Specialty Lab Testing In HIV Care

Association of Nurses in AIDS Care
2012 Conference

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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

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

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

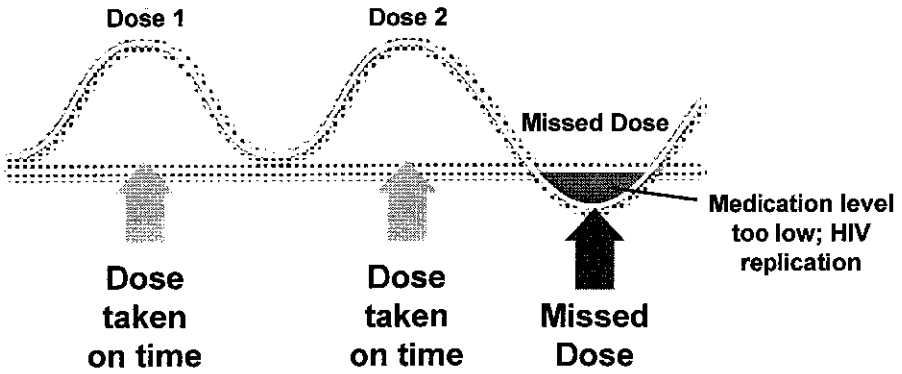
Disadvantages

- 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 my 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
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- Unable to detect minority variants (<10-25% of viral sample)
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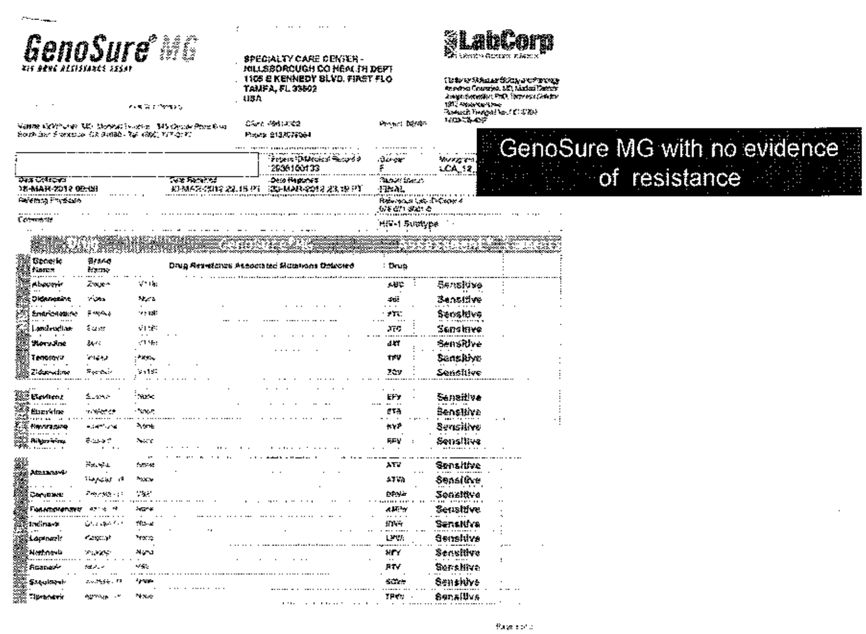
GenoSure® *MG*

- 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



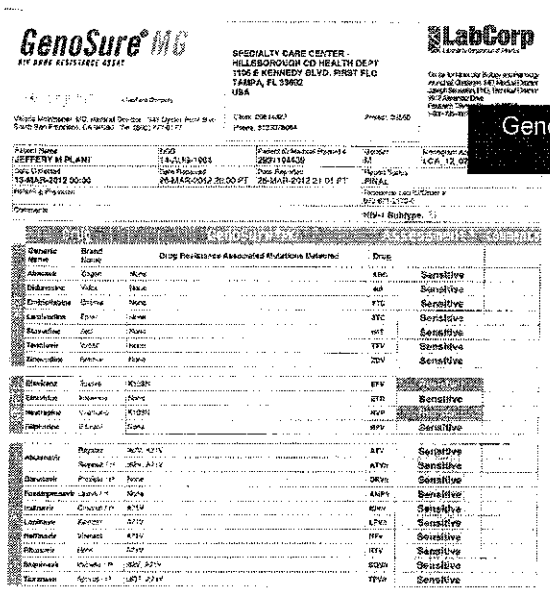
GenoSure® MG
HIV DRUG RESISTANCE ASSAY

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

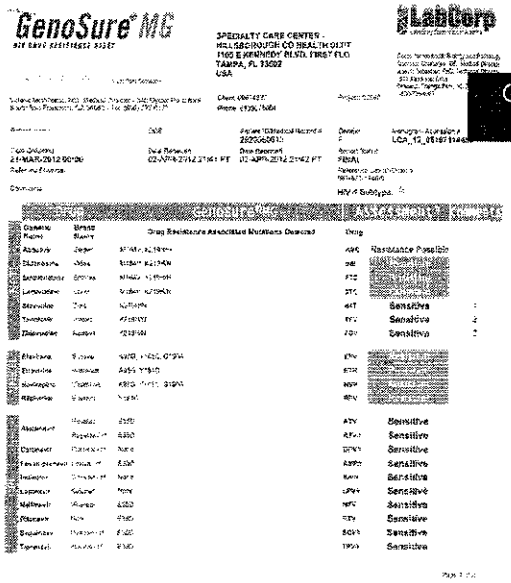
LabCorp

Patient ID/Medical Record #: 2936100133
Gender: F
Date Collected: 16-MAR-2012 06:08
Date Received: 20-MAR-2012 22:15 PT
Date Reported: 23-MAR-2012 23:19 PT
Report Status: FINAL
Referring Physician
Comments
HIV-1 Subtype

Generic Name	Brand Name		Drug Resistance Associated Mutations Detected	Drug	
Abacavir	[illegible]	[illegible]		ABC	Sensitive
Didanosine	[illegible]	[illegible]		ddI	Sensitive
Emtricitabine	[illegible]	[illegible]		FTC	Sensitive
Lamivudine	[illegible]	[illegible]		3TC	Sensitive
Stavudine	[illegible]	[illegible]		d4T	Sensitive
Tenofovir	[illegible]	[illegible]		TFV	Sensitive
Zidovudine	[illegible]	[illegible]		ZDV	Sensitive
Efavirenz	[illegible]	[illegible]		EFV	Sensitive
Etravirine	[illegible]	[illegible]		ETR	Sensitive
Nevirapine	[illegible]	[illegible]		NVP	Sensitive
Rilpivirine	[illegible]	[illegible]		RPV	Sensitive
Atazanavir	[illegible]	[illegible]		ATV	Sensitive
	[illegible]	[illegible]		ATV/r	Sensitive
Darunavir	[illegible]	[illegible]		DRV/r	Sensitive
Fosamprenavir	[illegible]	[illegible]		AMP/r	Sensitive
Indinavir	[illegible]	[illegible]		IDV/r	Sensitive
Lopinavir	[illegible]	[illegible]		LPV/r	Sensitive
Nelfinavir	[illegible]	[illegible]		NFV	Sensitive
Ritonavir	[illegible]	[illegible]		RTV	Sensitive
Saquinavir	[illegible]	[illegible]		SQV/r	Sensitive
Tipranavir	[illegible]	[illegible]		TPV/r	Sensitive

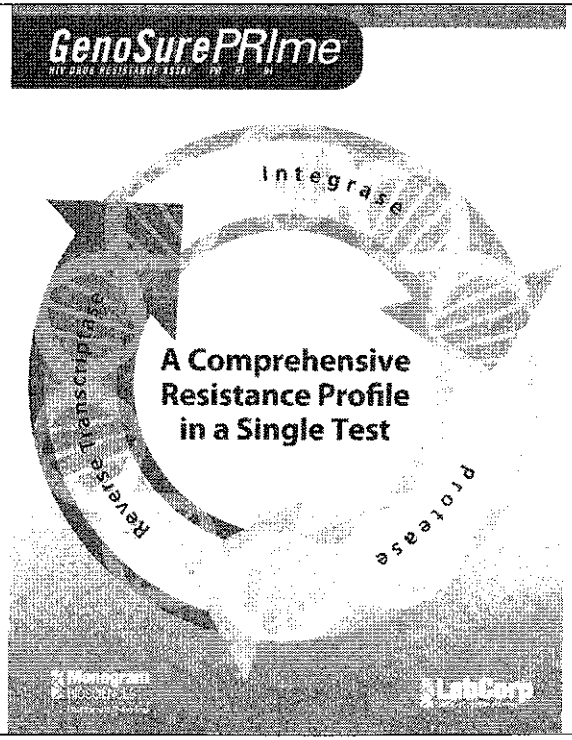


GenoSure MG with significant NNRTI resistance



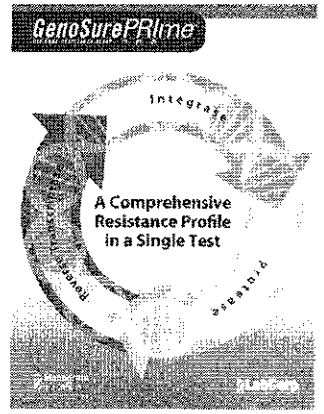
GenoSure MG with significant NRTI and NNRTI resistance

GenoSurePRIme



GenoSurePRIme

- Single genotypic assay providing a comprehensive picture of resistance
  - NRTIs
  - NNRTIs
  - PIs
  - INIs



