the natural and most powerful form of HIV — called the “wild type” — that dominates the population. However, once the

wild-type virus is destroyed by the offending drug, the drug-resistant form can reproduce and eventually become the dominant strain, sometimes within as little as a few days. Thus, only the “fittest” survive, as in the Darwinian understanding of natural selection.

What's with all the strange numbers?

A lot of medical information available to both healthcare providers and people living with HIV frequently concerns specific mutations. One example is the classic Efavir (lamivudine; 3TC) mutation: M184V.

The 184 refers to the amino acid position on the reverse transcriptase enzyme. The M — which stands for methionine — is the amino acid at position 184 of a wild-type (drug-sensitive) virus' reverse transcriptase enzyme. The V — which stands for valine — refers to the mutation that results in drug resistance. In other words, the amino acid methionine at position 184 has been replaced by a valine. This change thus prevents an antiretroviral drug from binding with the enzyme to prevent the virus from replicating.

How is drug resistance measured?

Over the past 10 years, a significant number of breakthroughs have been made in understanding the power of antiretroviral drugs against HIV. With the development and availability of viral load tests, we can

determine from a blood sample how much virus is replicating in the body. If viral load increases substantially while a person is on a combination of antiretroviral drugs, the most likely culprit is drug resistance.

Unfortunately, viral load tests cannot determine whether or not HIV is resistant to one drug in particular or the entire combination. Moreover, in a person with drug-resistant HIV, these tests cannot determine which drug or combination of drugs is likely to be the most effective in the future.

Two general approaches are now used for measuring resistance to HIV drugs. The first is called genotypic testing. Genotypic tests can help determine whether specific genetic mutations are causing drug resistance and drug failure. The second method, called phenotypic resistance testing, is a more direct measure of resistance and, more specifically, of the sensitivity of a person's HIV to particular antiretroviral drugs.

Can you explain more about genotypic tests?

Genotypic resistance testing examines HIV taken from a patient, looking for the presence of specific genetic mutations that are known to cause resistance to certain drugs. For example, it has been well documented by researchers that Efavirenz is not effective against strains of HIV that have a mutation at a particular position — known as M184V — in their reverse transcriptase enzyme (see “What’s with all the strange numbers?”).

Genotypic resistance testing examines HIV taken from a patient, looking for the presence of specific genetic mutations that are known to cause resistance to certain drugs.

If a genotypic resistance test discovers a mutation at position M184V, chances are that the person's HIV is resistant to Efavirenz and is not likely to respond to the drug.

For many drugs — for example, Zidovudine (AZT) and protease inhibitors — complex patterns of mutations are required for resistance to occur. Interpretation of these complex patterns can be difficult and incomplete in determining whether or not the virus is sensitive to particular drugs.

A number of laboratories in the United States and Europe offer genotypic resistance testing. The most common method of testing uses PCR technology to make many copies of, or “amplify,” the HIV genetic material. Once amplified, the genetic sequences of particular viral enzymes — such as reverse transcriptase and protease — can be examined carefully for mutations at any of their positions. Depending on the type and number of mutations found, the laboratory may be able to determine whether someone has developed resistance to a specific drug, since almost all drugs follow a set pattern of mutations. Some drugs have a single pattern of mutation, but others have complex and unpredictable patterns.

For genotypic tests to be accurate, they generally require the use of a blood sample from a person who is

actively taking antiretroviral medication and has a viral load higher than 1,000 copies/mL. In the absence of therapy, the wild-type virus may outgrow the mutant virus. In turn, the results may not show any drug-resistant mutations; but the mutant virus may still remain at very low numbers in the person's body and may quickly increase when therapy with the same drugs is restarted.

What are the advantages and limitations of genotypic resistance testing?

Genotypic resistance testing has a few advantages over phenotypic testing, most notably the relative simplicity and speed with which the test can be performed. The testing can take as little as a few days to complete, and because it is less complex, it is somewhat cheaper to perform.

Still, some limitations of genotypic resistance testing are worth noting. Most important, it may be difficult to translate the results of a genotypic resistance test into a meaningful conclusion about the resistance of the virus to drugs. We have learned a lot about the various genetic mutations that result in antiretroviral drug resistance, but it is also true that we don't know everything about these mutations. It is possible that we have both over- and

underestimated the importance of specific mutations and their role in causing drug resistance. Moreover, some genetic mutations have yet to be identified by researchers. Such is the case with older drugs like Videx (didanosine; ddl) and Zerit (stavudine; d4T), and newer drugs like Kaletra (lopinavir/ritonavir) and Viread (tenofovir DF). In people who take these drugs, resistance certainly does occur. However, researchers are continuing to learn more about the mutations that cause HIV to become resistant to these drugs.

