

# Specialty Lab Testing In HIV Care

Association of Nurses in AIDS Care  
2012 Conference

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Clinical Assistant Professor, USF College of Medicine  
Nurse Practitioner, Hillsborough County Health Department

## HIV Resistance Testing

### Why does resistance occur?

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

### Drug Resistance Testing: DHHS Recommendations

- All HIV-infected individuals entering care, regardless of whether therapy will be initiated
- If therapy is deferred
  - Repeat testing prior to initiating antiretroviral therapy
- All patients on therapy with virologic failure
- All pregnant women prior to initiation of therapy
  - Those entering pregnancy with detectable HIV RNA levels while on therapy

## Drug Resistance Assays: DHHS Recommendations

- Genotypic assay is preferred
  - Treatment-naïve patients (acute or chronic)
  - First or second virologic failure
    - May not be useful if therapy discontinued for >4 weeks
  - Consider adding INSTI-specific genotype test
    - Concern about transmitted INSTI resistance
    - Failing INSTI-based regimen

DHHS. Available at: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.  
Revision March 27, 2012.

## Drug Resistance Assays: DHHS Recommendations

- Phenotype or combined phenotype/genotype
  - High-level resistance to NRTIs or PIs on genotype
  - Multiple regimen failure with limited treatment options
- Virtual phenotype (interpretation of genotype data, predicted phenotypic)
  - May be used in settings where phenotypic testing would be preferred

DHHS. Available at: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.  
Revision March 27, 2012.

## Genotype

- Most genotype assays involve sequencing RT and protease genes to detect mutation
  - Sequence of bases (A,C,T,G) coding for amino acids that comprise viral proteins
  - Expressed as the coded amino acid and position (eg, M184)
  - If mutated, the change is indicated after the position (eg, M184V)
- Results available within 1 to 2 weeks
- Interpretation of results usually requires a specialist

DHHS. Available at: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.  
Revision March 27, 2012.

## Common HIV Resistance Tests

- Genotype
  - GenosureMG
  - VircoType
  - GeneSeq Integrase
  - Genosure Prime
- Phenotype
  - PhenoSense GT
  - PhenoSense Entry

## Limitations of Resistance Testing

- High cost compared with other tests routinely used in HIV care
- Cannot be reliably performed when HIV RNA <500-1,000 copies
- May not be able to detect minority populations of resistant virus (<20%)
  - Especially common after drug discontinuation
- Resistant strains in viral reservoirs are not detected

## General Limitations and Caveats of Resistance Testing

- Consider etiologies for failure other than resistance (adherence, PK)
- Be familiar with quality of testing lab
- RT does not replace patient treatment history

## Advantages and Disadvantages of Genotype Testing

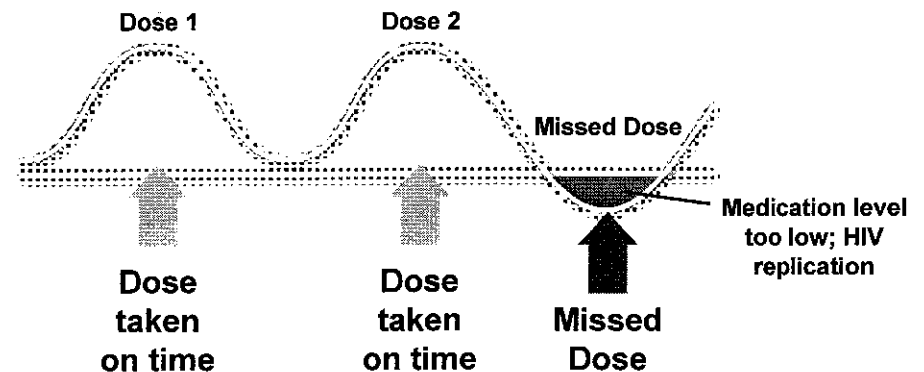
### Disadvantages

- 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

### Advantages

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

## Missed Doses Can Lead to the Development of Resistance



# List of Tests

- HIV Resistance Tests
  - Phenotype
  - Genotype
  - Phenotype and Genotype
  - Integrase Inhibitors
- Tropism Assays
  - Trofile
  - Trophile DNA
  - Quest Test
- Tuberculosis Testing
- HCV related testing

# Advantages and Disadvantages of Genotype Testing

- Indirect measure of resistance
- Reliance of some routine tests
- Unable to detect minority variants (<20% of viral genome)
- Complex regional patterns may be difficult to interpret
- Indirect measure of resistance
- Reliance of some routine tests
- Unable to detect minority variants (<20% of viral genome)
- Complex multi drug patterns may be difficult to interpret

# GenoSure® MG

- Genotypic HIV drug resistance test
- Performed using state-of-the-art technology
- Re results are determined using the most recently updated HIV mutation information and information from scientific research and Monogram's resistance experts
- Report form includes drug resistance information for all of the approved NRTIs, NNRTIs, and PIs

Source: Monogrambio.com

**GenoSure<sup>®</sup> MG**  
HIV DRUG RESISTANCE TEST

**LabCorp**  
UNIVERSITY MICROFILMS

SPECIALTY CARE CENTER -  
HILLSBOROUGH CO HEALTH DEPT  
1105 S KENNEDY BLVD, FIRST FLO  
TAMPA, FL 33603  
USA

DATE COLLECTED: 12/14/2012 09:00  
PATIENT NAME: [REDACTED]  
REFERRING PHYSICIAN: [REDACTED]

DATE REPORTED: 12/14/2012 22:19:17  
TEST REFERENCE: [REDACTED]

DRUG RESISTANCE ASSOCIATED SUBSTANCES ORDERED

Drug	Resistance	Associated Substances	Order	Result
Abacavir	Zalcitabine	None	ATV	Sensitive
Didanosine	Nucleoside	None	ddI	Sensitive
Zalcitabine	Nucleoside	None	FTC	Sensitive
Lamivudine	Nucleoside	None	JTD	Sensitive
Stavudine	Nucleoside	None	4dT	Sensitive
Tenofovir	Nucleoside	None	RPV	Sensitive
Zidovudine	Nucleoside	None	3TC	Sensitive
Efavirenz	Nucleoside	None	EFV	Sensitive
Etravirine	Nucleoside	None	ETN	Sensitive
Nevirapine	Nucleoside	None	NVP	Sensitive
Rilpivirine	Nucleoside	None	RPV	Sensitive
Atazanavir	Nucleoside	None	ATV	Sensitive
Darunavir	Nucleoside	None	DRV	Sensitive
Emtricitabine	Nucleoside	None	FTC	Sensitive
Lamivudine	Nucleoside	None	JTD	Sensitive
Lapatinib	Nucleoside	None	LPZ	Sensitive
Maraviroc	Nucleoside	None	MRV	Sensitive
Raltegravir	Nucleoside	None	RTV	Sensitive
Saqaviroc	Nucleoside	None	SCV	Sensitive
Tiplaxtenor	Nucleoside	None	TPI	Sensitive

Page 1 of 2

**GenoSure MG with no evidence of resistance**

**GenoSure<sup>MG</sup>**  
 HIV DRUG RESISTANCE ASSAY

**LabCorp**  
 SPECIALTY CARE CENTER  
 HILLSBOROUGH CO HEALTH DEPT  
 1105 E KENNEDY BLVD, FIRST FLO  
 TAMPA, FL 33602  
 USA

GenoSure<sup>MG</sup> with significant NNRTI resistance

Special Name: JEFFREY M PLANT  
 Date Collected: 15-MAR-2012 00:00  
 Patient ID: 18-18-1001  
 Date Received: 26-MAR-2012 00:00 PT  
 Patient Name: JEFFREY M PLANT  
 Date of Birth: 26-MAR-2012 21 01 PT  
 Patient ID: 18-18-1001  
 Date of Birth: 26-MAR-2012 21 01 PT

GenoSure Name	Brand Name	Drug Resistance Associated Mutations Detected	Drug	Resistance
Abacavir	Calyma	None	ABC	Sensitive
Didanosine	Didac	None	DDI	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Lamivudine	Epivir	None	3TC	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Tenofovir	Vemco	None	TDF	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Abacavir	Calyma	None	ABC	Sensitive
Didanosine	Didac	None	DDI	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Lamivudine	Epivir	None	3TC	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Tenofovir	Vemco	None	TDF	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
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Abacavir	Calyma	None	ABC	Sensitive
Didanosine	Didac	None	DDI	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
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**GenoSure<sup>MG</sup>**  
 HIV DRUG RESISTANCE ASSAY

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Zalcitabine	Ziagen	None	FTC	Sensitive
Tenofovir	Vemco	None	TDF	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive
Zalcitabine	Ziagen	None	FTC	Sensitive

**GenoSurePRime**  
 HIV DRUG RESISTANCE ASSAY

**GenoSurePRime**

A Comprehensive Resistance Profile in a Single Test

Integrase  
 Reverse Transcriptase  
 Protease

**LabCorp**

**GenoSurePRime**

Single genotypic assay providing a comprehensive picture of resistance

- NRTIs
- NNRTIs
- PIs
- INIs

**GenoSurePRime**

A Comprehensive Resistance Profile in a Single Test

**LabCorp**



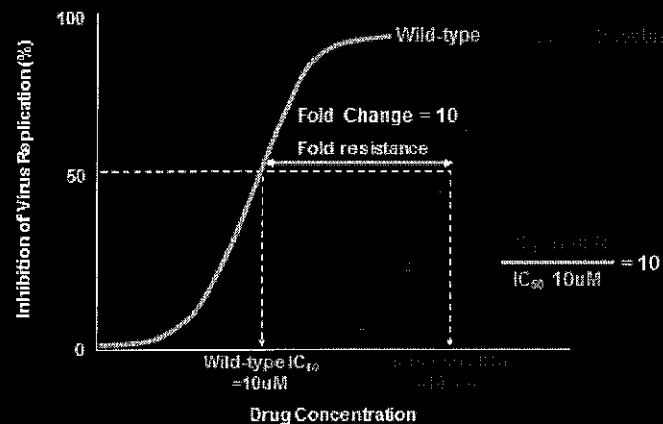
## HIV Resistance Testing: Phenotype

## Phenotype

- 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 resistance 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