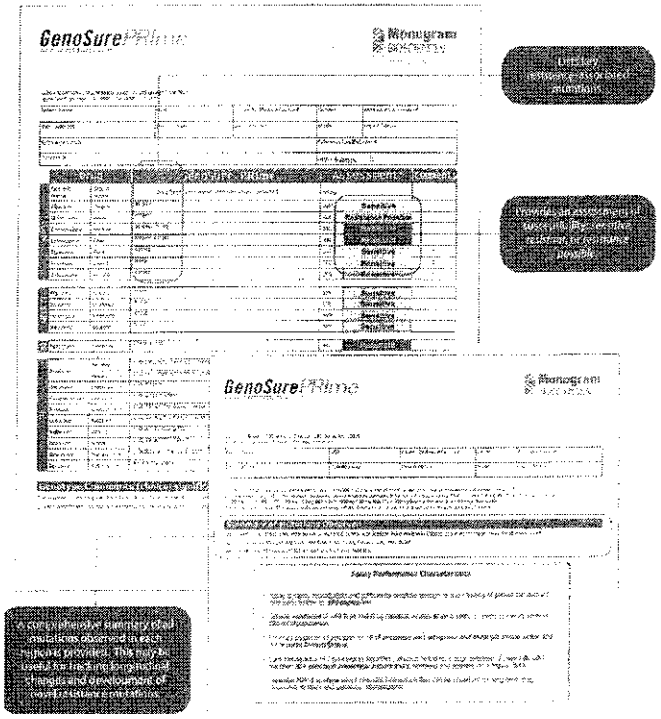
Features of GenoSurePRIme

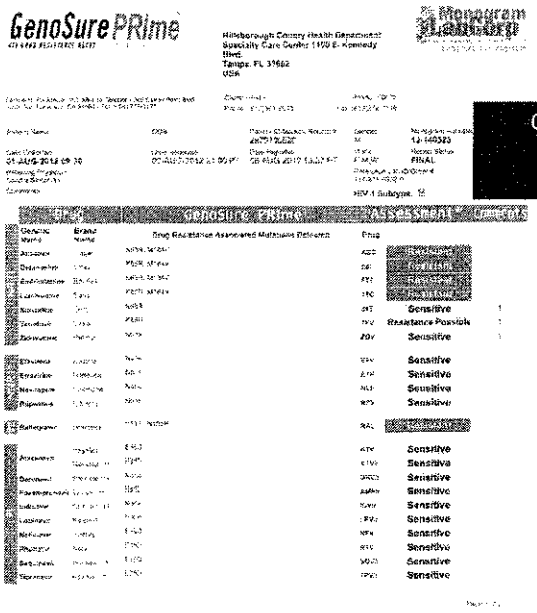
- The first HIV-1 genotype to provide susceptibility information for four drug classes in a single report
- Uses Monogram's proprietary HIV-1 genotyping algorithm which is based on a database of greater than 100,000 matched HIV-1 genotype-phenotype results
- Drug resistance algorithms are updated on a regular basis
- Available as a reflex from a quantitative viral load

Monogram Biosciences

Features of GenoSurePRime

- Provides an assessment of drug susceptibility
  - Sensitive
  - Resistance possible
  - Resistant
- Viral load ≥ 500 copies/mL
- TAT: 7-10 days
- Available as a reflex from a viral load



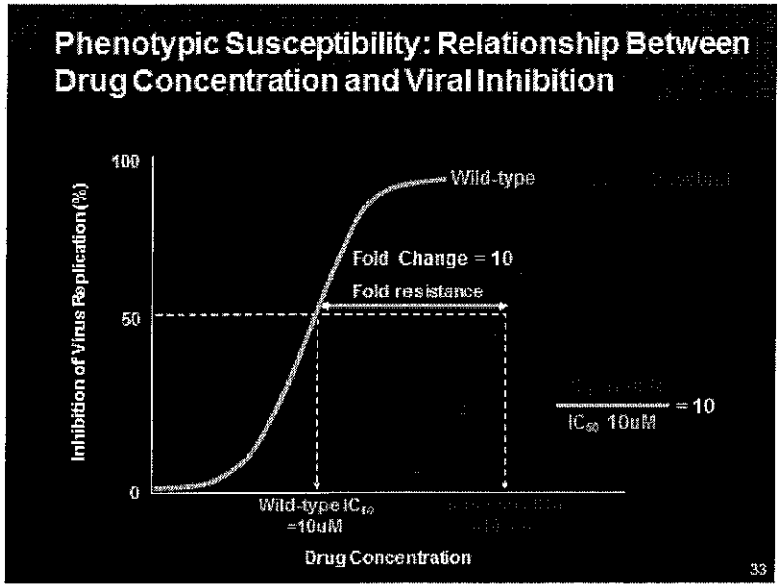


HIV Resistance Testing: Phenotype

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

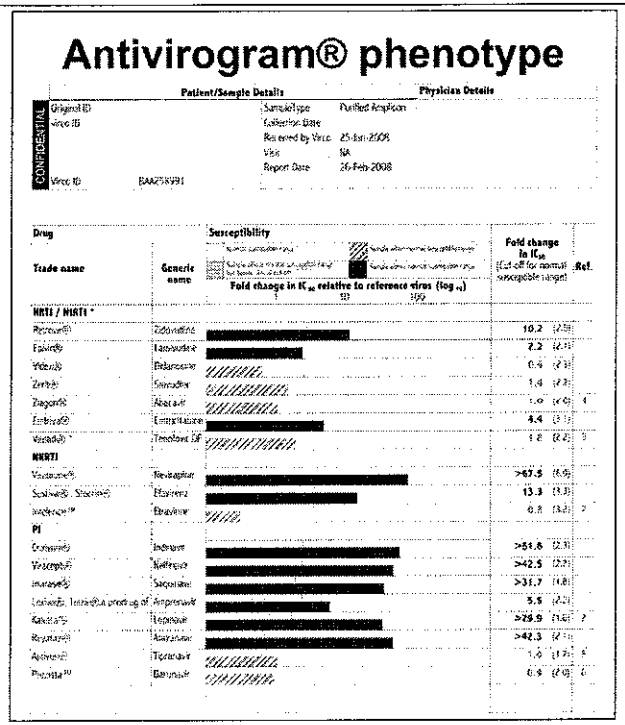
Phenotype



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

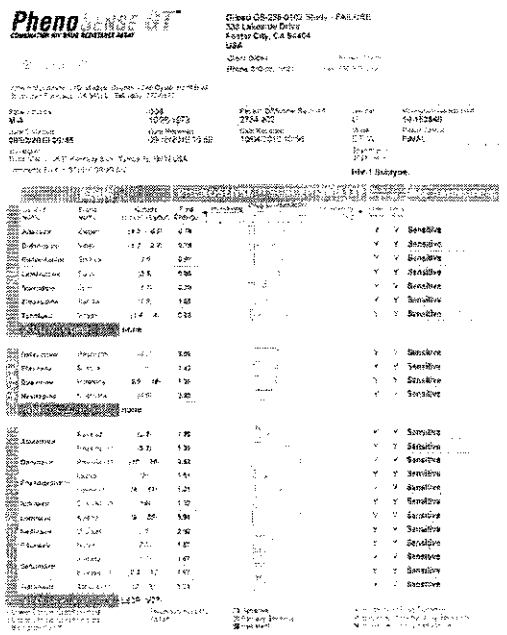


Phenosense

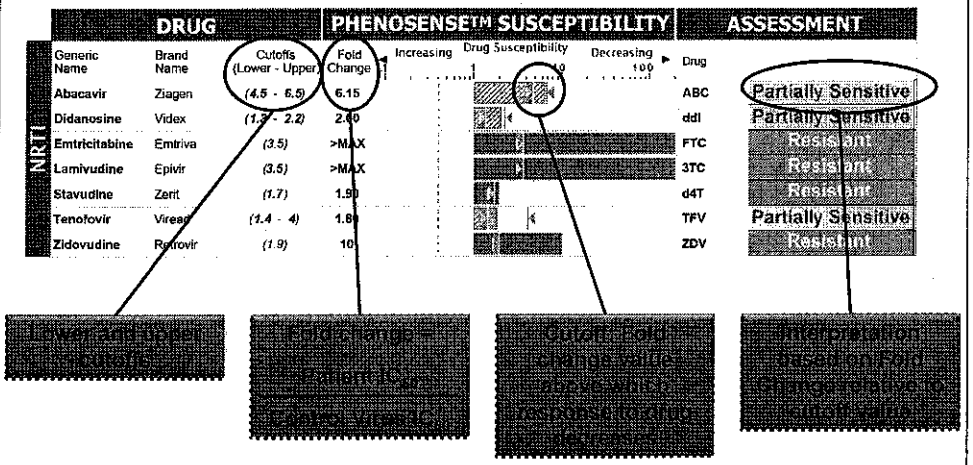
	DRUG			PHENOSENSE™ SUSCEPTIBILITY	ASSESSMENT	
	Generic Name	Brand Name	Cutoffs (Lower - Upper)	Fold Change	Drug	
NRTI	Abacavir	Ziagen	(4.5 - 6.5)	6.15	ABC	Partially Sensitive
	Didanosine	Videx	(1.3 - 2.2)	2.00	ddI	Partially Sensitive
	Emtricitabine	Emtriva	(3.5)	>MAX	FTC	Resistant
	Lamivudine	Epivir	(3.5)	>MAX	3TC	Resistant
	Stavudine	Zerit	(1.7)	1.90	d4T	Resistant
	Tenofovir	Viread	(1.4 - 4)	1.80	TFV	Partially Sensitive
	Zidovudine	Retrovir	(1.9)	10	ZDV	Resistant
NNRTI	Delavirdine	Rescriptor	(6.2)	0.55	DLV	Sensitive
	Efavirenz	Sustiva	(3)	0.72	EFV	Sensitive
	Nevirapine	Viramune	(4.5)	1.82	NVP	Sensitive
PI	Atazanavir	Reyataz	(2.2)	33	ATV	Resistant
		Reyataz / r[1]	(5.2)	33	ATV/r	Resistant
	Darunavir	Prezista / r[2]	(10 - 90)	1.71	DRV/r	Sensitive
	Fosamprenavir	Lexiva	(2)	4.18	AMP	Resistant
		Lexiva / r[1]	(4 - 11)	4.18	AMP/r	Partially Sensitive
	Indinavir	Crixivan	(2.1)	2.82	IDV	Resistant
		Crixivan / r[1]	(10)	2.82	IDV/r	Sensitive
	Lopinavir	Kaletra	(9 - 55)	2.36	LPV/r	Sensitive
	Nelfinavir	Viracept	(3.6)	66	NFV	Resistant
	Ritonavir	Norvir	(2.5)	1.66	RTV	Sensitive
	Saquinavir	Invirase	(1.7)	8.33	SQV	Resistant
		Invirase / r[1]	(2.3 - 12)	8.33	SQV/r	Partially Sensitive
	Tipranavir	Aptivus / r[1]	(2 - 8)	0.70	TPV/r	Sensitive