This may also be the case with Retrovir and Epivir. For example, a genotypic resistance test may demonstrate that a person's HIV has several genetic mutations that confer resistance to Retrovir and to Epivir. However, the Epivir mutation M184V is known to increase the sensitivity of HIV to Retrovir and thus can "balance out" the effect of Retrovir mutations. A genotypic resistance test may not accurately reflect the degree of Retrovir resistance in this case.

There is another disadvantage of genotypic resistance testing. The technology used to perform the test does not normally evaluate the genetic structure of small HIV populations found in a blood sample. These small populations — called subpopulations — can contain genetic mutations that do confer drug resistance. For example, there might be a subpopulation of HIV that contains a mutation at position M184V. Unless this particular strain accounts for more than 10% to 20% of the HIV population found in a blood sample, chances are that it will not be recognized.

It is possible that we have both over- and underestimated the importance of specific mutations and their role in causing drug resistance.

What about phenotypic testing? How does it work?

Unlike genotypic testing, which looks for particular genetic mutations that confer drug resistance, phenotypic testing directly measures the actual sensitivity of a patient's HIV to particular drugs. To do this, phenotypic tests measure the concentration of a drug required to inhibit HIV replication in the test tube by a defined amount such as 50% or 95%. This is called IC_{50} or IC_{95} ; IC stands for "inhibitory concentration." Interestingly, this is the method used by researchers to determine whether a drug might be effective against HIV before using it in human clinical trials.

Phenotypic resistance testing of HIV is conceptually very similar to methods used to measure antibiotic resistance in bacteria. Sometimes, as in the case of tuberculosis, phenotypic testing of the bacterium determines whether there are any drugs to which it will not respond. This procedure has dramatically improved the ability of healthcare providers to treat such infections effectively. Phenotypic testing has now also become a feasible method for measuring resistance to antiviral drugs, as a few companies have developed new tests in which the key portions of HIV genetic material are "inserted" into the shells of laboratory-derived reference strains of HIV. Sensitivity testing is then performed relatively quickly and under more standard conditions.

Phenotypic testing directly measures the actual sensitivity of HIV to particular drugs.

One of the developers of the newer phenotypic technology takes things a step further by replacing part of the HIV shells with a gene for the enzyme luciferase. With this enzyme in place, the infected cells glow when the virus successfully reproduces in the laboratory test. Using light sensors, the laboratory can then measure the amount of light produced by the virus in the presence or absence of drugs. Depending on the amount of light produced — when compared to that of a wild-type strain of HIV — the laboratory can determine both the IC_{50} and IC_{95} of the drug.

The concentration of drug necessary to inhibit virus replication is expressed in units called nanomoles (nM). For example, if the IC_{50} of the wild-type virus is 100 nM and that of the test virus is 400 nM, the test virus is considered to be four-fold resistant to the drug being tested. In other words, HIV in the patient is four-fold less sensitive to the drug. Similar to genotypic tests, phenotypic resistance tests generally can be performed on samples with viral loads around 1,000 copies. Also like genotypic testing, it is recommended that patients be taking antiretroviral therapies at the time of the test.

Using both genotypic and phenotypic tests in combination offers more comprehensive information about drug resistance.

What are the advantages and limitations of phenotypic resistance testing?

The results of phenotypic tests can be easier to interpret than genotypic tests. Because phenotypic testing directly measures the sensitivity of the virus to particular drugs, many researchers and healthcare providers have suggested that these tests are more comprehensive and trustworthy than genotypic tests. Phenotypic resistance testing procedures are relatively complex, however, and can take longer than genotypic tests to produce accurate results — from 10 days to several weeks. The intricacy of these tests also makes them more expensive.

Like genotypic testing, phenotypic tests are limited in their ability to assess the drug sensitivity of HIV subpopulations. Again, this may prevent such tests from producing an accurate estimate of HIV's ability to respond — especially over long periods of time — to specific antiretroviral drugs.

Another challenge with phenotypic testing is understanding what level of resistance translates into a failure of treatment. For example, a five-, six-, or seven-fold reduction in the sensitivity of HIV to a protease inhibitor may be considered “moderate.” But is there a

significant difference between a five-fold reduction and a seven-fold reduction? Research in drug resistance, specifically in clinical trials of antiretroviral drugs, is ongoing to improve the understanding of these levels.

Can phenotypic and genotypic testing be used together?

Yes. Using both tests in combination offers more comprehensive information about drug resistance. Having results from both tests helps address some of the limitations of using each test individually and represents a more complete picture of the virus. Using the tests together is usually most useful after failure of a second treatment regimen.