## Phenotype

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
- Clinical "Cut-off" values refer to the fold-change of virus susceptibility above which the drug has less activity *in-vivo*

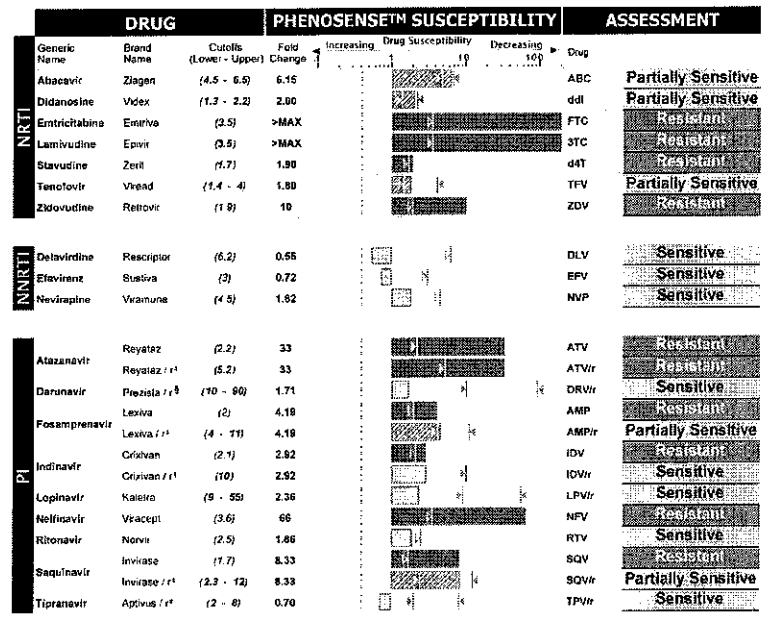
# Antivirogram® phenotype

Patient/Sample Details		Physician Details	
Signal ID	Sample type	Physician Name	
Spec ID	Collection Date	Referral by	
	Referral Date	Visit	
	Report Date		

Drug	Trade name	Generic name	Susceptibility	Fold change in I <sub>50</sub> (I <sub>50</sub> of test virus relative to reference virus (log <sub>2</sub> ))	Ref. susceptible range
<b>NRTI / NRTI*</b>					
Abacavir	Ziagen	Abacavir	██████████	10.2	12-25
Didanosine	Videx	Didanosine	██████████	2.2	12-25
Zalcitabine	Didanosine	Zalcitabine	██████████	0.5	12-25
Lamivudine	Epivir	Lamivudine	██████████	1.4	12-25
Stavudine	Zeniv	Stavudine	██████████	1.0	12-25
Tenofovir	Viread	Tenofovir	██████████	4.4	12-25
Zidovudine	Retrovir	Zidovudine	██████████	1.0	12-25
<b>NNRTI</b>					
Delavirdine	Rescriptor	Delavirdine	██████████	>47.5	16-50
Efavirenz	Sustiva	Efavirenz	██████████	13.3	13-33
Nevirapine	Viramune	Nevirapine	██████████	0.8	13-27
<b>PI</b>					
Atazanavir	Reyataz	Atazanavir	██████████	>54.6	12-31
Darunavir	Prezista	Darunavir	██████████	>42.5	12-31
Fosamprenavir	Lexiva	Fosamprenavir	██████████	>31.7	11-31
Indinavir	Crixivan	Indinavir	██████████	5.5	12-21
Lopinavir	Kaletra	Lopinavir	██████████	>78.0	11-27
Nelfinavir	Viracept	Nelfinavir	██████████	>42.3	12-31
Ritonavir	Norvir	Ritonavir	██████████	1.0	11-21
Saquinavir	Invirase	Saquinavir	██████████	6.4	12-31
Tipranavir	Aptivus	Tipranavir	██████████		

# Phenosense



# Combination Genotype and Phenotype PhenoSense GT

PhenoSense GT  
COMBINATION GENOTYPE AND PHENOTYPE

Client: 08-228-0102 Study: FAILURE  
233 Lakewood Drive  
Foster City, CA 94024  
USA

Client Name: [REDACTED]  
Phone: [REDACTED]

Study ID: 08-228-0102  
Study Name: 08-228-0102  
Study Site: 08-228-0102  
Study Start: 08-228-0102  
Study End: 08-228-0102

Drug	Trade Name	Generic Name	Fold Change	Assessment
Abacavir	Ziagen	Abacavir	6.15	Sensitive
Didanosine	Videx	Didanosine	2.00	Sensitive
Emtricitabine	Emtriva	Emtricitabine	>MAX	Sensitive
Lamivudine	Epivir	Lamivudine	>MAX	Sensitive
Stavudine	Zeniv	Stavudine	1.90	Sensitive
Tenofovir	Viread	Tenofovir	1.80	Sensitive
Zidovudine	Retrovir	Zidovudine	10	Sensitive
Delavirdine	Rescriptor	Delavirdine	0.55	Sensitive
Efavirenz	Sustiva	Efavirenz	0.72	Sensitive
Nevirapine	Viramune	Nevirapine	1.82	Sensitive
Atazanavir	Reyataz	Atazanavir	33	Sensitive
Darunavir	Prezista	Darunavir	1.71	Sensitive
Fosamprenavir	Lexiva	Fosamprenavir	4.18	Sensitive
Indinavir	Crixivan	Indinavir	2.92	Sensitive
Lopinavir	Kaletra	Lopinavir	2.36	Sensitive
Nelfinavir	Viracept	Nelfinavir	66	Sensitive
Ritonavir	Norvir	Ritonavir	1.86	Sensitive
Saquinavir	Invirase	Saquinavir	8.33	Sensitive
Tipranavir	Aptivus	Tipranavir	0.70	Sensitive

# Phenosense™ HIV Test Report

DRUG	PHENOSENSE™ SUSCEPTIBILITY		ASSESSMENT
	Cutoffs (Lower - Upper)	Fold Change	
Abacavir	(4.5 - 6.9)	6.15	Partially Sensitive
Didanosine	(1.7 - 2.2)	2.00	Partially Sensitive
Emtricitabine	(3.5)	>MAX	Resistant
Lamivudine	(3.5)	>MAX	Resistant
Stavudine	(1.7)	1.90	Resistant
Tenofovir	(1.4 - 4)	1.8	Partially Sensitive
Zidovudine	(1.9)	10	Resistant

Report ID: Medical Report A 2012092801  
 Patient ID: 12-124268  
 Date Collected: 11-MAR-2012 07:30  
 Date Report: 14-MAR-2012 11:52 P.M.  
 Date Expires: 22-MAR-2012 13:00 P.M.  
 Patient Name: [REDACTED]  
 HIV-1 Subtype: 11

Drug	IC50	Fold Change	Assessment
Abacavir	14.5	4.6	1.2
Didanosine	11.2	2.8	1.0
Emtricitabine	10.0	1.0	1.0
Lamivudine	10.0	1.0	1.0
Stavudine	11.7	1.0	1.0
Zidovudine	11.9	1.0	1.0
Zalcitabine	11.4	4.0	1.0

Drug	IC50	Fold Change	Assessment
Abacavir	14.5	4.6	1.2
Didanosine	11.2	2.8	1.0
Emtricitabine	10.0	1.0	1.0
Lamivudine	10.0	1.0	1.0
Stavudine	11.7	1.0	1.0
Zidovudine	11.9	1.0	1.0
Zalcitabine	11.4	4.0	1.0

Page 1 of combination genotype and phenotype with extensive NNRTI resistance and resistance to ATV

Report ID: [REDACTED]  
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Emtricitabine	10.0	1.0	1.0
Lamivudine	10.0	1.0	1.0
Stavudine	11.7	1.0	1.0
Zidovudine	11.9	1.0	1.0
Zalcitabine	11.4	4.0	1.0

## PhenoSense Entry: Enfuvirtide

Report ID: [REDACTED]  
 Patient ID: [REDACTED]  
 Date Collected: 11-MAR-2012 07:30  
 Date Report: 14-MAR-2012 11:52 P.M.  
 Date Expires: 22-MAR-2012 13:00 P.M.  
 Patient Name: [REDACTED]  
 HIV-1 Subtype: 11

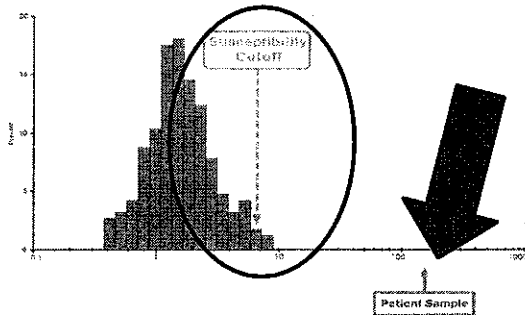
Fold Change in IC50 of Patient Virus in relation to distribution of enfuvirtide-naïve viral isolates:

# PhenoSense Entry

Comments:

DRUG		PHENOSENSE SUSCEPTIBILITY			ASSESSMENT	
Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility	Decreasing Drug Susceptibility	Drug
Enfuvirtide	Fuzoan	6.04125	157			ENF