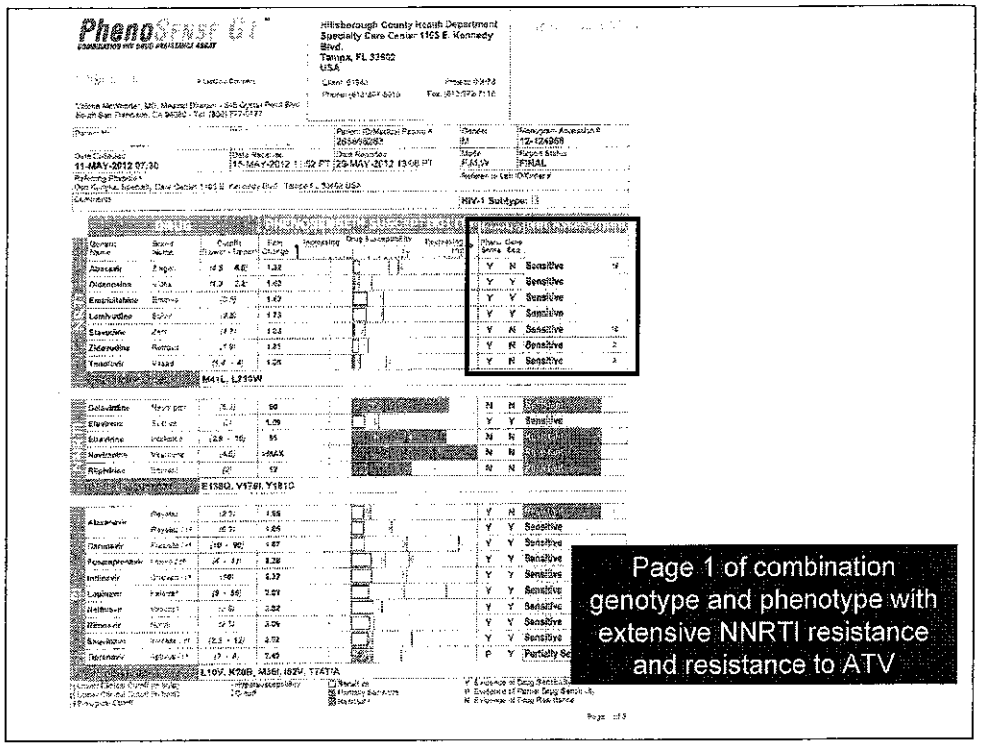
Combination Genotype and Phenotype

PhenoSense GT

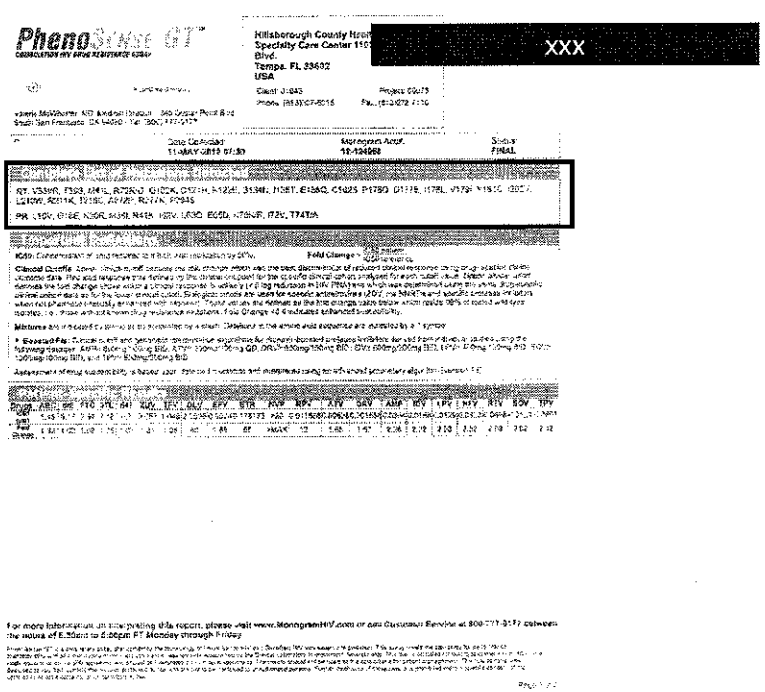


Phenosense™ HIV Test Report

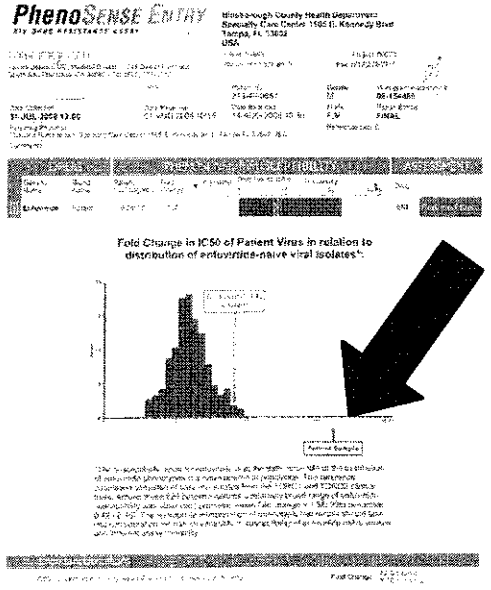




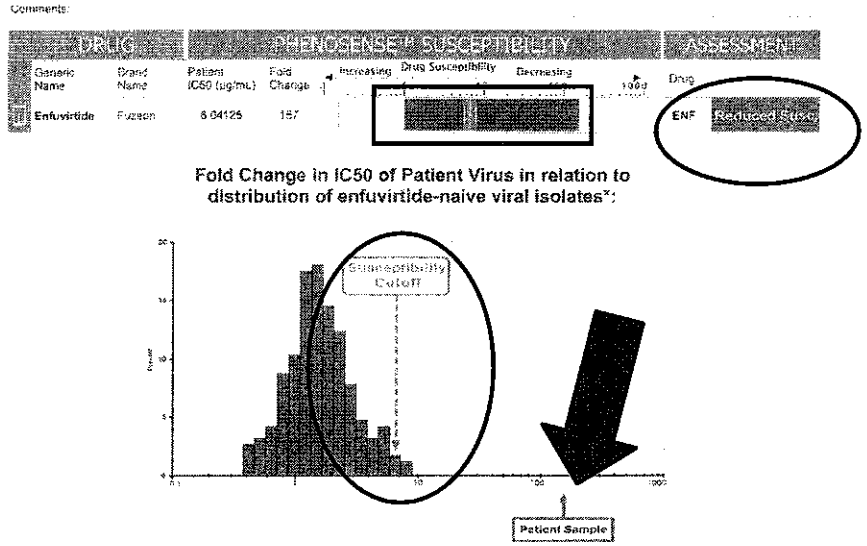
Page 1 of combination genotype and phenotype with extensive NNRTI resistance and resistance to ATV