Both tests are slightly limited in their ability to measure subpopulations of potentially drug-resistant HIV. Using both tests together may not necessarily overcome this obstacle. However, as technology improves, this problem will be diminished. Some companies have already discovered new ways to make tests more sensitive, increasing the usefulness of resistance testing at all stages of treatment.

How can these tests help decide on an initial treatment regimen?

Because drug-resistant HIV can still be passed from one person to another, resistance testing can be used to evaluate drug resistance in recently infected or newly diagnosed people. The results can help a healthcare provider work with an individual to put together an initial treatment regimen that is more likely to be effective for a longer period of time and that avoids using drugs that the individual may already be resistant to.

Recent research has found that HIV is either partially or fully resistant to one or more of the available anti-HIV drugs in 20% to 30% of newly infected people. In addition, the latest studies have shown that drug-resistant virus can linger or persist in a newly infected person for up to three years without treatment initiation.

As a result, recently released federal guidelines now recommend that resistance testing be used to select an initial treatment regimen.

How can these tests help in choosing a new treatment regimen when an old one fails?

Drug failure is loosely defined as an increase in viral load, a decrease in T-cell counts, and/or signs of physical disease progression in people who are on combination antiretroviral therapy. Although drug failure can also be used to describe the experience of people who must stop their medication because of intolerable side effects,

it is most often associated with the presence of genetic mutations and decreased drug sensitivity.

When a person no longer benefits from his or her HIV treatment and viral load is increasing, drug resistance testing helps determine which drugs are no longer effective and which ones are still likely to work. This allows healthcare providers to develop a new treatment plan that will be more effective.

Would it be necessary to switch every drug?

Several years ago, it was recommended that anyone who appeared to be failing a particular combination should switch to an entirely new batch of drugs. This, of course, was frustrating, as many HIV-positive people did not have three or more untried drugs from which to choose. It was also a potentially wasteful decision for those who did have several remaining options. Why toss out a drug that may, in fact, still be effective against HIV? With drug resistance testing, it is possible to weed out the ineffective drug or drugs in a given combination and keep using the drugs that are still working.

With drug resistance testing, it might be possible to weed out the ineffective drug or drugs in a given combination.

Drug resistance tests have become accepted as standard of care in HIV management. Virtually all insurance companies and other third-party reimbursement programs will pay for the tests.

Do most experts recommend these tests?

Yes. Two important groups of medical experts now recommend that drug resistance tests be used in helping HIV-positive people plan their treatment regimens, especially if a switch in therapies is needed. One group that recommends drug resistance testing is the US Department of Health and Human Services (DHHS), a branch of the federal government that oversees public health.

A second group that recommends these tests is the International AIDS Society-USA (IAS-USA), a private medical organization made up of many leading HIV/AIDS experts in the United States and elsewhere.

Where can I get these tests?

Resistance tests are prescribed by healthcare professionals and are performed by various clinical laboratories and hospitals. Your healthcare provider usually orders these tests through the laboratory he or she uses to check your viral load and T-cells, or directly through a resistance testing laboratory.

Will insurance cover the cost of resistance testing?

Yes. However, insurance coverage varies depending on where you live. Medicaid, Medicare, ADAP and most private insurance providers have coverage policies for drug resistance testing. Your healthcare provider or local AIDS service organization can help obtain information about the availability of government-funded programs to pay for resistance tests.

Because drug resistance tests have been recommended by the DHHS and IAS-USA and have become accepted as standard of care in HIV management, it is likely that virtually all insurance companies and other third-party reimbursement programs will pay for the tests.

GLOSSARY

Adherence: The degree to which a patient exactly follows a prescribed treatment regimen. Poor adherence may negatively impact a drug's effectiveness. Compliance is an alternate term.

Amino Acid: A nitrogen-containing molecule that serves as a building block for proteins, including enzymes, muscles, and structural molecules.

Antiretroviral: A substance that stops or suppresses the activity of a retrovirus such as HIV, AZT, ddC, ddI and d4T are examples of antiretroviral drugs.

Assay: A test.

Cross-Resistance: The phenomenon by which HIV and other disease-causing organisms become resistant to more than one drug after a single therapy. For example, people who develop resistance from taking one protease inhibitor are likely to be cross-resistant to other drugs in the same class.

DNA (Deoxyribonucleic Acid): A double-stranded molecule that makes up the chromosomes in the center of a cell and carries information in the form of genes.

Enzyme: A cellular protein whose shape allows it to hold together several other molecules in close proximity to each other. Enzymes also induce chemical reactions in other substances.

