

"Resistance Testing: What Is It, Who Pays For It, and How Does It Benefit ADAP?"

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Agenda

- Review lab tests associated with HIV treatment
- Resistance and how it develops
- Mechanics and prevalence of resistance
- Review guidelines related to resistance testing
- Examine different types of RT
- Limitations of RT

Laboratory Studies In the Treatment of HIV Disease

- CD4 lymphocyte count / percentage
- HIV RNA (viral load)
- HIV Resistance Testing
 - Genotype
 - Phenotype
 - Genotype/Phenotype
 - Predicted Phenotype (from Genotype)
- HIV Tropism / Co-receptor

CD4 Cell Count

- White blood cells derived from the thymus gland
- Indications
 - Stage HIV disease and treatment
 - Starting therapy and prophylaxis
 - Immunologic failure
 - AIDS diagnosis: < 200 cells or percent < 14
 - Assess prognosis for progression to AIDS or death
 - Formulate differential diagnosis in a symptomatic patients
- Mean values: 800-1050 (range 500-1400)

CD4 Cell Count

- Great variability in test results
 - Analytical variation
 - Seasonal and diurnal variation
 - Intercurrent illnesses
 - Corticosteroids

CD4 Cell Percentage

- Sometimes used in preference to absolute count due to less variation in a single measurement
- Approximate CD4 / CD4% equivalents

CD4 Cell Count	Percent CD4
>500 mm ³	>29%
200-500 mm ³	14 – 28%
<200 mm ³	<14%

HIV RNA (Viral Load)

Quantitative measurement of plasma HIV RNA

- Several techniques / methodologies
- Cost: \$100-150 per assay
- Indications
 - Therapeutic monitoring
 - Diagnose acute HIV infection
 - Predict the rate of progression in chronically infected patients

Quantitative Plasma HIV RNA Techniques

Quantitative Plasma HIV RNA Techniques

Technique	Test Name	Manufacturer	Dynamic Range
HIV RNA PCR (RT-PCR)	Amplicor HIV-1 Monitor Test version 1.5	Roche	< 50 – 750,000 copies/ml
Branched chain DNA (bDNA)	Versant HIV-1 RNA 3.0	Bayer	< 75 – 500,000 copies/ml
Nucleic acid sequence-based amplification (NASBA)	NASBA or NucliSens HIV-1 QT	bioMerieux	< 80 – 3,500,000 copies/ml

Adapted from Bartlett & Gallant (2005)

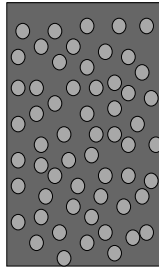
What is Resistance?

What is Resistance?

- The reduced susceptibility of a patient's viral isolate to suppression by an antiviral drug
- A change that improves viral replication in the presence of an inhibitor
- The point at which an ARV agent can no longer effectively inhibit viral reproduction

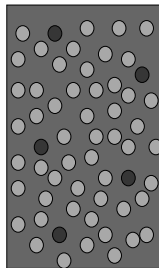
Wild Type Virus

- Non-mutant, drug-susceptible virus
- No previous effect from medication
- Reference virus



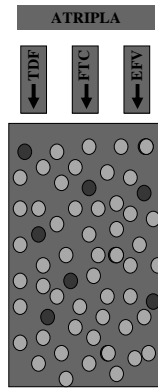
Quasi-Species

- Viral isolates are composed of various groups of virus
 - Wild Type (Sensitive)
 - Mutated (Resistant)
 - Mutated (Non-resistant)
- Acquired resistance
- Selective pressure from current medication



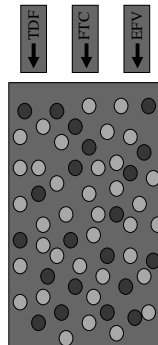
Selective Drug Pressure

- Drug pressure drives selective forces for genetic changes in the viral genome
- Mutations arising under ART allow virus to escape from the inhibitory effect of the drug
- Mutations that develop are associated with ARV agents being administered
- "Minority variants" - <20% of population