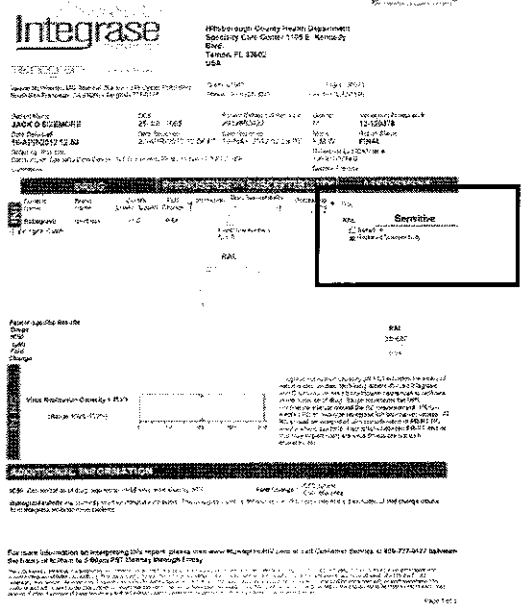
PhenoSense Entry: Enfuvertide



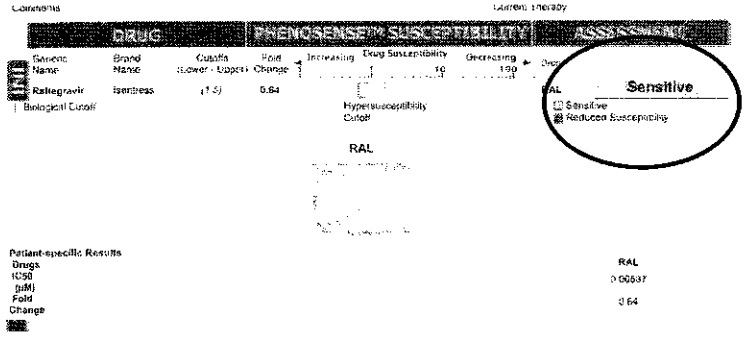
PhenoSense Entry



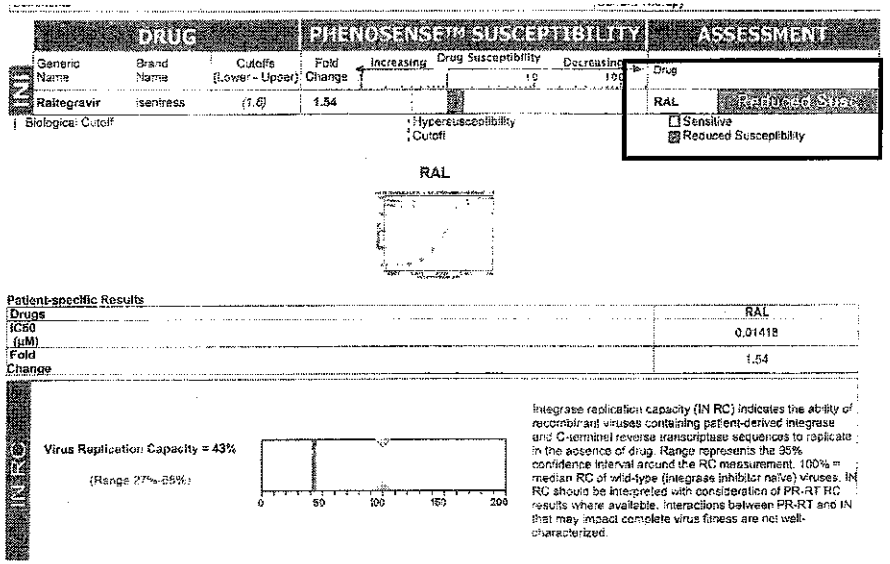
PhenoSense Integrase



PhenoSense Integrase



PhenoSense Integrase



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

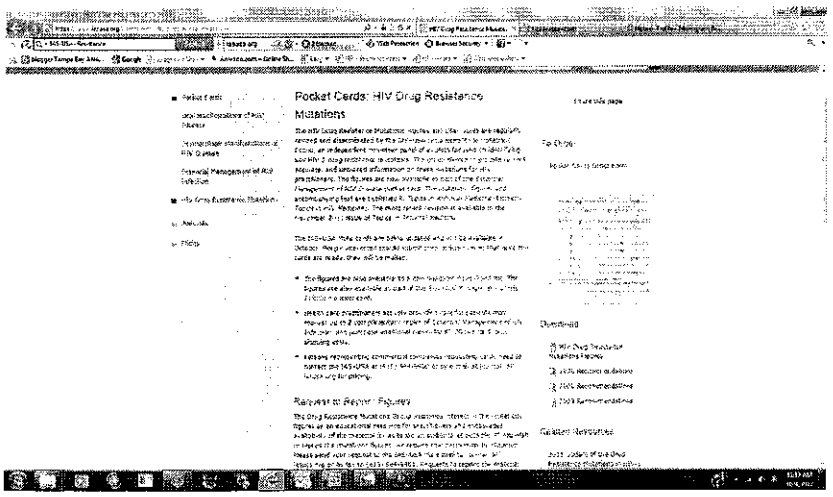
IAS-USA Search

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 pocket-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 "at" iasusa.org or call the IAS-USA at (415) 544-9400



International AIDS Society—USA* Drug Resistance Mutations Group

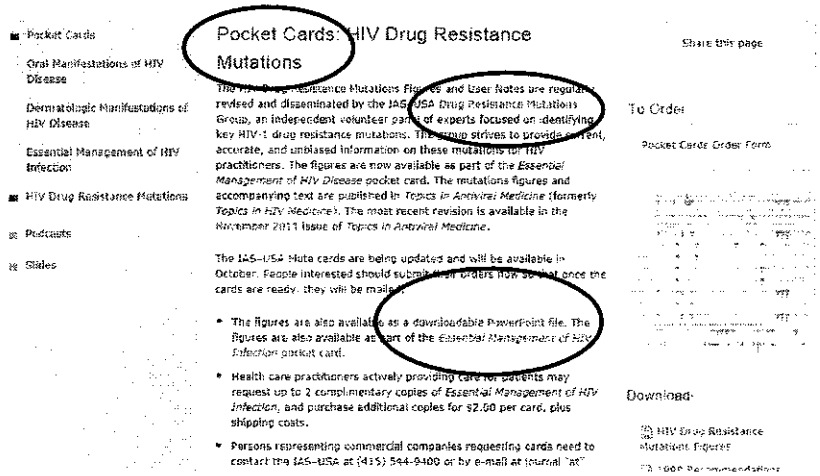
International AIDS Society–USA Topics in HIV Medicine

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

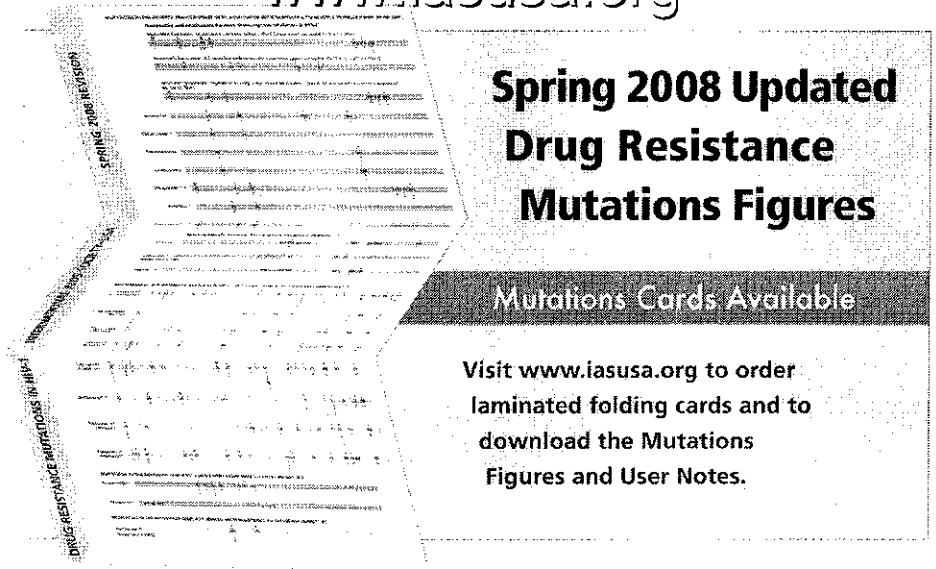
*The International AIDS Society—USA (IAS—USA) is a not-for-profit, HIV clinical specialist-education organization. It is entirely different from and not affiliated with the International AIDS Society (Stockholm, Sweden).

ISA-USA



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

www.iasusa.org



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		SampleType	Purified Amplicon	
virco ID		Collection Date		
		Received by Virco	25-Jan-2008	
		Visit	NA	
		Report Date	26-Feb-2008	
Virco ID	BAA256991			

CONFIDENTIAL

Drug: Trade name	Generic name	Susceptibility: Fold change in IC_{50} relative to reference virus (log_{10}) 1 / 10 / 100	Fold change in IC_{50} (Cut-off for normal susceptible range)	Ref.
NRTI / NtRTI				
Retrovir®	Zidovudine		10.2 (2.5)	
Epivir®	Lamivudine		2.2 (2.5)	
Videx®	Didanosine		0.6 (2.3)	
Zerit®	Stavudine		1.4 (2.2)	
Ziagen®	Abacavir		1.0 (2.0)	4
Emtriva®	Emtricitabine		4.4 (3.1)	
Viread® *	Tenofovir DF		1.6 (2.2)	3
NNRTI				
Viramune®	Nevirapine		>67.5 (6.0)	
Sustiva®, Stocrin®	Efavirenz		13.7 (3.3)	
Intelence™	Etravirine		0.5 (3.7)	7
PI				
Crixivan®	Indinavir		>51.8 (2.3)	
Viracept®	Nelfinavir		>42.5 (2.2)	
Invirase®	Saquinavir		>31.7 (1.8)	
Lexiva®, Telzir® a prodrug of	Amprenavir		5.5 (2.2)	
Kaletra®	Lopinavir		>28.9 (1.6)	2
Reyataz®	Atazanavir		>42.3 (2.1)	
Aptivus®	Tipranavir		1.0 (1.5)	5
Prezista™	Darunavir		0.9 (2.0)	6

virco®TYPE HIV-1 V4.2.01

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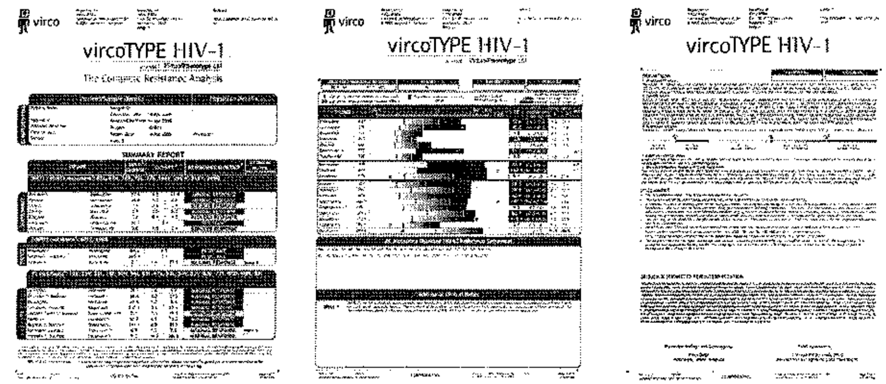
Summary Report	Detailed Report	Definitions and Disclaimers