Fold Change in IC50 of Patient Virus in relation to distribution of enfuvirtide-naive viral isolates\*:



# PhenoSense Integrase

## Integrase

PHENOSENSE SUSCEPTIBILITY

Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility	Decreasing Drug Susceptibility	Drug
Raltegravir	Isentress	(1.0)	1.54			RAL

Assessment:  Sensitive,  Reduced Susceptibility

Biological Cutoff

Hyper-susceptibility Cutoff

Additional Information: Virus Replication Capacity = 43% (Range 27%-65%)

# PhenoSense Integrase

PHENOSENSE SUSCEPTIBILITY

Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility	Decreasing Drug Susceptibility	Drug
Raltegravir	Isentress	(1.0)	0.64			RAL

Assessment:  Sensitive,  Reduced Susceptibility

Biological Cutoff

Hyper-susceptibility Cutoff

Additional Information: Virus Replication Capacity = 43% (Range 27%-65%)

# PhenoSense Integrase

PHENOSENSE SUSCEPTIBILITY

Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Increasing Drug Susceptibility	Decreasing Drug Susceptibility	Drug
Raltegravir	Isentress	(1.0)	1.54			RAL

Assessment:  Sensitive,  Reduced Susceptibility

Biological Cutoff

Hyper-susceptibility Cutoff

Additional Information: Virus Replication Capacity = 43% (Range 27%-65%)

Integrase replication capacity (IN RC) indicates the ability of recombinant viruses containing patient-derived integrase and C-terminal reverse transcriptase sequences to replicate in the absence of drug. Range represents the 95% confidence interval around the RC measurement. 100% = median RC of wild-type (integrase inhibitor naive) viruses. IN RC should be interpreted with consideration of PR-RT RC results where available. Interactions between PR-RT and IN that may impact complete virus fitness are not well-characterized.

## Key Points

- 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 measures the dominant species at the time the test is performed

## General Limitations and Caveats of Resistance Testing

- Consider etiologies for failure other than resistance (adherence, PK)
- Be familiar with quality of testing lab
- RT does not replace patient treatment history
- 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 measures the dominant species at the time the test is performed



www.iasusa.org  
**IAS-USA**  
 International AIDS Society—USA

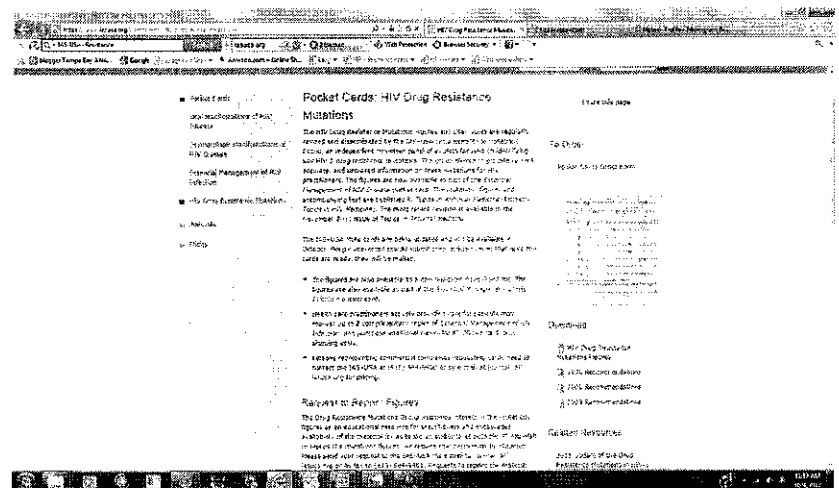
IAS-USA Search

### 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 identifying key HIV-1 drug resistance mutations. The group strives to provide current, accurate, and unbiased information on these mutations for HIV practitioners. The mutations figures and accompanying text are published in *Topics in HIV Medicine*. The most recent revision is available in the December 2008 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 a downloadable PowerPoint file. The figures are also available in packet-sized folding cards. To request folding cards, complete the card request form and return via fax at (415) 544-9401, or by mail to the address shown on the form. You may also send an e-mail to resistance2008@iasusa.org or call the IAS-USA at (415) 544-9400.



# International AIDS Society—USA\* Drug Resistance Mutations Group

International AIDS Society—USA *Topics in HIV Medicine*

## Update of the Drug Resistance Mutations in HIV-1: December 2008

Victoria A. Johnson, MD, Françoise Brun-Vézinet, MD, PhD, Bonaventura Clotet, MD, PhD, Huldrych F. Günthard, MD, Daniel R. Kuritzkes, MD, Deenan Pillay, MD, PhD, Jonathan M. Schapiro, MD, and Douglas D. Richman, MD

\*The International AIDS Society—USA (IAS—USA) is a not-for-profit, HIV clinical specialist-education organization. It is entirely different from and not affiliated with the International AIDS Society (Stockholm, Sweden).

## ISA-USA

### ■ Pocket Cards

#### ■ Oral Manifestations of HIV Disease

#### ■ Dermatologic Manifestations of HIV Disease

#### ■ Essential Management of HIV Infection

### ■ HIV Drug Resistance Mutations

#### ■ Podcasts

#### ■ Slides

### Pocket Cards: HIV Drug Resistance Mutations

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The IAS—USA Muta cards are being updated and will be available in October. People interested should submit their orders now so that once the cards are ready, they will be mailed.

- The figures are also available as a downloadable PowerPoint file. The figures are also available as part of the *Essential Management of HIV Infection* pocket card.
- Health care practitioners actively providing care at events may request up to 2 complimentary copies of *Essential Management of HIV Infection*, and purchase additional copies for \$2.50 per card, plus shipping costs.
- Persons representing commercial companies requesting cards need to contact the IAS—USA at (415) 544-9400 or by e-mail at [journal@iasusa.org](mailto:journal@iasusa.org)

<https://www.iasusa.org/content/hiv-drug-resistance-mutations>

[www.iasusa.org](http://www.iasusa.org)

## Spring 2008 Updated Drug Resistance Mutations Figures

Mutations Cards Available

Visit [www.iasusa.org](http://www.iasusa.org) to order laminated folding cards and to download the Mutations Figures and User Notes.

## Conventional 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 resistance 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

# Antivirogram® phenotype

Patient/Sample Details		Physician Details	
Original ID Viro ID	Sample Type Collection Date	Purified Amplifier Received By Viro	Physician Name
Viro ID: 9AAZ56591	25 Jun 2008	18A	
	Report Date	26 Feb 2008	

Drug	Trade name	Generic name	Susceptibility	Fold change in IC <sub>50</sub>	Ref.
			Fold change in IC <sub>50</sub> relative to reference virus (log <sub>2</sub> )	(Call off for normal susceptible range)	
<b>NNRTI / NNRTI</b>					
Retrovir®	Zalcitabine	[REDACTED]	[REDACTED]	10.2 (2.5)	
Triavir®	Lamivudine	[REDACTED]	[REDACTED]	2.2 (2.1)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	1.6 (2.1)	
Zenpep®	Didanosine	[REDACTED]	[REDACTED]	1.4 (2.2)	
Zenpep®	Zalcitabine	[REDACTED]	[REDACTED]	1.0 (2.0)	
Zenpep®	Zalcitabine	[REDACTED]	[REDACTED]	4.4 (3.1)	
Zenpep®	Zalcitabine	[REDACTED]	[REDACTED]	1.6 (2.0)	
<b>NNRTI</b>					
Viread®	Abacavir	[REDACTED]	[REDACTED]	>67.5 (3.0)	
Sustiva®	efavirenz	[REDACTED]	[REDACTED]	13.3 (3.5)	
Sustiva®	efavirenz	[REDACTED]	[REDACTED]	0.5 (2.7)	
<b>PI</b>					
Viread®	Abacavir	[REDACTED]	[REDACTED]	>51.8 (2.3)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	>42.5 (2.2)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	>11.7 (1.8)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	5.5 (2.2)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	>29.9 (1.4)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	>42.3 (2.3)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	1.0 (1.7)	
Viread®	Abacavir	[REDACTED]	[REDACTED]	0.9 (2.0)	

# virco®TYPE HIV-1 V4.2.01

Page 1 Summary Report      Page 2 Detailed Report      Page 3 Definitions and Disclaimers

# GenoSure® MG

Monogram Biosciences Clinical Development  
 345 Oyster Point Blvd.  
 South San Francisco, CA 94080  
 USA

Patient Name: [REDACTED]      DOB: [REDACTED]      Patient ID/Referral Number: [REDACTED]      Gender: [REDACTED]      Monogram Submission ID: [REDACTED]

Drug	Generic Name	Brand Name	Drug Resistance Assessed Mutations Detected	Drug	Assessment	Comments
NRTI	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, A319S	ABC	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, T319F	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A	DDI	Resistant	
	Didanosine	Didanosine	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L	DDI	Resistant	
NRTI	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S, A319V	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S, A319V, T319A	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S, A319V, T319A, I319L	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I	ABC	Resistant	
	Abacavir	Abacavir	M41L, E44D, D47N, V75I/N, S116V, L319W, T318I, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C, R319K, G319S, A319V, T319A, I319L, V319I, Y319C	ABC	Resistant	

# MDR HIV

- Gather all resistance data for patient
- Consider Stanford Data Base
- Utilize colleagues, local experts, national experts, AETC

