DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
HIV DIAGNOSTICS AND REFERENCE LABORATORY
9100 Brookville Rd, BLDG 508
Silver Spring, MD 20910

REPLY TO
ATTENTION OF

MCMR-UWA-A

23 March 2015

MEMORANDUM FOR HIV Diagnostics and Reference Laboratory (HDRL) Customers

SUBJECT: Changes to HIV-1 Genotype Assay, Effective 01 April 2015

1. The purpose of this memo is to notify customers of upcoming changes to the HIV-1 Genotype Assay performed by HDRL (2 pages).

2. Effective 01 April 2015, HDRL will switch from the Siemens TruGene HIV-1 Genotype Assay to the Abbott ViroSeq HIV-1 Genotype Assay.

3. HIV-1 viral loads **must now be ≥2000** copies/ml and have been performed within 30 days for the assay to be performed. Note: if viral load is >1000 - <2000 copies/ml, testing may be performed, but a resistance profile may not be generated.

4. The new genotype reports will look similar to the previous reports.

5. Customers will receive nucleoside reverse transcriptase, non- nucleoside reverse transcriptase and protease inhibitor class resistance, as well as the HIV-1 subtype and applicable mutations.

6. The drugs tested for resistance in the ViroSeq genotype assay are as follows:

a. **EMTRIVA® (emtricitabine, FTC)**
b. **EPIVIR® (lamivudine, 3TC)**
c. **RETROVIR® (zidovudine, ZDV)**
d. **VIDEX® (didanosine, ddI)**
e. **VIREAD® (tenofovir, TDF)**
f. **ZERIT® (stavudine, d4T)**
g. **ZIAGEN® (abacavir, ABC)**
h. **EDURANT® (rilpivirine, RPV)**
i. **INTELENCE® (etravirine, ETR)**
j. **SUSTIVA® (efavirenz, EFV)**
k. **VIRAMUNE® (nevirapine, NVP)**
l. **APTIVUS® (tipranavir, TPV)**
m. **CRIXIVAN® (indinavir, IDV)**
n. **FORTOVASE/INVIRASE® (saquinavir, SQV)**
o. **KALETRA® (lopinavir+ritonavir, LPV)**
p. **LEXIVA® (fosamprenavir, FPV)**
q. **PREZISTA® (darunavir, DRV)**

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SUBJECT: Changes to HIV-1 Genotype Assay, Effective 01 April 2015

r. **REYATAZ® (atazanavir, ATV)**
s. **VIRACEPT® (nelfinavir, NFV)**

7. The switch from the Siemens HIV-1 TruGene Genotype assay to the Abbott HIV-1 ViroSeq genotype assay is due to the manufacturer's discontinuation of the TruGene kit.

8. HDRL is in the process of developing and validating an Integrase genotype assay. More information will be made available in memo format closer to implementation.

9. Please retain a copy of this memorandum for your records.

10. Point of contact is the undersigned at (301) 319-3173 or jstewart@hivresearch.org.

Encls
1. Sample Report (6 pages)

JULIAN M. STEWART
CPT, USA
Laboratory Manager
HIV Diagnostics and Reference Laboratory

ViroSeq® HIV-1 Antiretroviral Drug Resistance Report

Patient ID:	-----	**Institution Name:**	HDRL
Accession Number:	-----	**Report Generated by:**	Administrator
Sample Name:	Sample Report	**Report Date & Time:**	Mar 27, 2015 8:22:09 AM

Drug Resistance:

NRTI Class	*Evidence of Resistance*
EMTRIVA® (emtricitabine, FTC)	None
EPIVIR® (lamivudine, 3TC)	None
RETROVIR® (zidovudine, ZDV)	None
VIDEX® (didanosine, ddI)	None
VIREAD® (tenofovir, TDF)	None
ZERIT® (stavudine, d4T)	None
ZIAGEN® (abacavir, ABC)	None

NNRTI Class	*Evidence of Resistance*
EDURANT® (rilpivirine, RPV)	None
INTELENCE® (etravirine, ETR)	None
SUSTIVA® (efavirenz, EFV)	None
VIRAMUNE® (nevirapine, NVP)	None