GenoSure® MG
HIV DRUG RESISTANCE ASSAY

monogram

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

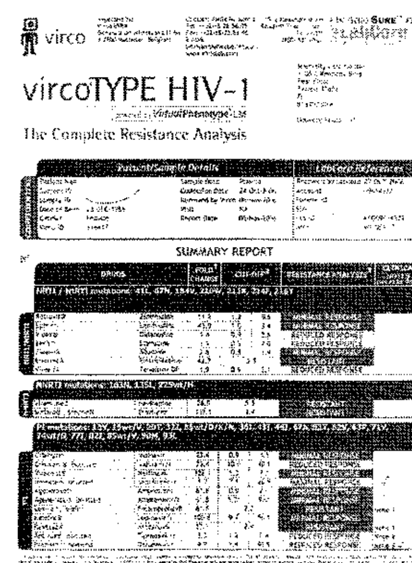
Patient Name	DOB	Patient ID/Medical Record #	Gender	Monogram Accession # 10-132004
Date Collected 06/03/2010 10:57	Date Received 06/03/2010 10:57	Date Reported 07/09/2010 12:41	M	Report Status FINAL

HIV-1 Subtype: B

Drug: Generic Name	Brand Name	GenoSure®MG: Drug Resistance Associated Mutations Detected	Drug	Assessment*	Comments
Abacavir	Ziagen	M41L, E44D, D67N, V75V/I/M, M184V, L210W, T215Y, K219R	ABC	Resistant	
Didanosine	Videx	M41L, E44D, D67N, V75V/I/M, M184V, L210W, T215Y	ddI	Resistant	
Emtricitabine	Emtriva	M41L, E44D, D67N, V75V/I/M, M184V, L210W, T215Y, K219R	FTC	Resistant	
Lamivudine	Epivir	M41L, E44D, D67N, V75V/I/M, M184V, L210W, T215Y, K219R	3TC	Resistant	
Stavudine	Zerit	M41L, E44D, D67N, T69D, V75V/I/M, L210W, T215Y, K219R	d4T	Resistant	1
Tenofovir	Viread	M41L, D67N, L210W, T215Y, K219R	TFV	Resistant	1
Zidovudine	Retrovir	M41L, E44D, D67N, V75V/I/M, L210W, T215Y, K219R	ZDV	Resistant	1
Delavirdine	Rescriptor	K103N, Y188L	DLV	Resistant	
Efavirenz	Sustiva	K103N, Y188L	EFV	Resistant	
Etravirine	Intelence	V179T, Y188L	ETR	Resistance Possible	
Nevirapine	Viramune	K103N, Y188L	NVP	Resistant	
Atazanavir	Reyataz	L10V, V32I, K43T, M46I, V82T	ATV	Resistant	
	Reyataz / r	L10V, V32I, K43T, M46I, V82T	ATV/r	Resistance Possible	
Darunavir	Prezista / r	V32I, K43T, M46I, I47A	DRV/r	Sensitive	
Fosamprenavir	Lexiva	L10V, V32I, K43T, M46I, I47A	AMP	Resistant	
	Lexiva / r	L10V, V32I, K43T, M46I, I47A	AMP/r	Resistant	
Indinavir	Crixivan / r	L10V, V32I, M46I, I47A, A71T, V82T	IDV/r	Resistant	
Lopinavir	Kaletra	L10V, V32I, E34Q, K43T, M46I, I47A, A71T, V82T	LPV/r	Resistant	
Nelfinavir	Viracept	L10V, V32I, M46I, A71T	NFV	Resistant	
Ritonavir	Norvir	L10V, V32I, K43T, A71T, V82T	RTV	Resistant	
Saquinavir	Invirase	L10V, K43T, A71T	SQV	Sensitive	
	Invirase / r	L10V, K43T, A71T	SQV/r	Sensitive	
Tipranavir	Aptivus / r	L10V, V32I, K43T, I47A, V82T	TPV/r	Resistant	

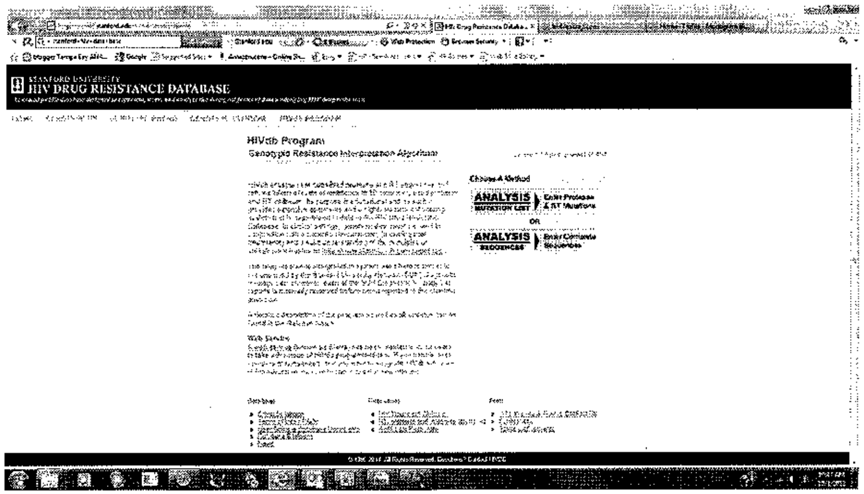
MDR HIV

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



Stanford University

HIV Drug Resistance Database



Reports you don't want to see!

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB

The HIV-1 GenoSure PRIme(SM) for this specimen has been completed.
GenoSure PRIme(SM)

HIV-1 Subtype: B

Drug Generic Name	Brand Name	Genotypic Assessment	Comments
NRTI			
Abacavir	Ziagen	Resistant	
RAMs: K65R, M184V			
Didanosine	Videx	Resistant	
RAMs: K65R, M184V			
Emtricitabine	Emtriva	Resistant	
RAMs: K65R, M184V			
Lamivudine	Epivir	Resistant	
RAMs: K65R, M184V			
Stavudine	Zerit	Sensitive	1
RAMs: K65R			
Tenofovir	Viread	Resistance Possible	1
RAMs: K65R			
Zidovudine	Retrovir	Sensitive	1
RAMs: None			
NNRTI			
Efavirenz	Sustiva	Sensitive	
RAMs: None			
Etravirine	Intelence	Sensitive	
RAMs: None			
Nevirapine	Viramune	Sensitive	
RAMs: None			
Rilpivirine	Edurant	Sensitive	
RAMs: None			
INI			
Raltegravir	Isentress	Resistant	
RAMs: [illegible]			

Viral Tropism Testing

Viral Tropism

- HIV enters cells by a complex process
  - Sequential attachment to the CD4 receptor
  - Binding to either CCR5 or CXC4R 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

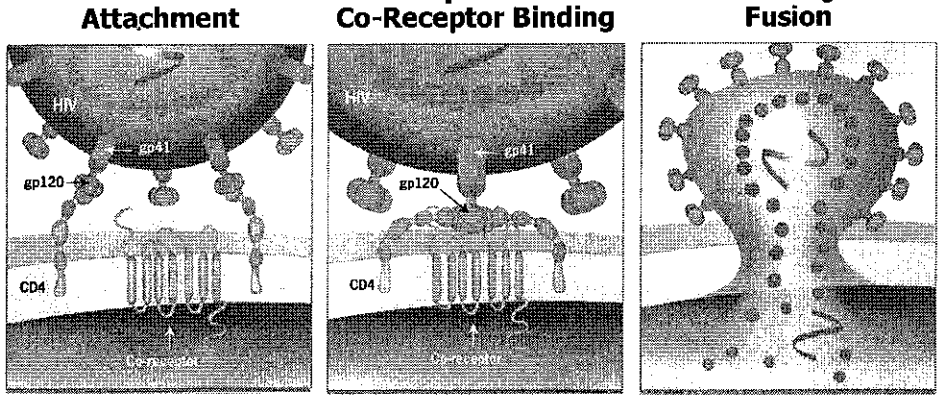
DHHS Treatment Guidelines, 2012

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



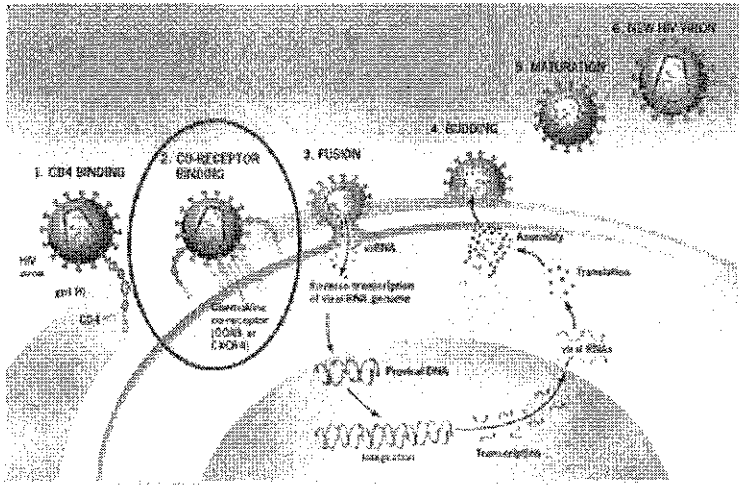
Viral gp120 protein binds to CD4 on host cell

gp120-CD4 complex binds to CCR5 or CXCR4 co-receptor on host cell

Virus fuses with host cell membrane, facilitating viral entry

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

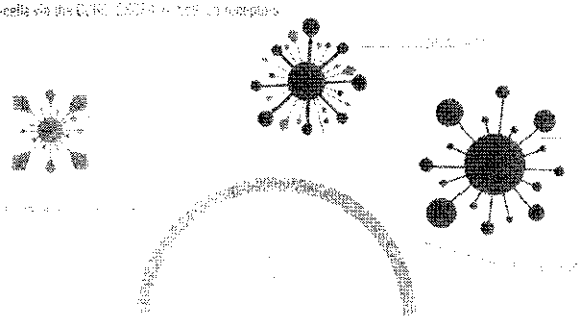
The HIV Life Cycle Provides Many Targets for Therapeutic Intervention