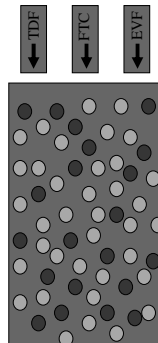
Selective Drug Pressure

- Ongoing replication under selective pressure...
- Increasing amount of resistant mutations are able to develop
- No longer minority variants



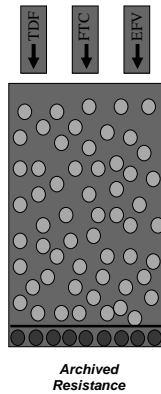
Selective Drug Pressure

- Ongoing replication under selective pressure...
- Increasing amount of resistant mutations are able to develop
- What happens if ARVs are discontinued?



Selective Drug Pressure

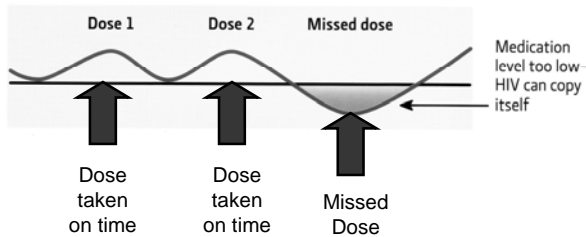
- In absence of ARV pressure, resistant clones are “overgrown” with Wild Type virus and fade to “undetectable”
- Resistance is a genetic characteristic, so it is “archived” and can be re-expressed rapidly
- Absence of resistance on RT does NOT guarantee susceptibility!
- Clinical history is critical



Why does resistance occur?

- Spontaneous mutation of the HIV genome
- Partial suppression of HIV replication promotes resistance
 - Suboptimal dosing of ARVs
 - Patient non-adherence
 - Drug-drug interactions
 - Malabsorption

Missed Doses Can Lead to the Development of Resistance



Adherence and Resistance

■ Meta-analysis

- Only 55% achieve $\geq 95\%$ ARV adherence
- 45% at risk for resistance due to suboptimal ARV exposure
- Development of drug resistance \rightarrow treatment failure

Mills et al. JAMA 2006, 296(6): 679-690

Types of Resistance Tests

■ Genotype

- GenoSure (LabCorp)
- GeneSeq (Monogram)

■ Phenotype

- Antivirogram (Virco)
- PhenoSense (Monogram)

■ Geno/Phenotype: PhenoSense GT (Monogram)

■ Predicted Phenotype:

- VircoType (Virco)
- GenoSure Plus (LabCorp)

HIV Resistance Testing Assays

RESISTANCE

- The (in)ability of HIV to replicate in the presence of Antiretroviral Drugs
- Caused by changes in relevant parts of the virus genome (mutations)

Genotyping Assay

- Indirect measure of the virus' susceptibility to Antiretroviral Drugs
- Based on sequence (mutations) of relevant parts of the viral genome
- Requires interpretation of sequence information

Initial: Virtual Phenotype

- Correlative database, frequently updated with GT and PT



Now: Virco@TYPE HIV-1

- PT fold change of virus is calculated from mutations in GT, with interpretation supported by clinical outcomes data base

Phenotyping Assay

- Direct measure of the ability of the virus to grow in the presence of antiretrovirals
- Compared to laboratory reference strain

Mechanics of Resistance: Example

- Primary resistance 3TC: M184V
- AA methionine (M) substituted by valine (V) at point 184 in AA chain
- Mutation allows HIV to reproduce in presence of 3TC
- High level of resistance to 3TC
- M184M / V / I

1 75 103 184 215 333 500

www.iasusa.org



News on IASUSA.org

October 5, 2007: Cases on the Web Presentation 19: Current Issues in HIV Disease and Substance Abuse, by Chirazee Cunningham, MD, and Hillary Kurins, MD, MPH, MS, is updated for 2007 and available again for CME credit

September 5, 2007: Cases on the Web presentation 23: HIV-associated Metabolic Complications, by Roger J. Bedimo, MD, MS is now available online! Improved interactive format!

August 24, 2007: The August/September 2007 issue (including the new drug resistance mutations figures [revised in August 2007] of Topics in HIV Medicine is now available online.

July 18, 2007: Registration for Fall 2007 New York is now open. Click here for more details.