# Stanford University HIV Drug Resistance Database

# Reports you don't want to see!

STRAT	STRAT	STRAT	STRAT	STRAT	STRAT	STRAT
The HIV-1 Genotype (A19101) for this specimen has been completed.						
Genotype Information						
HIV-1 Subtype: B						
Drug	Generic Name	Brand Name	Genotypic Management	Comments		
-----						
NRTI						
Abacavir	Abacavir	Ziagen	Sensitive			
	STRAT: R51R	Z184V				
Zidovudine	Zidovudine	Retrovir	Resistant			
	STRAT: K259L	N184Y				
Non-nucleoside Reverse Transcriptase Inhibitors						
Emtricitabine	Emtricitabine	Emtriva	Resistant			
	STRAT: Y185L	M184V				
Lamivudine	Lamivudine	Epivir	Resistant			
	STRAT: K103R	M184V				
Integrase Inhibitors						
Raltegravir	Raltegravir	Isentress	Sensitive			
	STRAT: K179	Y181F				
Protease Inhibitors						
Didanosine	Didanosine	Didan	Resistance Possible			
	STRAT: R157					
Zalcitabine	Zalcitabine	Didan	Sensitive			
	STRAT: None					
-----						
NRTI						
Emtricitabine	Emtricitabine	Emtriva	Sensitive			
	STRAT: None					
Protease Inhibitors						
Maraviroc	Maraviroc	Jintana	Sensitive			
	STRAT: None					
Non-nucleoside Reverse Transcriptase Inhibitors						
Emtricitabine	Emtricitabine	Emtriva	Sensitive			
	STRAT: None					
Lamivudine	Lamivudine	Epivir	Sensitive			
	STRAT: None					
Integrase Inhibitors						
Raltegravir	Raltegravir	Isentress	Sensitive			
	STRAT: None					

# Viral Tropism Testing

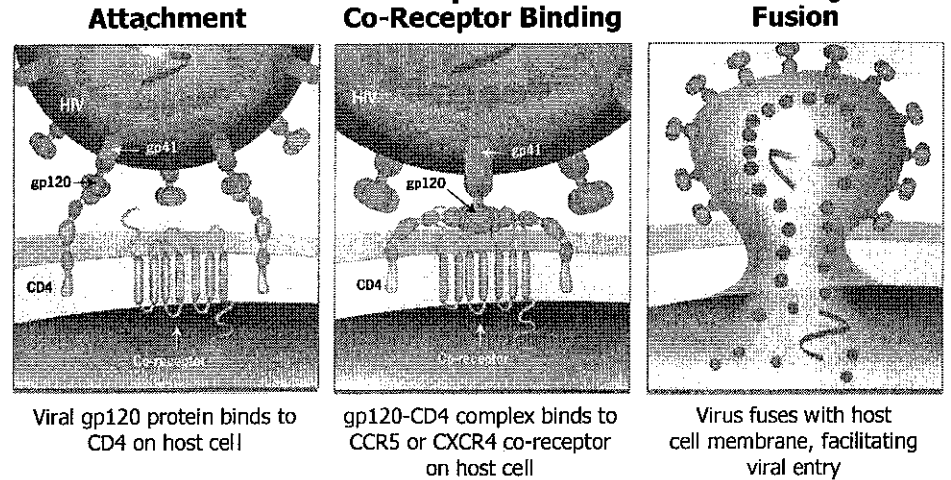
- HIV enters cells by a complex process
  - Sequential attachment to the CD4 receptor
  - Binding to either CCR5 or CXCR4 molecules
  - Fusion of the viral and cellular membranes
- CCR5 inhibitors prevent HIV entry into target cells by binding to the CCR5 receptor
- Before using a CCR5 antagonist, R5 tropic virus must be confirmed
- Maraviroc (MVC): only approved CCR5 antagonist

# Viral Tropism

- Viral tropism: the specific co-receptor that a particular HIV-1 virus uses to enter CD4-cells
- HIV-1 enters CD4 cells via:
  - CCR5 (R5)
  - CXCR4 (X4)
  - Viral mixtures using R5 and X4: dual or mixed tropic

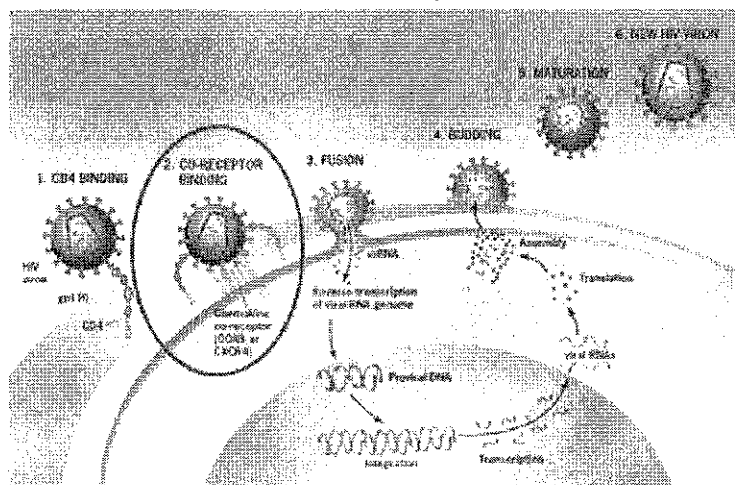
DHHS Treatment Guidelines, 2012

# Co-Receptor Binding is an Essential Step for Viral Entry



Adapted from Moore J, et al. *Proc Natl Acad Sci U S A.* 2003;100:10598-10602.

# The HIV Life Cycle Provides Many Targets for Therapeutic Intervention

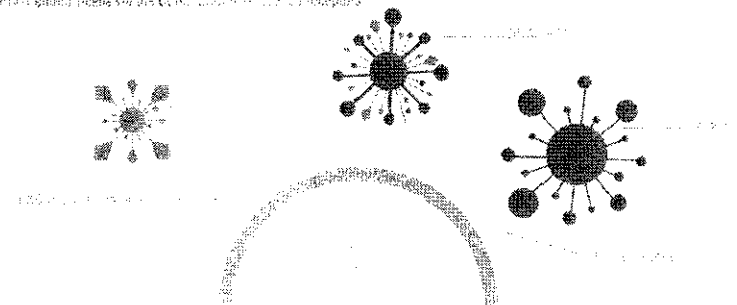


Adapted from Janeway C, et al. *Immunobiology*, 5<sup>th</sup> ed., New York, NY Garland Publishing, 2001: 456-457.

# Viral Tropism

**Viral Tropism**  
Creating a more complete HIV profile

- HIV-1 needs a co-receptor on the surface of target cells to enter and infect them
- Viral tropism refers to the specific co-receptor that HIV-1 virus uses to enter T-cells
- HIV-1 enters T-cells via the CD4, CCR5 or CXCR4 co-receptors



## Why test for tropism?

- Laboratory or clinical adverse events necessitating a change in regimen
- Patient intolerance to current regimen
- Concern regarding the long-term effects of a current regimen
- Desire to use MVC while tropism is still CCR5 tropic

DHHS Treatment Guidelines, 2012

## Viral Tropism

- R5-tropic virus: more common in treatment-naïve patients
- X4-tropic virus:
  - 13% of recent sero-converters
  - 50% of treatment experienced patients and those with advanced disease
  - X4 virus may emerge over time in patients initially infected with R5 virus

Wilkin TJ et al. Clin Infect Disease. 2007;44:591-595

## HIV Uses R5, X4, or a Combination of Co-receptors to Infect CD4 Cells

Co-receptor	Description	Prevalence
R5 (CCR5 tropic)	R5 virus infects CD4+ T-cells only through the CCR5-co-receptor	73% in treatment-naïve patients 37% highly treatment-experienced patients
X4 (CXCR4 tropic)	X4 virus infects CD4+ T-cells only through the CXCR4-co-receptor	0.3% in treatment naïve patients 4% in highly treatment-experienced patients
Dual (Dual tropic)	Dual tropic virus infects CD4+ T-cells only the through either the CCR5 or CXCR4-co-receptor	Unknown
D/M (Dual/Mixed tropic)	Dual/Mixed viral populations contain a combination of R5 and/or X4 and/or dual tropic viruses	27% in treatment naïve patients 59% in highly treatment-experienced patients

## Tropism Testing: DHHS

- Most patients have R5 virus during acute and recent infection
- Patients may have a shift in coreceptor tropism from R5 to CXCR4 or both R5 and X4 (dual mixed)
- Extensive drug resistance: more likely to have X4 or dual/mixed
- CD4 < 100 associated with greater risk for X4 or dual/ mixed

DHHS Treatment Guidelines, 2012

# Tropism Assays

- Phenotypic
  - Monogram Biosciences
    - Trofile®
    - Trofile DNA®
- Genotypic
  - Quest Diagnostics
    - HIV-1 Coreceptor Tropism with Reflex to Ultradeep Sequencing

# Tropism Testing

- Original Trofile® Test
- Single-cycle, recombinant virus assay (phenotype)
- Isolate viral RNA from circulating HIV virions in plasma
- Viral load: >1,000 copies /mL
- Cost: expensive
- Voucher: possible
- TAT: 2-3 weeks
- Results: CCR5, CXCR4, or D/M tropic

trofile

**trofile**  
CD-RECEPTOR TROPISM ASSAY

**ACTIVITY OF CCR5 ANTAGONIST ANTICIPATED?**  
 YES  
 NO

**ACTIVITY OF CCR5 ANTAGONIST ANTICIPATED?**  
 YES  
 NO

Trofile X4

**trofile**  
CD-RECEPTOR TROPISM ASSAY

**ACTIVITY OF CCR5 ANTAGONIST ANTICIPATED?**  
 YES  
 NO

**ACTIVITY OF CCR5 ANTAGONIST ANTICIPATED?**  
 YES  
 NO

# Trofile® DNA HIV-1 Co-Receptor Tropism Assay

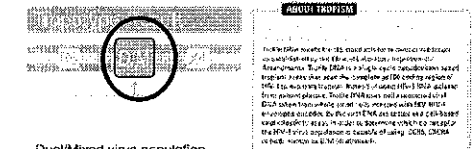
- Single-cycle, recombinant phenotypic virus similar Trofile®
- Trofile® DNA isolates viral DNA from HIV infected blood cells in whole blood
- Viral load: < 1,000 copies / mL
- TAT: 3 weeks
- Cost: expensive
- Vouchers: possible
- Results: CCR5, CXCR4, or D/M tropic

# trofileDNA

trofileDNA  
HIV-1 CO-RECEPTOR TROPISM ASSAY

THE UNIVERSITY OF CHICAGO  
Center for HIV/AIDS Research, Prevention, and Control  
608 S. EAST ASIAN BLVD. CHICAGO, IL 60607-7173

Client Name: [Redacted]      Date: 09/27/06  
 Facility: [Redacted]      Date: [Redacted]  
 Referring Physician: [Redacted]      Date: [Redacted]  
 Comments: [Redacted]      HIV Envelope Subtype: B



Dual/Mixed virus population can use CXCR4 and/or CCR5 co-receptors to enter the CD4+ cell.