Gene: A unit of DNA in the chromosomes that determines the structure of a specific protein or enzyme. Genes regulate the metabolism of individual cells and the development and specialization of body cells and tissues.

Genotype: The genetic makeup of an individual organism, determined by the sequence of nucleotides in its genes. *See also* Phenotype.

Genotypic Assay: A blood test that determines the genetic sequences of an organism. Frequently performed in HIV to establish whether certain mutations conferring drug resistance are present. *See also* Phenotypic Assay; Resistance.

IC (Inhibitory Concentration): The amount of drug in the blood needed to suppress the reproduction of a disease-causing microorganism such as HIV. For example, IC_{95} is the drug level needed to block 95% of HIV's normal replication; IC_{50} is the drug level needed to block 50% of HIV's normal replication.

NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor): A member of a class of compounds — including efavirenz, delavirdine, and nevirapine — that act directly to combine with and block the action of HIV's reverse transcriptase to prevent viral RNA from

being converted into DNA and integrated into the uninfected cell's nucleus. NNRTIs have suffered from HIV's ability to mutate rapidly and become resistant to their effects.

Nucleoside: The molecular units that serve as the building blocks of DNA and RNA, the genetic material found in living organisms.

PCR (Polymerase Chain Reaction): A technique that amplifies DNA. PCR is a critical part of tests for viral load, genotyping, and phenotyping.

Phenotype: The functional capabilities and outward appearance of a microorganism. It is the physical expression of the genotype.

Phenotypic Assay: A test that measures the sensitivity of HIV to specific antiretroviral drugs. It is considered a more direct measure of HIV drug resistance than genotypic tests. *See also* Genotypic Assay.

Protease: An enzyme that triggers the breakdown of proteins. HIV's protease enzyme breaks apart long strands of viral protein into the separate proteins constituting the viral core and the enzymes it contains. HIV protease acts as new virus particles are budding off a cell membrane.

Protease Inhibitor (PI): A drug that binds to and blocks HIV protease from working, thus preventing the production of new functional viral particles. Examples include saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir.

Protein: Large molecules made up of long sequences of amino acids. Some hormones and all enzymes and cellular structural components are proteins.

Resistance: Reduction in an organism's sensitivity to a particular drug. Resistance is thought to result mainly from a genetic mutation. In HIV, such mutations can change the structure of viral enzymes and proteins so that an antiviral drug can no longer bind with them. Resistance detected by searching a pathogen's genetic makeup for mutations believed to confer lower susceptibility is called genotypic resistance. Resistance found by successfully growing laboratory cultures of the pathogen in the presence of a drug is called phenotypic resistance.

RNA (Ribonucleic Acid): A single-stranded molecule composed of nucleotide sequences. It is similar in basic structure to half of the double-stranded DNA. In cells, RNA transmits the code from the DNA-based genes that instructs the cells' chemical machinery to produce structural proteins and enzymes. In retroviruses, RNA is the sole repository of the viral genes.

Sensitivity: The degree to which an organism is affected by a drug. *See also* Resistance.

Wild-Type Virus: Naturally occurring HIV with an optimal genetic makeup and no laboratory-induced mutational defects. The term also refers to HIV that has not been exposed to antiviral drugs and therefore has not accumulated mutations conferring drug resistance.

RESOURCE GUIDE

To learn more about HIV drug resistance testing, contact one of the national AIDS/HIV referral organizations listed below. These organizations can either provide information directly or refer you to a local AIDS/HIV service organization that specializes in healthcare counseling. Moreover, several of the listed organizations produce free or low-cost treatment-related publications for people with AIDS/HIV.

National STD and AIDS Hotline

1-800-342-AIDS

Provides information and referrals on all aspects of HIV/AIDS treatment and services. Open 24 hours a day, 365 days a year.

AIDS Education Global Information System (AEGIS)

www.aegis.org

Provides comprehensive, up-to-date news and information about HIV/AIDS from around the world.

AIDSmeds.com

www.AIDSmeds.com

Provides complete and up-to-date information about HIV treatment and topics surrounding treatment, including resistance testing, treatment failure, structured treatment interruptions, and more.

The Body

www.thebody.com

Provides a variety of up-to-date information about HIV and AIDS, including general HIV education, treatment, policy and activism, and more.

HIV and Hepatitis.com

www.HIVandHepatitis.com

Provides accurate and timely information about treatment for HIV/AIDS, chronic hepatitis B and hepatitis C, and co-infection with HIV/HCV and HIV/HBV.

HIV Drug Resistance.com

www.HIVDrugResistance.com

Provides a comprehensive overview of drug resistance, as well as resources for working with healthcare providers and insurance carriers to obtain resistance tests and maximize the benefits of HIV treatment.