Welcome to the International AIDS Society-USA

HIV Physician Education: Bridging Clinical Research and Patient Care

The mission of the International AIDS Society-USA is to improve the treatment, care, and quality of life for HIV-infected people through high-quality, relevant, balanced, and needs-oriented education and information for practitioners who

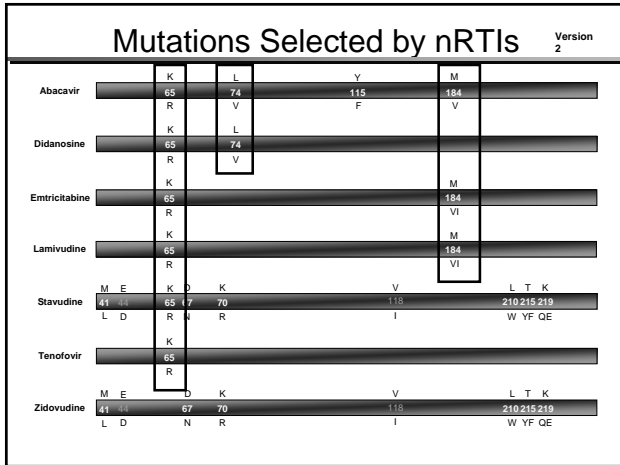


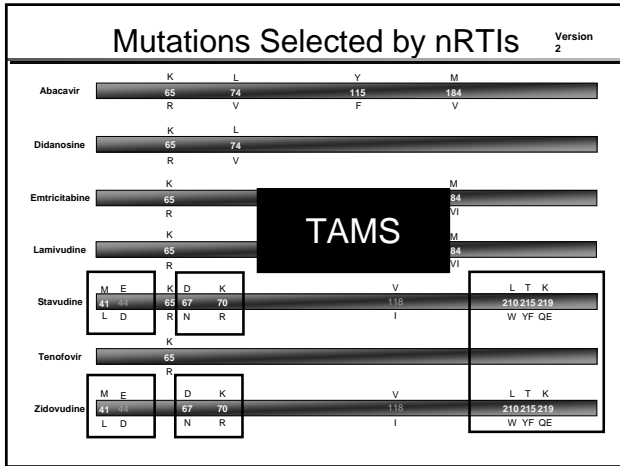
HIV Drug Resistance Mutations

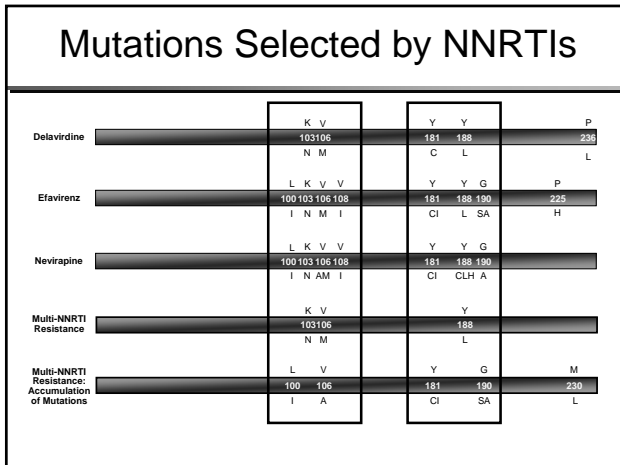
The HIV Drug Resistance Mutations Figures and User Notes are regularly revised and disseminated by the IAS-USA Drug Resistance Mutations Group, an independent volunteer panel of experts focused on studying HIV drug resistance mutations. The group strives to provide current, accurate, and unbiased information on these mutations to the HIV community. The mutations figures and accompanying text are published in Topics in HIV Medicine. The most recent revision will be made available in the August/September 2007 issue.

The IAS-USA has recently compiled a list of resources related to HIV drug resistant mutations. This list will be expanded in the upcoming months.

The Mutations Figures and User Notes are available as downloadable PowerPoint file. The figures will soon be available in pocket-sized folding cards, available in both Spanish and English. To request folding cards, complete the card request form and return via fax at (415) 544-9401, or mail to the address shown on the card. You may also send an e-mail to resistance2007@iasusa.org, or call the IAS-USA at (415) 544-9400.