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

Viral Tropism

Viral Tropism – Creating a more complete HIV profile



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-naive 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

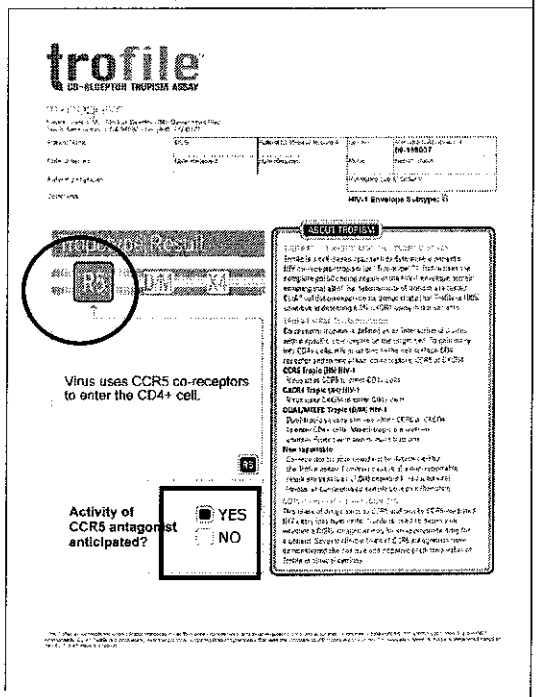
Tropism Assays

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

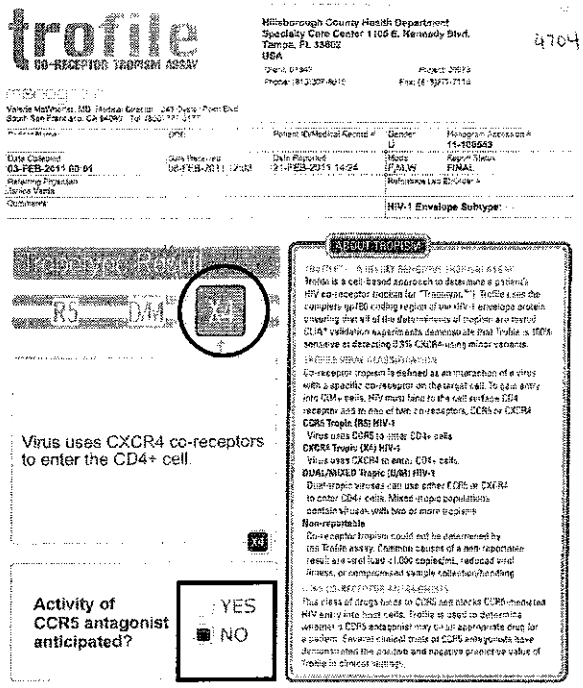
Tropism Testing

- 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
CO-RECEPTOR TROPISM ASSAY
Tropism Result
R5
DM
X4
Virus uses CCR5 co-receptors to enter the CD4+ cell.
Activity of CCR5 antagonist anticipated?
YES
NO
ABOUT TROPISM

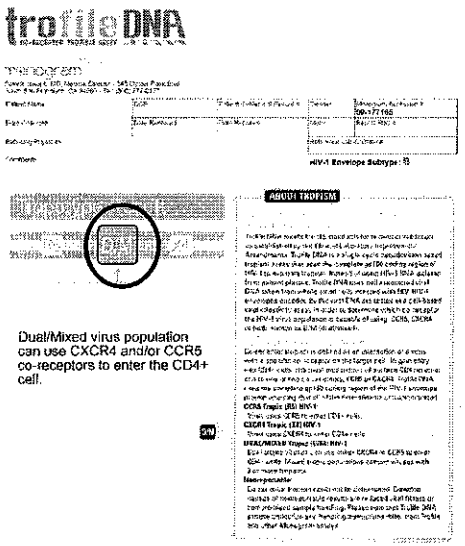
Trofile X4


trofile
CO-RECEPTOR TROPISM ASSAY
Hillsborough County Health Department
Specialty Care Center 1105 E. Kennedy Blvd.
Tampa, FL 33602
USA
4704
Tropism Result
R5
DM
X4
Virus uses CXCR4 co-receptors to enter the CD4+ cell.
Activity of CCR5 antagonist anticipated?
YES
NO
ABOUT TROPISM

Trofile® DNA HIV-1 Co-Receptor Tropism Assay

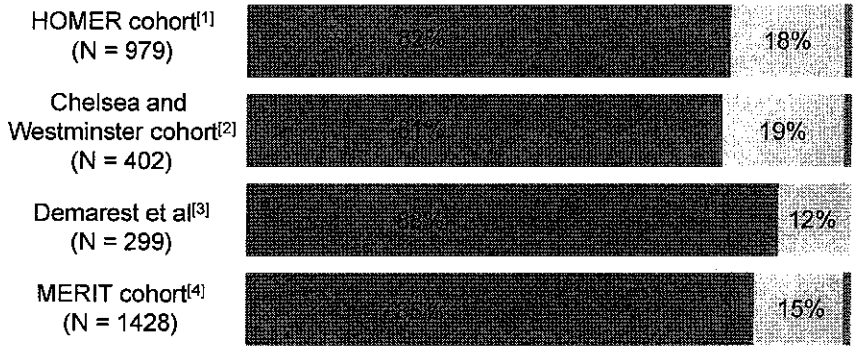
- Single-cycle, recombinant phenotypic virus similar Trofile®
- Trofile® DNA isolates viral DNA from HIV infected blood cells in whole blood
- Viral load: < 1,000 copies / mL
- TAT:3 weeks
- Cost: expensive
- Vouchers: possible
- Results: CCR5, CXCR4, or D/M tropic

trofileDNA



HIV Tropism in Antiretroviral-Naive Populations

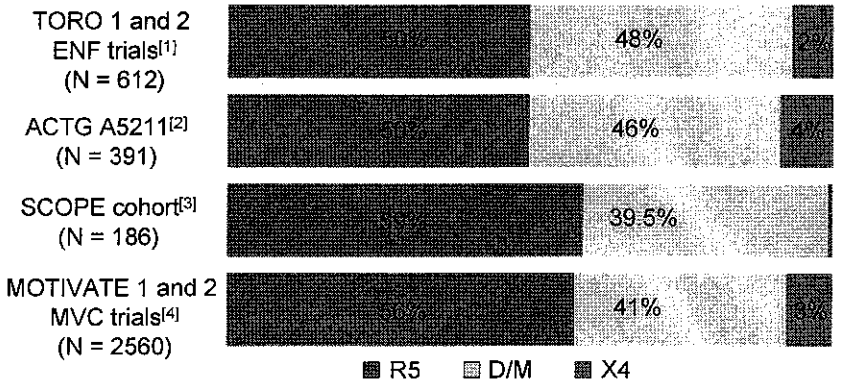
- R5-only virus in 80% to 90% of patients, with D/M or X4 virus in remainder



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



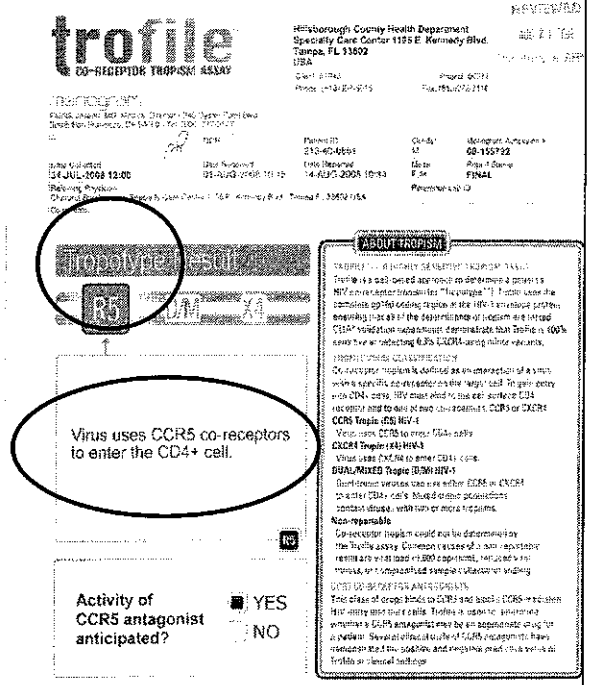
1. Melby J, et al. J Infect Dis. 2006;194:238-246. 2. Wilkin TJ, et al. Clin Infect Dis. 2007;44:591-595. 3. Hunt PW, et al. J Infect Dis. 2006;194:926-930. 4. Coakley E, et al. Intl Wkshp on Targeting HIV Entry 2006. Abstract 8.