PI+ Class	*Evidence of Resistance*
APTIVUS® (tipranavir, TPV)	None
CRIXIVAN® (indinavir, IDV)	None
FORTOVASE® / INVIRASE® (saquinavir, SQV)	None
KALETRA® (lopinavir + ritonavir, LPV)	None
LEXIVA® (fosamprenavir, FPV)	None
PREZISTA® (darunavir, DRV)	None
REYATAZ® (atazanavir, ATV)	None
VIRACEPT® (nelfinavir, NFV)	None

*	NOTE: At least one mutation used to determine Evidence of Resistance for this drug has not been fully validated.
**	NOTE: At least one mutation used to determine Evidence of Resistance for this drug has not been clinically verified.
***	NOTE: For at least one mutation used to evaluate Evidence of Resistance for this drug, both notes above apply.
+	Evidence of Resistance for Protease Inhibitors estimates response to ritonavir-boosted regimens. Refer to section titled "Notes on Evidence of Resistance"

Notes on Evidence of Resistance:

Resistance	Mutations present constitute a high level of genetic evidence for viral resistance
Possible Resistance	Mutations present suggest the possibility of viral resistance
None	There is insufficient evidence for viral resistance

The protease inhibitor (PI) evidence of resistance interpretations were developed to estimate the expected virological response to standard doses of protease inhibitors with pharmacokinetic boosting by ritonavir. This has become the most common method of administering each of the protease inhibitors, except nelfinavir (ref. 1), to ensure adequate drug levels in all patients. Boosted PIs are more active in the presence of resistance than non-boosted PIs. (ref. 2,3)

Patient ID:	-----	**Institution Name:**	HDRL
Accession Number:	-----	**Report Generated by:**	Administrator
Sample Name:	Sample Report	**Report Date & Time:**	Mar 27, 2015 8:22:09 AM

Drug Resistance Mutations Identified:

NRTI Class:

None

NNRTI Class:

None

PI Class:

None

Additional Mutations:

Additional Mutations: The following amino acids differing from the reference sequence (HXB-2, accession number K03455) at the indicated codon positions were identified and may be useful as a baseline determination of virus genotype.

Protease

V3I, I15V, G16E, M36I, R57K, I72V

RT

T39S, E122P, I142V, F171Y, K173A, Q174K, R211K, L214F, V245I, E248N, D250E, R277K, I293V, Q334Y

Patient Information:

Accession Number	-----	Patient Information	
Assay Operator Name	-----	ID	-----
Institution Name	-----	First Name	-----
Physician	-----	Middle Name	-
Field 1	-----	Last Name	-----
Field 2	-----	Gender	Not Available
Date Drawn		Date of Birth	

Site Information:

Institution Name	HDRL
Department Name	-
Lab Director	Sheila Peel
Address 1	9100 Brookville Rd
Address 2	BLDG 508
Mail Stop	-
City	Silver Spring
State/Province	MD
ZIP / Postal Code	20910
Country	-
Phone	301-319-3123
Fax	301-319-3502
Contact Email	-
Website URL	HIVRESEARCH.ORG

Comments:

-

Review & Release of Results:

Signature / Date: _____ Name(Print) / Title: _____

Notes: _____

HIV-1 Resistance Mutation List:

NRTI Class	*Mutations Included within the Algorithm*
EPIVIR® (lamivudine, 3TC)	{M184V,M184I**} *[69ins**,K65R,Q151M]* (V75I*,69del***,K65N***,K219Q,K219R***,L210W,D67T***,D67S***,D67N*,Q151L***,D67E,D67H***,D67G***,K70T***, A62V,T215Y,F116Y,M41L,F77L,K219E,K70E***,67del***,K70G***,K70Q***,K70R,K70S***,K219N***,K70N***,T215F)
EMTRIVA® (emtricitabine, FTC)	{M184V,M184I**} *[69ins**,K65R,Q151M]* (V75I*,69del***,K65N***,K219Q,K219R***,L210W,D67T***,D67S***,D67N*,Q151L***,D67E,D67H***,D67G***,K70T***, A62V,T215Y,F116Y,M41L,F77L,K219E,K70E***,67del***,K70G***,K70Q***,K70R,K70S***,K219N***,K70N***,T215F)
VIDEX® (didanosine, ddI)	{K65R,L74I***,L74V,Q151M,69ins**} *[K65N***,69del***,Q151L***,67del***,V75M***,T69D,K70E***,K70G***,V75T***]* (K219Q,K219R***,A62V,T215S***,F116Y,T215Y,T215V***,M41L,T215L***,T215N***,T215E***,T215D***,T215C***, T215I***,T215F,V75I*,T69A***,T215A***,V75S***,L210W,D67T***,D67S***,D67N*,T69N***,V75A***,D67E,T69S***, D67H***,D67G***,K70T***,M184V,F77L,K219E,M184I**,K70Q***,K70R,K70S***,K219N***,K70N***)
ZERIT® (stavudine, d4T)	{Q151M,T215Y,T215F,69ins**,V75M***,V75T***} *[K65R,69del***,Q151L***,T215S***,T215V***,T215L***,67del***,T215N***,T215E***,T215D***,T215C***,T215I***, T215A***,V75S***,V75A***,K70E***,K70R]* (K65N***,K219Q,K219R***,A62V,F116Y,M41L,V75I*,T69A***,T69D,L210W,D67T***,D67S***,D67N*,T69N***,D67E, T69S***,D67H***,D67G***,K70T***,F77L,K219E,K70Q***,K70S***,K219N***,K70N***) <M184V,M184I**>
ZIAGEN® (abacavir, ABC)	{69ins**,K65R,Q151M,Y115F*} *[L74I***,69del***,K65N***,L74V,Q151L***,K70E***,67del***,K70G***]* (V75I*,T215A***,K219Q,K219R***,L210W,D67T***,D67S***,D67N*,D67E,D67H***,D67G***,K70T***,M184V,A62V, T215S***,T215Y,F116Y,T215V***,M41L,T215L***,F77L,K219E,T215N***,V75T***,T215E***,M184I**,T215D***,K70Q***, T215C***,K70R,K70S***,T215I***,K219N***,K70N***,T215F)
VIREAD® (tenofovir, TDF)	{69ins**,K65R} *[K65N***,Q151M,K70E***,K70G***]* (V75I*,L74I***,69del***,T215A***,K219Q,K219R***,L210W,D67T***,D67S***,D67N*,Q151L***,D67E,D67H***,D67G***, K70T***,A62V,T215S***,T215Y,F116Y,T215V***,M41L,T215L***,F77L,K219E,T215N***,67del***,T215E***,T215D***, K70Q***,T215C***,K70R,K70S***,Y115F*,T215I***,K219N***,K70N***,T215F) <M184V,M184I**>
RETROVIR® (zidovudine, ZDV)	{69ins**,Q151M,T215Y,T215F} *[T215A***,Q151L***,T215S***,T215V***,T215L***,T215N***,67del***,T215E***,T215D***,T215C***,K70R,T215I***]* (V75I*,T69A***,K219Q,T69D,K219R***,L210W,D67T***,D67S***,D67N*,T69N***,D67E,D67H***,T69S***,D67G***,A62V, F116Y,M41L,F77L,K219E,K219N***) <K65R,K65N***,M184V,K70E***,M184I**>
NNRTI Class	***Mutations Included within the Algorithm***
INTELENCE® (etravirine, ETR)	{Y181V***,Y181I*} *[M230L***,L100I*,K101P***,G190Q***,F227C***,Y181C,G190E***]* (E138G***,V106I***,H221Y***,E138A***,V179D***,V179E***,V179F***,Y188L**,M230I***,E138K***,V179L***,V179T***, K101H***,L100V***,A98G***,K101E*,G190T***,G190S*,G190V***,E138R***,G190A*,E138Q***,V90I***,G190C***)

NNRTI Class	*Mutations Included within the Algorithm*

SUSTIVA® (efavirenz, EFV)