Protease Mutations: The Simple and Complex

- I50L
- Selected by ATV
- Leads to ATV resistance
- Increases *in vitro* susceptibility to other PIs
- I50V
- Selected by fAPV
- Cross resistance to LPV/r and DRV/r
- Increases susceptibility to TPV

Interactions Between Genotypic Mutations

- Some mutations increase drug resistance
 - M184V → 3TC or FTC or ABC
- Some mutations also increase drug susceptibility
 - M184V → ZDV or TDF
- For some drugs, this gets complex

Genotypic Mutations Impacting Lamivudine Activity

- 184V or 184I
- 65R
- 70E
- 215Y or F
- Insertions at position 69
- 151M MDR complex
- 95 additional RT mutations and mutation pairs

Advantages and Disadvantages of Genotype Testing

Advantages

- Rapid turnaround (1-2 weeks)
- Less expensive
- Detection of mutations may precede phenotypic resistance
- Widely available
- More sensitive than phenotype for detecting mixtures of resistant and wild-type virus

Disadvantages

- Indirect measure of resistance
- Relevance of some mutations unclear
- Unable to detect minority variants (<20-25% of viral sample)
- Complex mutational patterns may be difficult to interpret

Points to Consider

- Resistance often develops initially to NNRTIs (EFV, NVP) and 3TC
- Protease inhibitors:
 - Resistance generally develops more slowly compared with other classes
 - RTV boosting significantly delays development of resistance to protease inhibitors
- Additional mutations are likely to develop if patients are left on failing regimens

HIV Resistance Testing Assays

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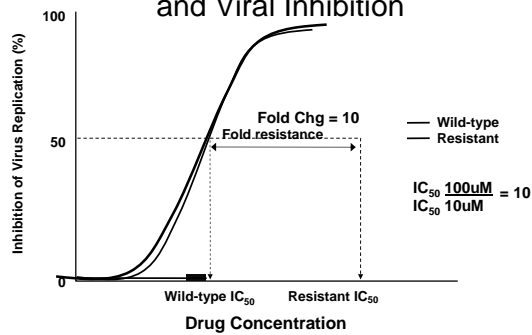
Phenotyping Assay

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Phenotype Testing

- Measures laboratory susceptibility of an HIV isolate to a given drug
- Measures the concentration of drug needed to inhibit the replication of a patient's virus
- Degree of resistant is quantified
 - Compares the fold-change in drug concentration required to inhibit the replication of the patient's virus compared to a representative, wild type, sensitive virus isolate

Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition



Fold Change

- Phenotypic tests compare the drug susceptibility of a lab / wild-type virus to a patient's virus
- "Cut-off" values refer to the fold-change of virus susceptibility above which the drug has less activity in-vivo

Cut-Offs

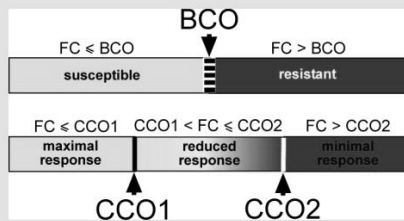
■ Biological Cut-offs

- Defines what is resistant and non-resistant based on how viruses respond to a drug in vitro

■ Clinical Cut-offs

- Defines what is resistant and non-resistant based on how viruses respond to a drug in vivo
- Typically generated through clinical trials

Terminology Used in the Resistance Analysis



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Advantages and Disadvantages of Phenotype Testing

Advantages

- Provides direct and quantitative measure of resistance
- Uses 2 clinical cutoffs (CCO) derived from clinical cohorts to define spectrum of resistance
 - CCO1: value below which reduced virologic response is likely
 - CCO2: value above which a virologic response is unlikely
- Indicates which drugs have partial activity
- Can assess interactions among mutations

Disadvantages

- Clinical cutoffs not defined for some agents
- May be unable to detect minority variants for some mutations (< 20% to 25% of viral sample)
- Complex technology with longer turnaround (~ 3 wks)
- More expensive than genotyping
- Limited laboratories perform testing