Caveats Related to Prevalence of CCR5 Tropic Virus

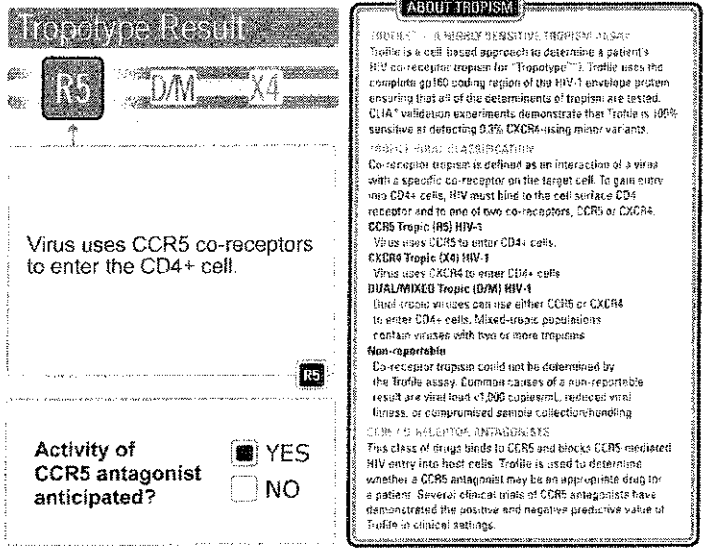
- Higher CD4+ T-Cell counts are generally associated with increased CCR5 prevalence
- ARV-naïve patients are more likely to have CCR5-tropic virus compared to treatment experienced patients

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



CCR5 Tropic Result

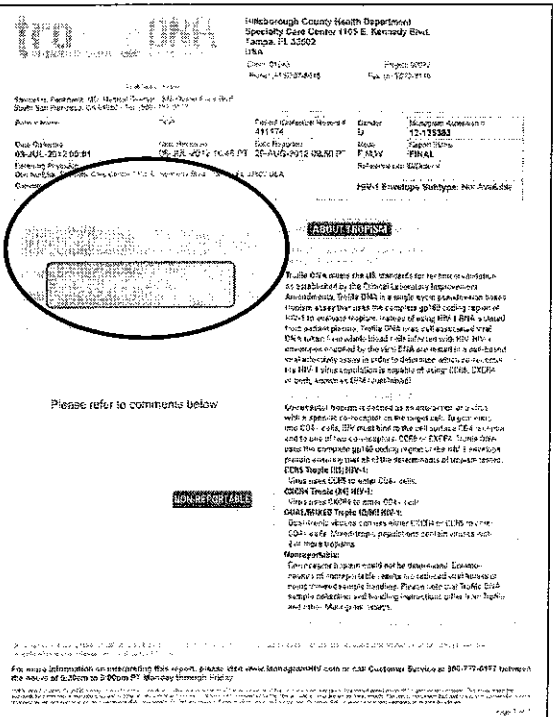


Trofile DNA

- 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



Please refer to comments below.

NON-REPORTABLE

ABOUT TROPISM

Trofile DNA meets the US standards for technical validation as established by the Clinical Laboratory Improvement Amendments. Trofile DNA is a single cycle pseudovirion based tropism assay that uses the complete gp160 coding region of HIV-1 to evaluate tropism. Instead of using HIV-1 RNA isolated from patient plasma, Trofile DNA uses cell associated viral DNA taken from whole blood cells infected with HIV. HIV-1 envelopes encoded by the viral DNA are tested in a cell-based viral infectivity assay in order to determine which co-receptor the HIV-1 virus population is capable of using: CCR5, CXCR4, or both, known as D/M (dual/mixed).

Co-receptor tropism is defined as an interaction of a virus with a specific co-receptor on the target cell. To gain entry into CD4+ cells, HIV must bind to the cell surface CD4 receptor and to one of two co-receptors, CCR5 or CXCR4. Trofile DNA uses the complete gp160 coding region of the HIV-1 envelope protein ensuring that all of the determinants of tropism tested.

CCR5 Tropic (R5) HIV-1:
Virus uses CCR5 to enter CD4+ cells.

CXCR4 Tropic (X4) HIV-1:
Virus uses CXCR4 to enter CD4+ cells.

DUAL/MIXED Tropic (D/M) HIV-1:
Dual-tropic viruses can use either CXCR4 or CCR5 to enter CD4+ cells. Mixed-tropic populations contain viruses with 2 or more tropisms

Nonreportable:
Co-receptor tropism could not be determined. Common causes of nonreportable results are reduced viral fitness or compromised sample handling. Please note that Trofile DNA sample collection and handling instructions differ from Trofile and other Monogram assays.

Specimen Collection

Proper Collection is Critical!

- Verify viral load ≥1,000 copies/mL
- Using 2 PPT or 2 lavender-top (EDTA) tubes, draw enough whole blood to provide at least 3 mL plasma
- Centrifuge immediately if drawn in lavender-top tube; transfer plasma to screw-cap tubes
- Samples should not be left in centrifuge
- Place tubes and test requisition form in specimen bag
- Freeze immediately and ensure sample is frozen before shipping (-20°C)
- Include LabCorp test number **829574** (LabCorp clients)

- Recommended for patients with **undetectable** viral loads
- Draw 4 mL of whole blood into an EDTA (lavender-top) test tube
- Do **NOT** centrifuge
- Place tube and test requisition form in specimen bag
- Freeze immediately and ensure sample is frozen before shipping (-20°C)
- Include LabCorp test number **829670** (LabCorp clients)

Proper Preparation is Critical!

Specimen Collection / Requisitions

- Prepare requisitions forms with care
  - Document VL, dates, etc to prevent DELAY

Quest Diagnostics: triplicate population sequencing with reflex to ultradeep sequencing
for patients with detectable viral loads only

Sample preparation overview

1. Using an EDTA (lavender-top) or PPT (white-top) tube, draw sufficient whole blood to provide 2 mL (preferred) to 0.6 mL (minimum) of plasma
2. Centrifuge within 6 hours after collection and transfer plasma to a separate plastic screw-cap tube
3. Freeze immediately and ensure sample is frozen before shipping

If the triplicate population sequencing result is CXCR4 NOT DETECTED, then ultradeep sequencing will be performed at an additional charge.

Contact a representative from Quest Diagnostics at 1-800-642-4657 for more information.

HIV-1 Coreceptor Tropism with Reflex to Ultradeep Sequencing

- Genotypic tropism assay
- Quest Diagnostics
- V3 loop of HIV-1 gene is sequenced
- Sensitivity for detection of minority X4 virus in dual/mixed viral populations is enhanced by ultradeep DNA sequencing which is performed if standard sequencing detects only R5 virus
- UDS performance is comparable to phenotypic tropism tests for predicting clinical response to MVC
- Comparable to high-sensitivity phenotypic test in distinguishing between virologic responders and nonresponders

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

- Results reported:
- Population sequencing: X4 detected or not
- Ultradeep sequencing X4
  - Analytical sensitivitiy
    - 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: i\Initial Testing
  - Tier 2: Additional testing for ultradeep sequencing if tropism is not detected on first round

CDC. http://www.cdc.gov

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

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

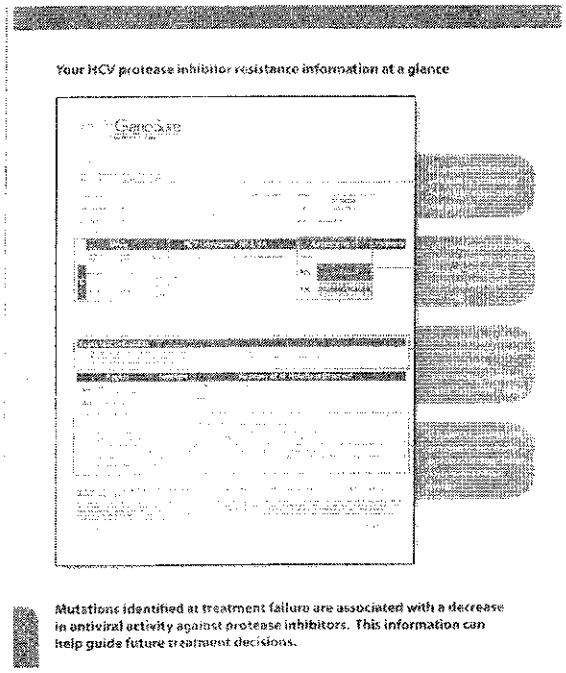
LabCorp/Monogram Biosciences

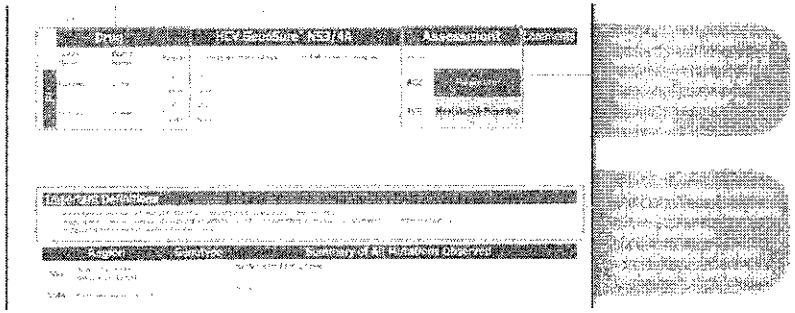
- Provides a comprehensive sequence-based analysis of drug resistance for HCV based direct-acting antiviral (DAA) therapies
- Available as a reflex test when VL $\geq$ 2000 IU/mL

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

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

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

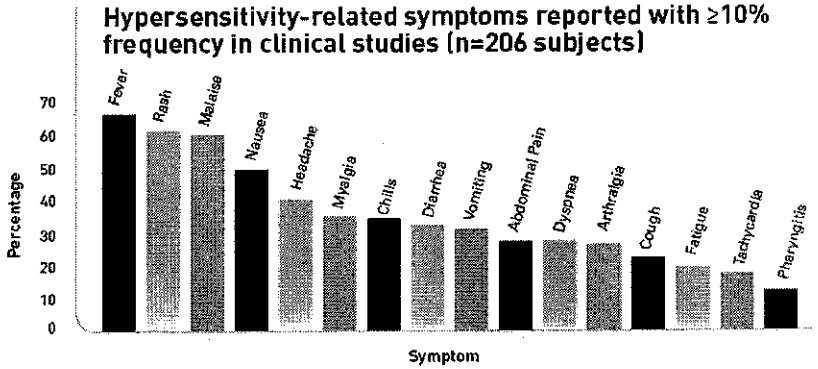
- Hypersensitivity to abacavir was reported in approximately 8% of 2,670 patients (n=206) in 9 clinical trials (range 2% to 9%) with enrollment from November 1999 to February 2002

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 Hypersentivity-Related Symptoms