{V106M***,Y188L**,K103N,K101P***,Y188C**,K103S***,G190Q***,K103H***,G190T***,G190S*,G190V***,G190C***,G190E***}
[M230L,Y181V***,V106A**,Y188H***,L100I*,K103T***,P225H***,F227C***,K238T***,Y181I*,G190A*,Y181C]***
(E138G***,V106I***,H221Y***,V179D***,V108I*,V179E***,V179F***,M230I***,E138K***,V179L***,K101Q***,V179T***,K101H***,L100V***,A98G***,Y318F***,K238N***,F227L***,K101E*,E138R***,E138Q***,V90I***)

VIRAMUNE® (nevirapine, NVP)

{M230L***,Y181V***,V106M***,Y188L**,V106A**,Y188H***,K103N,K101P***,Y188C**,K103T***,K103S***,G190Q***,K103H***,G190T***,G190S*,F227C***,G190V***,Y181I*,G190A*,G190C***,G190E***,Y181C}
[M230I,L100I*,Y318F***,P225H***,F227L***,K101E*,K238T***]***
(E138G***,V106I***,H221Y***,V179D***,V108I*,V179E***,V179F***,E138K***,V179L***,K101Q***,V179T***,K101H***,L100V***,A98G***,K238N***,E138R***,E138Q***,V90I***)

EDURANT® (rilpivirine, RPV)

{Y181V***,Y188L**,K101P***,F227C***,Y181I*}
[M230L,M230I***,E138K***,L100I*,G190Q***,Y181C,G190E***]***
(E138G***,V106I***,H221Y***,E138A***,V179D***,V179E***,V179F***,V179L***,V179T***,K101H***,L100V***,A98G***,K101E*,G190T***,G190S*,G190V***,E138R***,G190A*,E138Q***,V90I***,G190C***)

PI Class	*Mutations Included within the Algorithm*

APTIVUS® (tipranavir, TPV)

[I84V,V82L,I84A***,V82T]***
(V82M**,V32I*,V82C***,L90M,V82F**,L33F*,I54A***,I47V*,L10I,K43T***,I54S***,T74P***,I54V,L10R**,I54T*,A71L***,I84C***,Q58E***,L10V*,I47A***,A71V,I54M***,L10Y***,A71T*,V82S**,M46L*,A71I***,N83D***,M46I)
<L76V***,L24I***,I50L***,I54L***,I50V**>

CRIXIVAN® (indinavir, IDV)

{I84A***}
[I84V,L90M,I84C,V82T,V82S**,L76V***,V82M**,V82A,V82C***,V82F**]***
(M46V***,G48A***,V32I*,G73A***,F53L*,I54A***,L24I***,I47V*,L10I,L10F*,I54S***,I54V,T74P***,I54T*,I54M***,I54L***,G48T***,G48S***,G48V,M46L*,G48Q***,G48L***,G48M***,M46I,N83D***,V82L***,N88S***,G73T***,G73S*,L10R**,A71L***,L10V*,I47A***,A71V,L10Y***,A71T*,G73C***,A71I***)
<I50L***>

FORTOVASE® / INVIRASE® (saquinavir, SQV)

{I84V,G48A***,I84C***,I84A***,G48T***,G48S***,G48V,G48Q***,G48L***,G48M***}
[L90M]
(V82A,V82C***,G73A***,V82F**,F53L*,L24I***,I54A***,L10I,I54S***,G73T***,T74P***,G73S*,I54V,L10R**,I54T*,A71L***,L10V*,A71V,I54M***,A71T*,L10Y***,I54L***,V82T,V82S**,G73C***,M46L*,A71I***,N83D***,M46I)
<L76V***,I50L***,I47A***>

KALETRA® (lopinavir + ritonavir, LPV)