**ABOUT TROPISM**

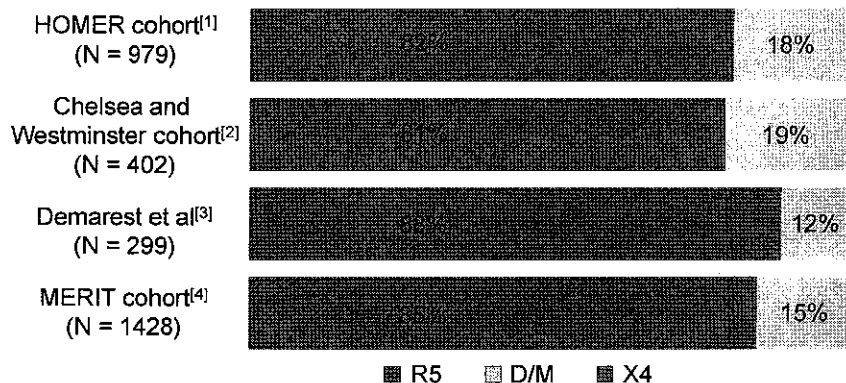
The trofileDNA assay is a single cycle phenotypic assay that uses a recombinant HIV-1 virus containing a reporter gene to determine the co-receptor usage of HIV-1. The assay is performed in a single cycle and the results are reported as R5, D/M, or X4.

Co-receptor usage is determined by the ability of HIV-1 to enter a target cell via either CCR5 or CXCR4. HIV-1 that uses CCR5 is referred to as R5. HIV-1 that uses CXCR4 is referred to as X4. HIV-1 that uses both CCR5 and CXCR4 is referred to as D/M.

The trofileDNA assay is a single cycle phenotypic assay that uses a recombinant HIV-1 virus containing a reporter gene to determine the co-receptor usage of HIV-1. The assay is performed in a single cycle and the results are reported as R5, D/M, or X4.

# HIV Tropism in Antiretroviral-Naive Populations

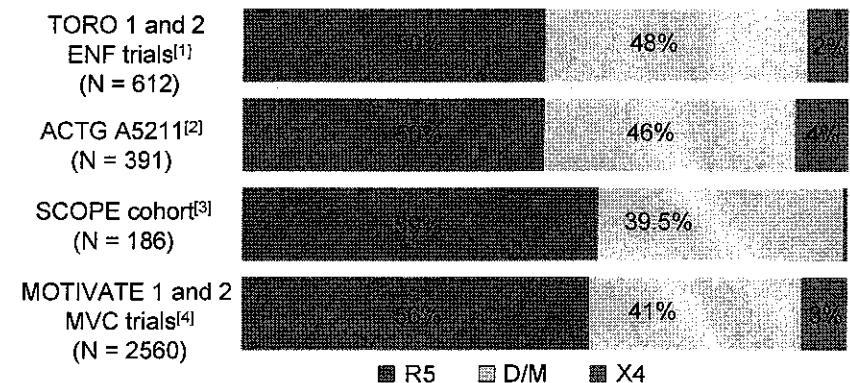
- R5-only virus in 80% to 90% of patients, with D/M or X4 virus in remainder



1. Brumme ZL, et al. J Infect Dis. 2005;192:466-474. 2. Moyle GJ, et al. J Infect Dis. 2005;191:866-872. 3. Demarest J, et al. ICAAC 2004. Abstract H-1136. 4. Coakley E, et al. Intl Wkshp on Targeting HIV Entry 2006. Abstract 8.

# HIV Tropism in Antiretroviral-Experienced Populations

- R5-only virus in 50% to 60% of patients, with D/M or X4 virus in remainder



1. Melby J, et al. J Infect Dis. 2006;194:238-246. 2. Wilkin TJ, et al. Clin Infect Dis. 2007;44:591-595. 3. Hunt PW, et al. J Infect Dis. 2006;194:928-930. 4. Coakley E, et al. Intl Wkshp on Targeting HIV Entry 2006. Abstract 8.

# Caveats Related to Prevalence of CCR5 Tropic Virus

- Higher CD4<sup>+</sup> T-Cell counts are generally associated with increased CCR5 prevalence
- ARV-naïve patients are more likely to have CCR5-tropic virus compared to treatment experienced patients

Wilkin T, et al. *Clin Infect Dis.* 2011;52:925- 928.  
 Hunt PW, et al. *J Infect Dis.* 2006;194:926-930.  
 Demarest J, et al. 44th ICAAC 2004; Poster H-1136.  
 Brumme Z, et al. *J Infect Dis.* 2005;192:466-474.

# R5 Tropism Result

**trofile**  
CO-RECEPTOR TROPISM ASSAY

Hillsborough County Health Department  
Specialty Care Center 1115 E. Kennedy Blvd.  
Tampa, FL 33602  
USA

Client: 0146  
Request: 02/18/2015  
Phone: 813-274-8415 Fax: 813-274-8414

Request ID: 02-183752  
Client ID: 02-183752  
Date: 02-18-2015  
Age of Specimen: FINAL

Specimen: HIV-1  
Patient: 0146

Tested: 02-18-2015 12:00  
Lab: HIV-1  
Lab Requested: 02-18-2015 10:00  
Lab Reported: 02-18-2015 10:00

Ordering Physician: [Redacted]  
Ordering Facility: [Redacted]

**Tropism Result**  
R5

**ABOUT TROPISM**  
Trofile is a cell-based approach to determine a patient's HIV co-receptor tropism (the "keyhole"). Trofile uses the complete gp120 coding region of the HIV-1 envelope protein, including the gp120 V3 loop, to determine if trofile is able to bind to and activate the CD4 co-receptor and to one of two co-receptors, CCR5 or CCR2. CCR5 Tropic (R5) HIV-1 Virus uses CCR5 to enter CD4+ cells. CCR2 Tropic (R2) HIV-1 Virus uses CCR2 to enter CD4+ cells. DUAL/MIXED Tropic (DM) HIV-1 Dually-tropic viruses can use either CCR5 or CCR2 to enter CD4+ cells. Mixed-tropic populations contain viruses with two or more tropisms. Non-reportable Co-receptor tropism could not be determined by the Trofile assay. Common causes of a non-reportable result are viral load <1,200 copies/mL, reduced viral fitness, or compromised sample collection/handling. Trofile is not applicable to HIV-1 strains with the following mutations: CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F, CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F.

**Activity of CCR5 antagonist anticipated?**  YES  NO

# CCR5 Tropic Result

**Tropotype Result**  
R5 DM X4

**ABOUT TROPISM**  
Trofile is a cell-based approach to determine a patient's HIV co-receptor tropism (the "keyhole"). Trofile uses the complete gp120 coding region of the HIV-1 envelope protein, including the gp120 V3 loop, to determine if trofile is able to bind to and activate the CD4 co-receptor and to one of two co-receptors, CCR5 or CCR2. CCR5 Tropic (R5) HIV-1 Virus uses CCR5 to enter CD4+ cells. CCR2 Tropic (R2) HIV-1 Virus uses CCR2 to enter CD4+ cells. DUAL/MIXED Tropic (DM) HIV-1 Dually-tropic viruses can use either CCR5 or CCR2 to enter CD4+ cells. Mixed-tropic populations contain viruses with two or more tropisms. Non-reportable Co-receptor tropism could not be determined by the Trofile assay. Common causes of a non-reportable result are viral load <1,200 copies/mL, reduced viral fitness, or compromised sample collection/handling. Trofile is not applicable to HIV-1 strains with the following mutations: CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F, CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F.

**Activity of CCR5 antagonist anticipated?**  YES  NO

# Trofile DNA

- Non-reportable: Co-receptor tropism could not be determined

- Common causes:
- Reduced viral fitness or compromised sample handling
  - Trofile DNA sample collection and handling instructions differ from Trofile and other Monogram assays

**trofile**  
CO-RECEPTOR TROPISM ASSAY

Hillsborough County Health Department  
Specialty Care Center 1115 E. Kennedy Blvd.  
Tampa, FL 33602  
USA

Client: 0146  
Request: 02/18/2015  
Phone: 813-274-8415 Fax: 813-274-8414

Request ID: 02-183752  
Client ID: 02-183752  
Date: 02-18-2015  
Age of Specimen: FINAL

Specimen: HIV-1  
Patient: 0146

Tested: 02-18-2015 12:00  
Lab: HIV-1  
Lab Requested: 02-18-2015 10:00  
Lab Reported: 02-18-2015 10:00

Ordering Physician: [Redacted]  
Ordering Facility: [Redacted]

**Tropism Result**  
Non-reportable

**ABOUT TROPISM**  
Trofile DNA uses the full sequence for the gp120 V3 loop, as established by the Clinical Laboratory Improvement Amendments. Trofile DNA is a single cycle quantitative assay that uses a real-time PCR to amplify the gp120 V3 loop region of the HIV-1 envelope protein. Trofile DNA uses a cell-based assay to determine if trofile is able to bind to and activate the CD4 co-receptor and to one of two co-receptors, CCR5 or CCR2. Trofile DNA is not applicable to HIV-1 strains with the following mutations: CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F, CCR5 Y188L, CCR5 Y188I, CCR5 Y188V, CCR5 Y188F.