When To Use Resistance Testing *Naïve Patients / Starting Therapy*

- RT is recommended when patients enter into care regardless of whether therapy will be initiated immediately
- *Early RT provides a record of all resistant variants: treatment can be selected appropriately when needed*
- Therapy deferred: consider repeat testing when starting ART
- Genotype usually preferred

DHHS Treatment Guidelines, January 29, 2008

Prevalence of Resistance in Acute/Recent HIV Infections

Study	N	Definition	Period	Prevalence
CATCH ¹	596	Clinical	1996-2002	10%
CDC ²	182	STARHS	1997-2001	12%
San Fran. ³	180	<1 year	2000-2002	26%
Canada (surveillance) ⁴	144	STARHS	1997-2001	10%
Montreal ⁵	170	<1 year	1996-2003	12%
France ⁶	296	<6 months	2001-2002	11%
UK ⁷	157	<18 months	1996-2003	17%
Madrid ⁸	74	<1 year	1997-2002	19%

1. XIII International HIV Drug Resistance Workshop, June 10-14, 2003, Los Cabos, Mexico. Abstract 117.
2. *Ibid.*, abstract 119. 3. *Ibid.*, abstract 120. 4. *Ibid.*, abstract 121. 5. *Ibid.*, abstract 122. 6. *Ibid.*, abstract 123. 7. *Ibid.*, abstract 124. 8. *Ibid.*, abstract 130.
Source: ICMER, 2003.

When To Use Resistance Testing *Naïve Patients / Starting Therapy*

- If resistance mutations are not detected, it is still possible that the patient has been infected with a drug-resistant strain
- The lack of drug pressure can cause the wild-type strain to dominate and minor resistant species may not be detected

When To Use Resistance Testing *On Therapy*

- Virologic failure: assist in selecting active drugs for next regimen
- Suboptimal viral load reduction
- Perform while patient is taking ARV agents or immediately after discontinuing therapy (within 4 weeks)
- All pregnant women prior to initiation of therapy and for those entering pregnancy with detectable viremia while on therapy

DHHS Treatment Guidelines, January 29, 2008

Types of Resistance Tests

- Genotype
 - HIV gene sequencing of the patient's virus to detect mutations known to confer drug resistance
- Phenotype
 - Measures ability of a recombinant virus derived from the patient sample to grow in different concentrations of ARV drugs
- Predictive phenotype (VircoType)
 - Use of genotype results to predict phenotypic susceptibility based on scores derived from linear modeling
- Combination genotype / phenotype

Advantages and Disadvantages of Predictive Phenotype Testing

Advantages

- Require less interpretation of complex genotypes
- Less expensive, quicker than conventional phenotyping
- Assess impact of interactions between mutations
- Equivalent virologic outcomes in clinical trials to conventional phenotyping
- Available from many reference labs
- Results usually available in minutes after genotype

Disadvantages


- Not an actual measured phenotype; a predicted phenotype based on genotypic information
- Reliability will depend on the accuracy of the genotype
- Available only from 1 vendor
- Slightly more expensive than genotype alone

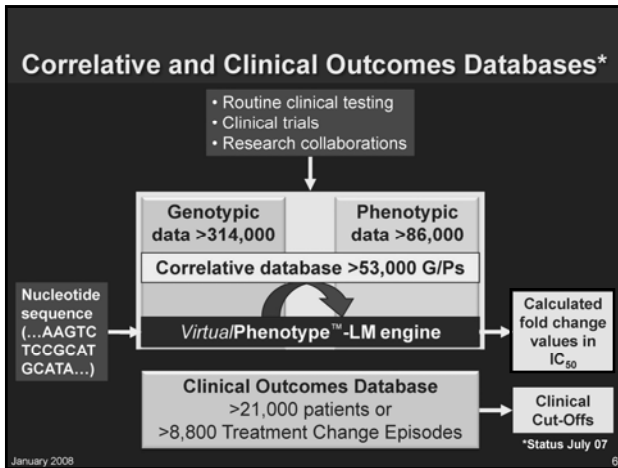
virco® TYPE HIV-1 V4.1.00

Page 1
Summary Report

Page 2
Detailed Report

Page 3
Definitions and Disclaimers

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Creating the Phenotype from the Genotype