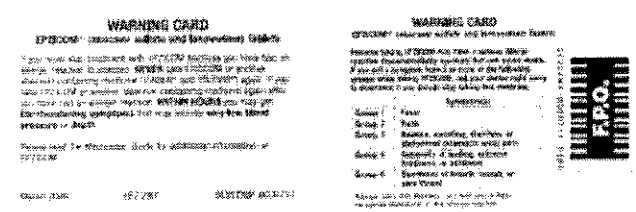
GSK

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 ⟹ *DNA extracted from cells*
- Buccal swabs
  - 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
Initial Process	Process whole blood within 16 hours	Process PBMCs within 8 hours, or if T-Cell Xtend is used, within 30 hours
Measurement	IFN-g concentration	Number of IFN-g producing cells (spots)
Possible Results	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)

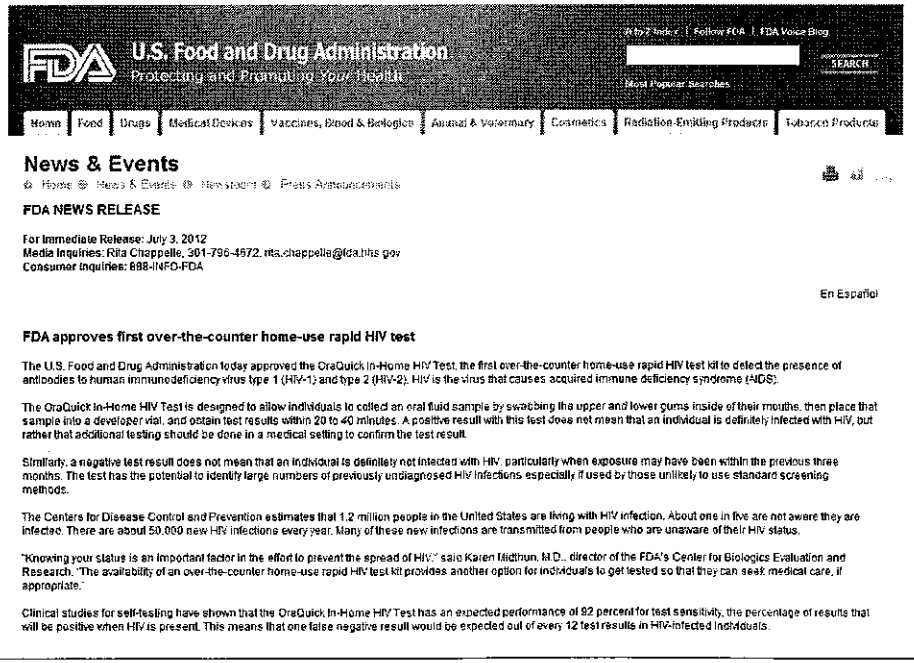
CDC. http://www.cdc.gov

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



U.S. Food and Drug Administration
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News & Events

FDA NEWS RELEASE

For Immediate Release: July 3, 2012
Media Inquiries: Rita Chappelle, 301-796-4672, 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 Midthun, 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

- Fast: Results in 20 minutes.

Learn More

http://www.oraquick.com/

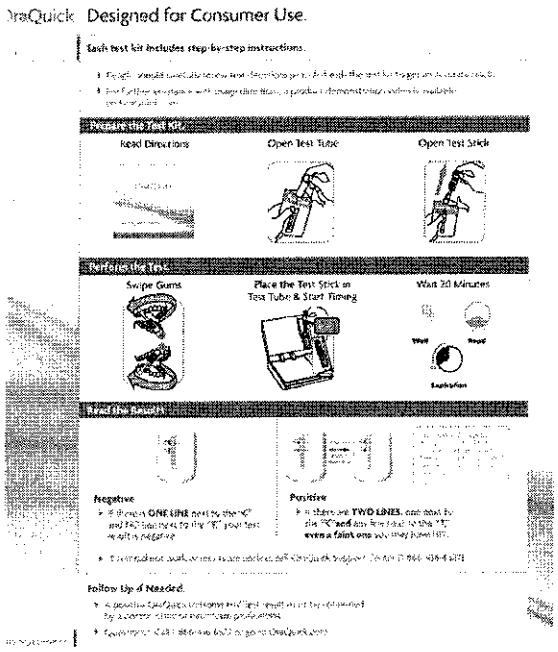
OraQuick In-Home HIV Test

- 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/

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

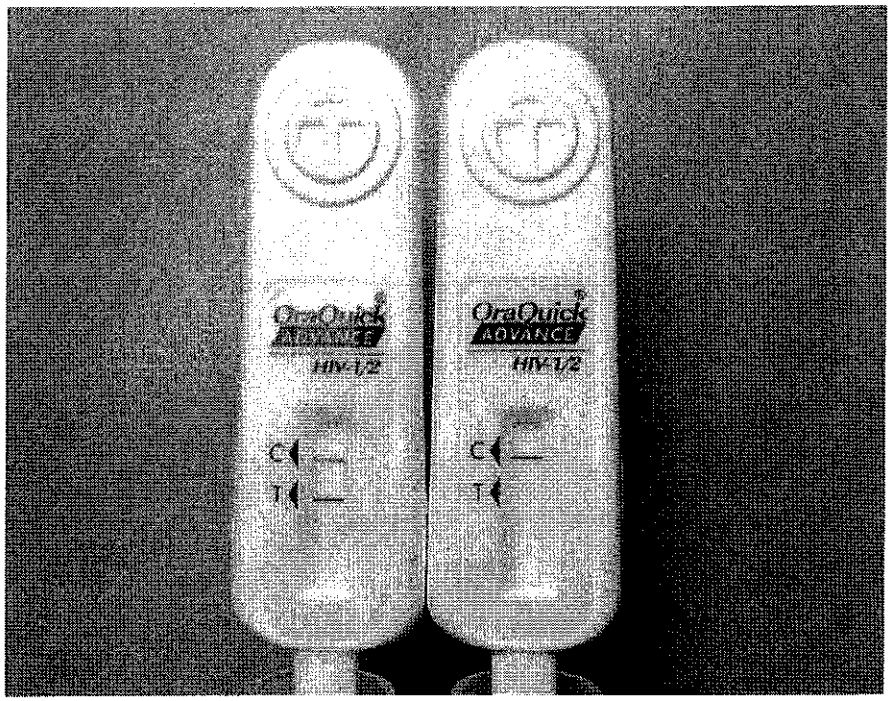


http://www.oraquick.com/

Home Testing: Oral Swab

- Pro
- More privacy
- 24 hour hotline
- Usually more confidential

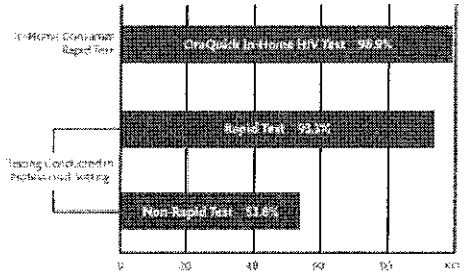
- Con
- More expensive
- Patient error in sampling / interpretation
- Patients may be testing too early
- Confidentiality could be compromised: kit in trash, credit card or store receipt
- No guarenteed counseling and education
- False negative results



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.



http://www.oraquick.com/

Overcome Barriers to Testing

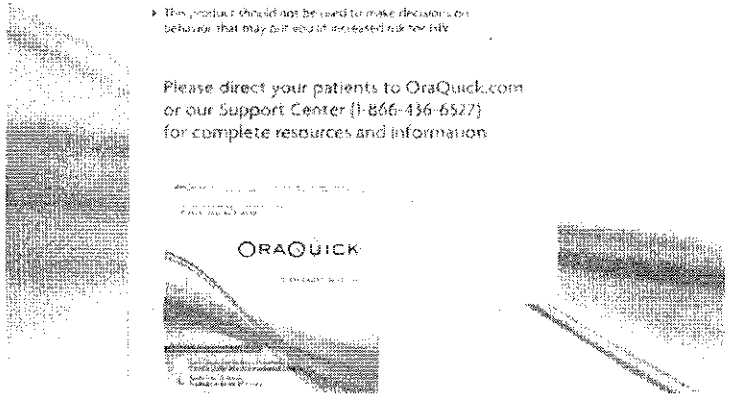
- Fear of a positive test result
- Privacy

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 at risk of increased risk for HIV

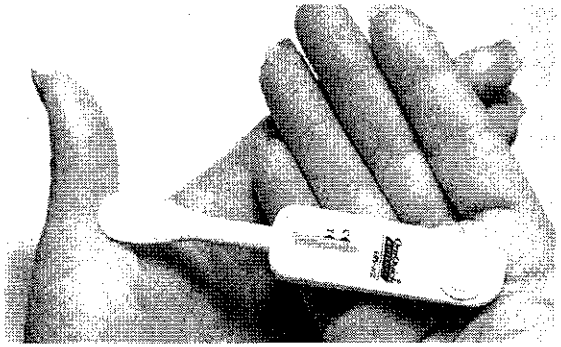
Please direct your patients to OraQuick.com or our Support Center (1-866-436-6527) for complete resources and information



http://www.oraquick.com/

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

For the first time, the Food and Drug Administration has approved over-the-counter HIV tests for consumer use. How effective are they, really?



The OraQuick HIV test promises to deliver results in as little as 40 minutes (Jason Grow/Reuters)

Last week the FDA approved the first at-home HIV testing kit for direct sales to consumers. With OraQuick, in a matter of minutes, most any adult might check for antibodies to the virus that causes AIDS. Already the device is in widespread use by health care professionals in the United States and some 35 other countries. The FDA's decision didn't follow a recent advance in medical science or technology. Rather, it reflects a shift in officials' attitudes about the disease -- and the capability of ordinary individuals to handle medical information.

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