**Activity of CCR5 antagonist anticipated?**  YES  NO



## Quest: HIV-1 Coreceptor Tropism with Reflex to Ultradeep Sequencing

- Results reported:
- Population sequencing: X4 detected or not
- Ultradeep sequencing X4
  - Analytical sensitivity
    - 12% for X4 virus in dual/mixed samples at 25,000 copies/mL
    - 5% X4 virus at 100,000 copies/mL

## Quest Genotypic Assay

- **Quicker TAT**
- **More cost effective**
- **Performs comparably to phenotypic testing**

## HIV-1 Coreceptor Tropism With Reflex to Ultradeep Sequencing (UDS)

- Quest Diagnostics
- Cost: 2 tiers
  - Tier 1: Initial Testing
  - Tier 2: Additional testing for ultradeep sequencing if tropism is not detected on first round

## HIV-1 Coreceptor Tropism with Reflex to Ultradeep Sequencing Interpretation

- X4 Detected:
  - CCR5 antagonists not recommended
- X4 Not Detected:
  - Consistent with eligibility for treatment with CCR5 antagonist

## **HIV Genotypic Sequencing Test performs comparably to standard phenotypic test in predicting potential**

### **response to CCR5 antagonist**

- Genotypic and phenotypic tests performed comparably at predicting response in patients undergoing therapy with maraviroc
- At week eight, the positive predictive value was 66 percent for the phenotypic test and 65 percent for the genotypic test, and negative predictive values were 59 percent for phenotyping and 58 percent for genotyping
- can provide results from the testing service in approximately a week for samples with a TPS result of X4 and in as little as 10 days for samples reflexed to UDS, compared to reported turnaround times of approximately 14 days for the phenotyping test used in the study.

Need to simplify and condense

Kagan RM et al. PLOS One. September 2012. [www.plos.one.org](http://www.plos.one.org)

## **Hepatitis C Testing**

## **Hepatitis C Resistance Testing**

- Rapid progression of HCV research and treatment agents
- HCV replicates to high levels and with poor fidelity and can rapidly accumulate mutations that may confer drug resistance
- Need to identify drug resistant mutations that may impact the efficacy of PI therapy

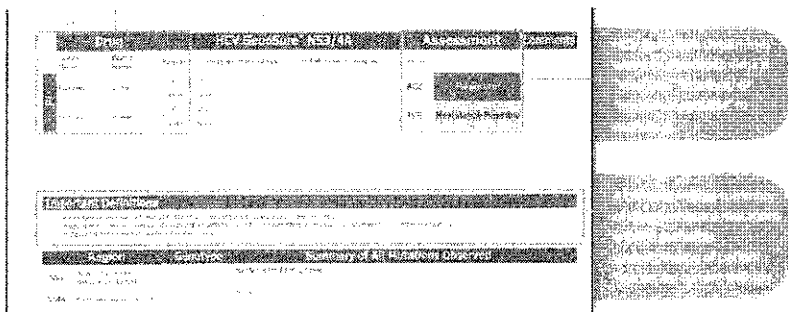
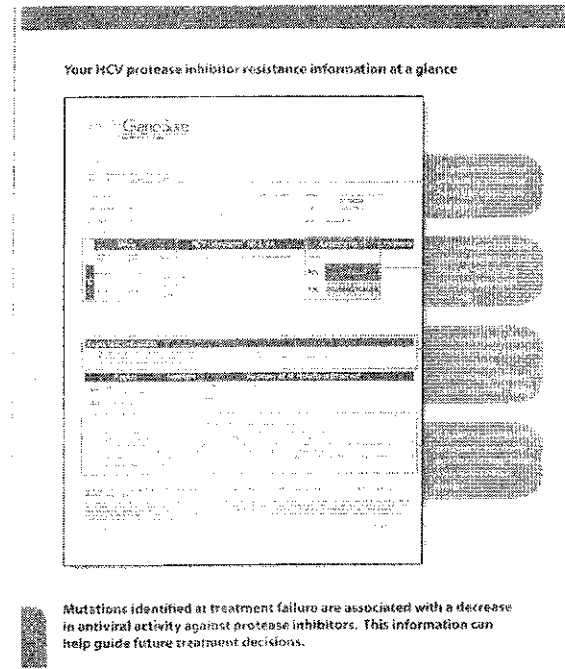
## **HCV GenoSure NS3/4A**

*LabCorp/Monogram Biosciences*

- Provides a comprehensive sequence-based analysis of drug resistance for HCV based direct-acting antiviral (DAA) therapies
- Available as a reflex test when VL  $\geq$  2000 IU/mL

## HCV GenoSure NS3/4A LabCorp/Monogram Biosciences

- Analyzes genetic sequence for nonstructural proteins NS3 and NS4A of HCV genotypes 1a and 1n
- Detects mutations in NS3 and NS4A and identifies drug-resistant variants for PIs boceprevir and telaprevir
- Sensitivity to detect minor variant levels as low as 10%
- Interpretation: *sensitive, resistant or resistance possible (for each drug)*



[www.monogrambio.com](http://www.monogrambio.com)

## Monogram Biosciences HCV Tests

Name	Clinical Use
HCV Viral Load	Quantifies amount of HCV viral RNA
HCV Genotype (subtype)	Determines viral genotype (subtype) 1-6
Interleukin 28B (IL28B) polymorphism	Determines IL28B polymorphism associated with response to peg IFN/ribavirin
HCV GenoSure NS3/4A Drug Resistance	Determines genotypic resistance for NS3/4A PIs
HCV FibroSURE	Assessment of fibrosis and necroinflammatory activity in liver

[www.monogrambio.com](http://www.monogrambio.com)

## HLA-B\*5701

## HLA-B\*5701 DHHS Guidelines

- Screen for HLA-B\*5701 before starting an abacavir (ABC) –containing regimen
  - Ziagen, Epzicom, Trizivir
- HLA-B\*5701 positive patients should NOT be prescribed ABC
- Record positive result as an allergy in medical record
- IF HLA-B\*5701 testing is not available, it is reasonable to initiate ABC with clinical counseling and monitoring for signs of HSR

DHHS Guidelines, 2012

## Incidence of HSR

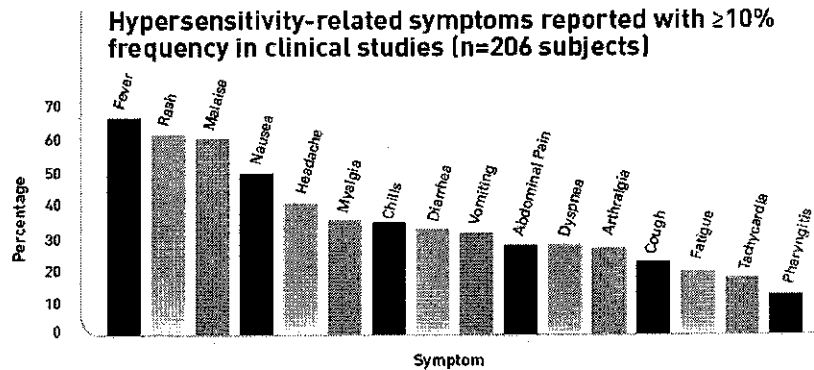
- Hypersensitivity to abacavir was reported in approximately 8% of 2,670 patients (n=206) in 9 clinical trials (range 2% to 9%) with enrollment from November 1999 to February 2002

RE- WORK SLIDE

## HLA-B\*5701 Genotyping

- Patients who carry the *HLA-B\*5701* allele are at high risk for experiencing a hypersensitivity reaction to abacavir
- Prior to initiating therapy with abacavir, screening for the *HLA-B\*5701* allele is recommended
  - This approach has been found to decrease the risk of a hypersensitivity reaction
- Screening is also recommended prior to reinitiation of abacavir in patients of unknown *HLA-B\*5701* status who have previously tolerated abacavir

## ABC Hypersensitivity-Related Symptoms

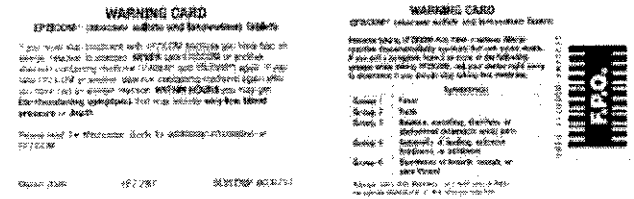


GSK  
CDC. <http://www.cdc.gov>

## Patient Warning Card Abacavir Containing Regimens

### Patient Warning Card

- Distributed at pharmacy each time prescription is filled



CDC. <http://www.cdc.gov>

## HLA-B\*5701 Specimen Collection

- Blood testing
- Buccal swabs  $\Rightarrow$  DNA extracted from cells
  - Buccal results may have higher rates of accuracy
  - Serum results could be affected by recent blood transfusion or bone marrow transplant
  - Ideal for geographically dispersed situations
- TAT: several days – 1 week
- Cost: varies
  - Limited or no insurance: voucher possible?