- Define mutations with impact on each drug
- Two factors in Virco G/P database:
 - The “Weight”: *How much* this mutation changes the phenotype
 - The “Direction”: Does this mutation lead to more resistance, or more drug susceptibility?
- Each mutation (single and pairs) analyzed for these two factors
- A total “score” is created from the sum of these factors → the “fold change”

Complex Resistance Interpretation: Tipranavir Mutations and Phenotype

Decrease in FC ← → Increase in FC

10F & 47V	22V & 84V	34Q & 84V	36L	46L & 82T	53L	54V	74S
10F & 82A	24I & 82A	35D & 36I	36L & 58E	47V & 54M	54A & 55R	54V & 70E	76V
10F & 84V	24I & 82T	35D & 54A	36L & 83D	47V & 54V	54A & 84V	54V & 74P	82A & 84V
10V	30N	35D & 54V	36L & 95F	47V & 83D	54L	54V & 84V	82C
10V & 33F	30N & 74S	35D & 58E	38W	47V & 84V	54L & 82A	58E & 84V	82L
10V & 88D	33F	35D & 73T	41K	48A & 71V	54M	60E	82T
13V & 69K	33F & 82A	35G & 71V	41T	48M & 53L	54M & 74S	60F	84V
13V & 71V	33I & 36I	35N & 84V	43T	48V	54M & 82A	69K	85V
13V & 82S	33M	36I & 47V	43T & 82T	48V & 54V	54S	71V & 73T	88D
13V & 84V	33V	36I & 54T	46L & 53L	50L	54S & 82T	71V & 95F	90M
20R	33V & 84V	36I & 84V	46L & 71V	50V	54V	74P & 82A	

Creating the Fold Change: First: Define the Genotypic Mutation Pattern For a Patient

PI Mutations:

10V, 13wt/V, 32I, 33F, 41K, 46I, 58E,
63P, 71wt/V, 73S, 77I, 84V, 89V, 90M, 93L

Can we use Tipranavir?

Complex Resistance Interpretation: Tipranavir Mutations and Phenotype

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10V	30N	35D & 54V	36L & 95F	47V & 83D	54L	54V & 84V	82C
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13V & 84V	33V	36I & 54T	46L & 53L	50L	54S & 82T	71V & 95F	90M
20R	33V & 84V	36I & 84V	46L & 71V	50V	54V	74P & 82A	

10V, 13wt/V, 32I, 33F, 41K, 46I, 58E, 63P, 71wt/V, 73S, 77I, 84V, 89V, 90M, 93L

Key Points

- Resistance testing most reliably identifies drugs that should be avoided
 - Less reliable at detecting drugs that are most likely to be active
- Testing is most reliable for indicating activity of drugs being given or recently given
 - Drugs discontinued → re-emergence and proliferation of wild-type virus
- RT measure the dominant species at the time the test is performed

LabTracker™

- Download VircoType™ results directly into LabTracker
- Populate mutation fields
- Print VircoType reports
- Query LabTracker for mutational information

Clinical Utility HIV Resistance Testing

- Value of resistance testing has been demonstrated in clinic trials and observational cohort studies
 - Viradapt
 - ARGENTA
 - GART
 - Havana
 - VIRAS001
- DHHS and other expert panels

Evaluating The Reliability of VircoTYPE HIV-1 as compared to PhenoSense GT in a Community-Based Setting

- 51 PhenoSense GT assays from a one urban Title I (Part A) clinic
 - 30 cases: treatment could have been selected by genotype alone
 - 21 cases: phenotype guided selection
- All drugs maintained or added by PSGT could have been selected by VircoType
- Potential benefit:
 - Faster TAT
 - Cost savings: \$25,000

Mounzer et. al., Clinical Virology Symposia 2007 Clearwater, FL April 28-May 2, 2007

General Limitations and Caveats of Resistance Testing

- Obtain plasma sample for testing while patient is on therapy
- Consider etiologies for failure other than resistance (adherence, PK)
- Be familiar with quality of testing lab
- **RT does not replace patient treatment history**

Questions?