{I47A***}
[I84A,I50V**,L76V***]***
(M46V***,I84V,G48A***,V32I*,L90M,G73A***,F53L*,L33F*,I54A***,L24I***,I47V*,L10I,L10F*,I54S***,I54V,T74P***,I54T*,I84C***,I54M***,I54L***,V82T,G48T***,V82S**,G48S***,G48V,M46L*,G48Q***,G48L***,G48M***,M46I,N83D***,V82M**,V82L***,V82A,V82C***,V82F**,G73T***,G73S*,L10R**,A71L***,L10V*,A71V,L10Y***,A71T*,G73C***,A71I***)
<I50L***>

PREZISTA® (darunavir, DRV)

(V11I***,L76V***,G73T***,I84V,T74P***,G73S*,I84C***,V32I*,I84A***,L89V***,I47A***,G73A***,I54M***,V82F**,L33F*,I54L***,I50V**,I47V*,G73C***,L10F*)
<I50L***,N88S***>

PI Class	*Mutations Included within the Algorithm*

REYATAZ® (atazanavir, ATV)

{I84V,I84C***,I50L***,I84A***,N88S***}
*[G48A***,G48T***,G48S***,G48V,G48Q***,G48L***,G48M***]*
(M46V***,V32I*,L90M,G73A***,F53L*,L33F*,I54A***,L24I***,L10I,I54S***,N88G***,I54V,T74P***,I54T*,I54M***,I54L***,V82T,V82S**,M46L*,M46I,N83D***,V82M**,V82L***,V82A,V82C***,V82F**,N88T***,G73T***,G73S*,L10R**,A71L***,L10V*,A71V,L10Y***,A71T*,G73C***,A71I***)
<L76V***>

VIRACEPT® (nelfinavir, NFV)

{I84V,L90M,D30N,I84C***,I84A***,N88D*,N88S***}
*[G48A***,I54A***,N88G***,I54S***,I54V,I54T*,I54M***,I54L***,V82T,G48T***,L23I***,V82S**,G48S***,G48V,M46L*,G48Q***,G48L***,G48M***,M46I,V82A,V82C***,V82F**,N88T***]*
(M46V***,V32I*,K20T***,G73A***,L24I***,I47V*,L10I,L10F*,T74P***,N83D***,V82M**,V82L***,G73T***,G73S*,L10R**,A71L***,L10V*,I47A***,A71V,L10Y***,A71T*,G73C***,A71I***)
<I50L***>

LEXIVA® (fosamprenavir, FPV)

{L76V***,I84V,I84C***,I84A***,I47A***,I54M***,I54L***,I50V**}
*[V82F**,I47V*]*
(M46V***,V11I***,V82M**,V82L***,V82A,V32I*,V82C***,L90M,G73A***,L33F*,I54A***,L10I,L10F*,I54S***,G73T***,T74P***,G73S*,I54V,L10R**,I54T*,A71L***,L10V*,L89V***,A71V,L10Y***,A71T*,V82T,V82S**,G73C***,M46L*,A71I***,N83D***,M46I)
<N88S***,I50L***>

Mutation Notations Key:

{Red Bold Curly Bracket}	Presence of this mutation alone confers viral resistance
[Blue Bold-Italics Square Bracket]	Presence of this mutation alone confers the possibility of viral resistance
(Black Parenthesis)	This mutation must appear with at least one other mutation to confer the possibility of viral resistance
<Green Angle Bracket>	This mutation counters resistance
*	NOTE: This mutation has not been fully validated
**	NOTE: This mutation has not been clinically verified
***	NOTE: For this mutation, both notes above apply

References:

1. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

2. M Boffito, E Acosta, D Burger, CV Fletcher, C Flexner, R Garaffo, G Gatti, M Kurowski, CF Perno, G Paytavin, M Regazzi and D Back. Current status and future prospects of therapeutic drug monitoring and applied clinical pharmacology in antiretroviral therapy. Antiviral Therapy, 2005, 10:375-392

3. N Shulman, A Zolopa, D Havir, A Hsu, C Renz, S Boller, P Jiang, R Rode, J Gallant, E Race, DJ Kempf and E Sun. Virtual inhibitory quotient predicts response to ritonavir boosting of indinavir-based therapy in human immunodeficiency virus-infected patients with ongoing viremia. Antimicrobial Agents Chemotherapy, 2002,46:3907-3916