CDC. <http://www.cdc.gov>

## Interpreting HLA-B\*5701 Results

- HLA-B\*5701 *Negative* Proceed with dosing
- HLA-B\*5701 *Positive*: Do not administer abacavir containing regimen to patient
  - Abacavir (Ziagen), Epzicom, Trizavir
  - Document allergy in chart and with pharmacies
- Inform patient; provide allergy alerts
- Provide counseling related to ABC HSR to patients testing negative

CDC. <http://www.cdc.gov>

# Quest Diagnostics Tropism Assay

HIV-1 Coreceptor Tropism  
With Reflex to Ultradeep  
Sequencing (UDS)

CDC. <http://www.cdc.gov>

# Tuberculosis Blood Tests

Interferon-Gamma  
Release Assays (IGRAs)

CDC. <http://www.cdc.gov>

## Interferon-Gamma Release Assays IGRA

- Measures how the immune system reacts to the bacteria that cause TB
- Measures how strong a person's immune system reacts to TB bacteria by testing the person's blood
- Does not help differentiate LTBI from TB disease

CDC. <http://www.cdc.gov>

## How Do IGRAs Work?

- Measure a person's immune reactivity to *M. tuberculosis*
- WBCs from most persons that have been infected with *M. tuberculosis* will release interferon-gamma (IFN-g) when mixed with antigens derived from *M. tuberculosis*
- Fresh blood samples are mixed with antigens and controls

CDC. <http://www.cdc.gov>

## FDA Approved IGRAs

- QuantiFERON®-TB Gold In-Tube test (QFT-GIT)
- T-SPOT®

CDC. <http://www.cdc.gov>

## Interpretation

- Positive IGRA
  - Patient has been infected with TB bacteria
  - Additional testing needed to determine if patient has latent TB infection or TB disease
- Negative IGRA
  - Person's blood did not react to the test
  - Latent TB or TB disease not likely
- *IGRAs are not affected by prior BCG vaccination and are not expected to give false positive results*

CDC. <http://www.cdc.gov>

## Differences in IGRAs

	QFT-GIT	T-Spot
<b>Initial Process</b>	Process whole blood within 16 hours	Process PBMCs within 8 hours, or if T-Cell Xtend is used, within 30 hours
<b>Measurement</b>	IFN-g concentration	Number of IFN-g producing cells (spots)
<b>Possible Results</b>	Positive, negative, indeterminate	Positive, negative, indeterminate, borderline

CDC. <http://www.cdc.gov>

## Advantages of IGRAs

- Requires a single patient visit to conduct the test
- Results can be available within 24 hours
- Does not boost responses measured by subsequent tests
- Prior BCG vaccination does not cause false positive IGRA test

CDC. <http://www.cdc.gov>

## IGRA Limitations and Disadvantages

- Blood samples must be processed within 8-30 hours after collection while WBCs are still viable
- Errors in collecting or transporting blood specimens or in running and interpreting the assay can decrease the accuracy
- Limited data on use of IGRAs to predict who will progress to TB disease in the future

CDC. <http://www.cdc.gov>

## IGRA Limitations and Disadvantages

- Limited data on the use of IGRAs for:
  - Children younger than 5 years of age
  - Persons recently exposed to *MTB*
  - Immuno-compromised persons
  - Serial testing
- Testing may be expensive

CDC. <http://www.cdc.gov>

## Interpreting IGRA Test

- Based on amount of IFN-g that's released or on number of cells that release IFN-g
- Qualitative interpretation: positive, negative, indeterminate
- Quantitative interpretation: Nil, TB, and Mitogen concentrations or spot counts

CDC. <http://www.cdc.gov>

## An Aid In Diagnosing TB

- Positive: suggests that MTB infection is likely
- Negative: suggests that MTB infection is unlikely
- Indeterminate: uncertain likelihood of MTB
- Borderline T-Spot: uncertain likelihood of MTB

CDC. <http://www.cdc.gov>

## LTBI Diagnosis

- Requires that TB disease be excluded by medical evaluation
  - Signs and symptoms suggestive of TB
  - Chest X-ray
  - Sputum (or other specimen samples)

CDC. <http://www.cdc.gov>

## When to Use IGRA Tests

- Used in place of TST in all situations in which CDC recommends TST as an aid in diagnosing MTB infection
  - Contact investigations
  - Testing during pregnancy
  - Screening of health care workers and others undergoing serial evaluation for MTB
  - *Consider in HIV infected patients who are non-adherent to TST (not a CDC recommendation)*

CDC. <http://www.cdc.gov>

## HIV Home Rapid Testing

The screenshot shows the top portion of the FDA website. At the top left is the FDA logo with the text "U.S. Food and Drug Administration" and "Protecting and Promoting Your Health". To the right are links for "Why? Index", "Follow FDA", and "FDA Voice Blog". Below this is a search bar with the text "What's New" and "SEARCH". A horizontal navigation menu contains the following items: Home, Food, Drugs, Medical Devices, Vaccines, Blood & Biologics, Animal & Veterinary, Cosmetics, Radiation-Emitting Products, and Tobacco Products.

### News & Events

Home News & Events Newsroom Press Announcements

#### FDA NEWS RELEASE

For Immediate Release: July 3, 2012  
Media Inquiries: Rita Chappelle, 301-796-4572, [rita.chappelle@fda.hhs.gov](mailto:rita.chappelle@fda.hhs.gov)  
Consumer Inquiries: 888-InfO-FDA

En Español

#### FDA approves first over-the-counter home-use rapid HIV test

The U.S. Food and Drug Administration today approved the OraQuick In-Home HIV Test, the first over-the-counter home-use rapid HIV test kit to detect the presence of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). HIV is the virus that causes acquired immune deficiency syndrome (AIDS).

The OraQuick In-Home HIV Test is designed to allow individuals to collect an oral fluid sample by swabbing the upper and lower gums inside of their mouths, then place that sample into a developer vial, and obtain test results within 20 to 40 minutes. A positive result with this test does not mean that an individual is definitely infected with HIV, but rather that additional testing should be done in a medical setting to confirm the test result.

Similarly, a negative test result does not mean that an individual is definitely not infected with HIV, particularly when exposure may have been within the previous three months. The test has the potential to identify large numbers of previously undiagnosed HIV infections, especially if used by those unlikely to use standard screening methods.

The Centers for Disease Control and Prevention estimates that 1.2 million people in the United States are living with HIV infection. About one in five are not aware they are infected. There are about 50,000 new HIV infections every year. Many of these new infections are transmitted from people who are unaware of their HIV status.

"Knowing your status is an important factor in the effort to prevent the spread of HIV," said Karen Midtrun, M.D., director of the FDA's Center for Biologics Evaluation and Research. "The availability of an over-the-counter home-use rapid HIV test kit provides another option for individuals to get tested so that they can seek medical care, if appropriate."

Clinical studies for self-testing have shown that the OraQuick In-Home HIV Test has an expected performance of 92 percent for test sensitivity, the percentage of results that will be positive when HIV is present. This means that one false negative result would be expected out of every 12 test results in HIV-infected individuals.

## OraQuick Test

- Close to 100% accurate when it indicates that someone is NOT infected, and in fact, is not infected
- 93% accurate when it says that someone is not infected and the person actually does have HIV, though the body is not yet producing antibodies

## Home HIV Testing

- Products now on the market
- Primary purpose: person can find out privately if they have HIV
- Secondary: 70% of clinical study patients stated they would screen for potential sexual partner
- Cost: \$40 per test
- Could result in unprotected sex: risk of HIV and other STIs

<http://www.oraquick.com/>

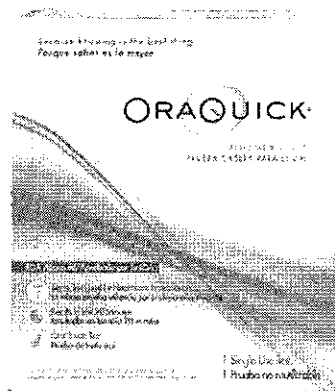
ORAQUICK

Home

What is OraQuick?

Taking the Test

About HIV



### The first in-home oral HIV test

- Trusted: The first and only in-home oral HIV test
- Fast: Results in 20 minutes
- Oral: No need for needles or blood samples
- Confidential: 24/7 support available
- Safe & Effective: Easy to use

Learn More

## OraQuick In-Home HIV Test

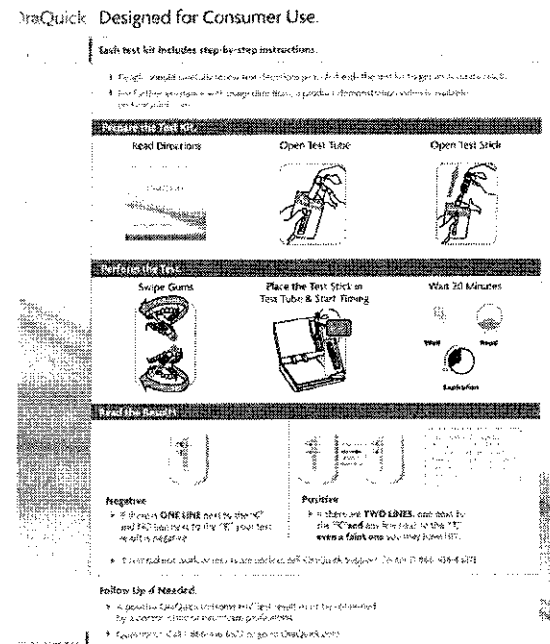
- Qualitative test that is designed to be visually read and tests for the presence of antibodies to HIV-1 and HIV-2
- Oral swab
- Results in 20 minutes
- FDA approved 7/3/12 for age 17+
- Toll free support available 24/7

<http://www.oraquick.com/>

<http://www.oraquick.com/>

# Ora-Quick In-Home HIV Test

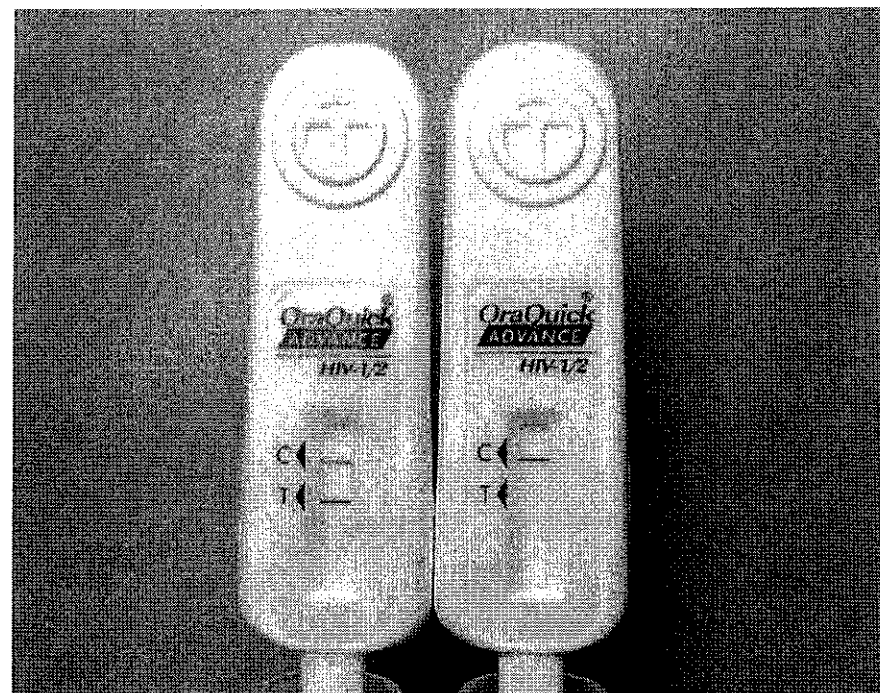
- **Specificity: 99.8%**
  - The percentage of results that will be negative when HIV is not present
  - One false positive would be expected out of every 5,000 tests in uninfected individuals
  
- **Sensitivity: 91.67%**
  - The percentage of results that will be positive when HIV is present
  - One false negative test would be expected out of every 12 test results in HIV-infected individuals



<http://www.oraquick.com/>

# Home Testing: Oral Swab

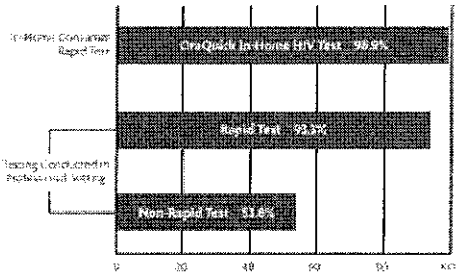
- |   |  |
|---|--|
| <ul style="list-style-type: none"> <li>■ Pro</li> <li>■ More privacy</li> <li>■ 24 hour hotline</li> <li>■ Usually more confidential</li> </ul> | <ul style="list-style-type: none"> <li>■ Con</li> <li>■ More expensive</li> <li>■ Patient error in sampling / interpretation</li> <li>■ Patients may be testing too early</li> <li>■ Confidentiality could be compromised: kit in trash, credit card or store receipt</li> <li>■ No guaranteed counseling and education</li> <li>■ False negative results</li> </ul> |
|---|--|



**OraQuick increases the number of people who benefit from knowing their HIV status.**

Less than 54% of patients who took non-rapid tests in a professional setting actually followed up to receive their test results. The fast results and confidential in-home testing environment of the OraQuick test increased the receipt of HIV test results to 98.9%.

98.9% of people received test results with in-home rapid testing compared to 54% who were tested using non-rapid laboratory tests.



1. Gendreau, A., Smith, J., & Martin, T. (2004). *Effect of pre-test counseling, self-administered, rapid HIV testing, and post-test counseling on HIV testing and diagnosis among injection drug users in the United States*. *Journal of Acquired Immune Deficiency Syndromes*, 36(2), 275-280.  
2. CDC. (2003). *Behavioral Risk Factor Surveillance System, National Health and Medical Examination Survey*. Atlanta, GA: CDC.

<http://www.oraquick.com/>

# Overcome Barriers to Testing

- Fear of a positive test result
- Privacy

<http://www.oraquick.com/>

## OraQuick: Warnings and Precautions.

- ▶ A positive result with this test does not mean that you are definitely infected with HIV, but rather that additional testing should be done in a medical setting.
- ▶ A negative result with this test does not mean that you are definitely not infected with HIV, particularly when exposure may have been within the previous 3 months.
- ▶ If your test is negative and you engage in activities that put you at risk for HIV on a regular basis, you should test regularly.
- ▶ This product should not be used to make decisions on behavior that may put you at increased risk for HIV.

Please direct your patients to [OraQuick.com](http://www.oraquick.com) or our Support Center (1-866-436-6527) for complete resources and information.



<http://www.oraquick.com/>

## This at-Home HIV Test Looks Simple, but Is It Accurate?

U.S. Pat. 6,265,211; 6,488,874; 6,488,875; 6,488,876; 6,488,877; 6,488,878; 6,488,879; 6,488,880; 6,488,881; 6,488,882; 6,488,883; 6,488,884; 6,488,885; 6,488,886; 6,488,887; 6,488,888; 6,488,889; 6,488,890; 6,488,891; 6,488,892; 6,488,893; 6,488,894; 6,488,895; 6,488,896; 6,488,897; 6,488,898; 6,488,899; 6,488,900; 6,488,901; 6,488,902; 6,488,903; 6,488,904; 6,488,905; 6,488,906; 6,488,907; 6,488,908; 6,488,909; 6,488,910; 6,488,911; 6,488,912; 6,488,913; 6,488,914; 6,488,915; 6,488,916; 6,488,917; 6,488,918; 6,488,919; 6,488,920; 6,488,921; 6,488,922; 6,488,923; 6,488,924; 6,488,925; 6,488,926; 6,488,927; 6,488,928; 6,488,929; 6,488,930; 6,488,931; 6,488,932; 6,488,933; 6,488,934; 6,488,935; 6,488,936; 6,488,937; 6,488,938; 6,488,939; 6,488,940; 6,488,941; 6,488,942; 6,488,943; 6,488,944; 6,488,945; 6,488,946; 6,488,947; 6,488,948; 6,488,949; 6,488,950; 6,488,951; 6,488,952; 6,488,953; 6,488,954; 6,488,955; 6,488,956; 6,488,957; 6,488,958; 6,488,959; 6,488,960; 6,488,961; 6,488,962; 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# Drug Resistance Testing: DHHS Recommendations

- All HIV-infected individuals entering care, regardless of whether therapy will be initiated
- If therapy is deferred
  - Repeat testing prior to initiating antiretroviral therapy
- All patients on therapy with virologic failure
- All pregnant women prior to initiation of therapy
  - Those entering pregnancy with detectable HIV RNA levels while on therapy

DHHS : <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>  
Revision March 27, 2012.

# Genotype

- Most genotype assays involve sequencing RT and protease genes to detect mutation
  - Sequence of bases (A,C,T,G) coding for amino acids that comprise viral proteins
  - Expressed as the coded amino acid and position (eg, M184)
  - If mutated, the change is indicated after the position (eg, M184V)
- Results available within 1 to 2 weeks
- Interpretation of results usually requires a specialist

DHHS. Available at: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>  
Revision March 27, 2012.

## trofile® and trofileDNA - Reports

**trofile**  
CCR5 RECEPTOR GENOTYPE ASSAY

Sample ID: 1234567890  
Patient Name: J. Smith  
Date of Birth: 01/01/1980  
Lab ID: 1234567890  
HIV-1 Genotype Subject ID

**CCR5 RECEPTOR GENOTYPE**

Genotype: **CCR5-Δ32/CCR5**

**Interpretation:**  
This patient is CCR5-Δ32/CCR5. This genotype is associated with resistance to HIV-1 infection. The CCR5-Δ32 mutation is a 32 base pair deletion in the CCR5 gene, which is a coreceptor for HIV-1. This mutation is associated with a lower viral load and a higher CD4+ T cell count. The CCR5-Δ32/CCR5 genotype is associated with a lower risk of HIV-1 infection and a higher rate of viral suppression.

**Activity of CCR5 antagonist anticipated?**  YES  NO

**trofileDNA**  
CXCR4/CCR5 GENOTYPE ASSAY

Sample ID: 1234567890  
Patient Name: J. Smith  
Date of Birth: 01/01/1980  
Lab ID: 1234567890  
HIV-1 Genotype Subject ID

**CXCR4/CCR5 GENOTYPE**

Genotype: **CXCR4/CCR5**

**Interpretation:**  
This patient is CXCR4/CCR5. This genotype is associated with susceptibility to HIV-1 infection. The CXCR4/CCR5 genotype is associated with a higher viral load and a lower CD4+ T cell count. The CXCR4/CCR5 genotype is associated with a higher risk of HIV-1 infection and a lower rate of viral suppression.

**Dual/Mixed virus population can use CXCR4 and/or CCR5 co-receptors to enter the CD4+ cell.**