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 \( \geq 2000 \) 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, ddl)
   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)
MCMR-UWA-A

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
Drug Resistance:

**NRTI Class**
- EMTRIVA® (emtricitabine, FTC)
- EPIVIR® (lamivudine, 3TC)
- RETROVIR® (zidovudine, ZDV)
- VIDEX® (didanosine, ddl)
- VIREAD® (tenofovir, TDF)
- ZERIT® (stavudine, d4T)
- ZIAGEN® (abacavir, ABC)

**Evidence of Resistance**
- None

**NNRTI Class**
- EDURANT® (rilpivirine, RPV)
- INTELENCE® (etravirine, ETR)
- SUSTIVA® (efavirenz, EFV)
- VIRAMUNE® (nevirapine, NVP)

**Evidence of Resistance**
- None

**PI+ Class**
- APTIVUS® (tipranavir, TPV)
- CRIXIVAN® (indinavir, IDV)
- FORTOVASE® / INVIRASE® (saquinavir, SQV)
- KALETRA® (lopinavir + ritonavir, LPV)
- LEXIVA® (fosamprenavir, FPV)
- PREZISTA® (darunavir, DRV)
- REYATAZ® (atazanavir, ATV)
- VIRACEPT® (nefﬁnavir, NFV)

**Evidence of Resistance**
- 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:**

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Mutations present constitute a high level of genetic evidence for viral resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible Resistance</td>
<td>Mutations present suggest the possibility of viral resistance</td>
</tr>
<tr>
<td>None</td>
<td>There is insufficient evidence for viral resistance</td>
</tr>
</tbody>
</table>

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 nefﬁnavir (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)
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

ViroSeq® HIV-1 Antiretroviral Drug Resistance Report

Patient Information:

Acceesion 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  

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:  

FOR IN VITRO DIAGNOSTIC USE
HIV-1 Resistance Mutation List:

<table>
<thead>
<tr>
<th>NRTI Class</th>
<th>Mutations Included within the Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIVIR® (lamivudine, 3TC)</td>
<td>[M184V,M184I]</td>
</tr>
<tr>
<td></td>
<td>[69ins*,K65R,Q151M]</td>
</tr>
<tr>
<td>EMTRIVA® (emtricitabine, FTC)</td>
<td>[M184V,M184I]</td>
</tr>
<tr>
<td></td>
<td>[69ins*,K65R,Q151M]</td>
</tr>
<tr>
<td>VIDEK® (didanosine, ddl)</td>
<td>[K65R,L74I*,L74V,Q151M,69ins*]</td>
</tr>
<tr>
<td>ZERIT® ( stavudine, d4T)</td>
<td>(Q151M,T215Y,T215F,69ins*,V75M**,V75I**)</td>
</tr>
<tr>
<td>ZIAGEN® (abacavir, ABC)</td>
<td>(69ins*,K65R,Q151Y,Y115F*)</td>
</tr>
<tr>
<td>VIREAD® (tenofovir, TDF)</td>
<td>(69ins*,K65R)</td>
</tr>
<tr>
<td></td>
<td>[K65N*,Q151M,K70E**,K70G**]</td>
</tr>
<tr>
<td>RETROVIR® (zidovudine, ZDV)</td>
<td>(69ins*,Q151M,T215Y,T215F)</td>
</tr>
<tr>
<td>&lt;M65R,K65N**,M184V,K70E**,M184I*&gt;</td>
<td></td>
</tr>
<tr>
<td>NNRTI Class</td>
<td>Mutations Included within the Algorithm</td>
</tr>
<tr>
<td>INTELENCE® (etravirine, ETR)</td>
<td>[Y181V**,Y181I**]</td>
</tr>
</tbody>
</table>
ViroSeq® HIV-1 Antiretroviral Drug Resistance Report

NNRTI Class

Mutations Included within the Algorithm

SUSTIVA® (efavirenz, EFV)


VIRAMUNE® (nevirapine, NVP)


EDURANT® (rilpivirine, RPV)


PI Class

Mutations Included within the Algorithm

APTVUS® (tipranavir, TPV)

{I84V*, V82L**, I84A**, V82T*}


{L76V**, L24**, I50L**, I54L**, I50V***}

CRIXIVAN® (indinavir, IDV)

{I84A***}


{L76V**, I50L**, I47A***}

FORTOVASE® / INVIRASE® (saquinavir, SQV)


{L90M}


{L76V**, I50L**, I47A***}

KALETRA® (lopinavir + ritonavir, LPV)

{I47A***}

{I84A***, I50V**, L76V**}


{L76V**, I50L**, I47A***}

PREZISTA® (darunavir, DRV)


{L76V**, N88S**}
**Pl Class**

**Mutations Included within the Algorithm**

**REYATAZ® (atazanavir, ATV)**

\{I84V, I84C\**, I50L\**, I84A\**, N88S\**\}

\{G48A\**, G48T\**, G48S\**, G48V, G48Q\**, G48L\**, G48M\**\}


\{L76V\**\}

**VIRACEPT® (nelфинavir, NFV)**

\{I84V, L90M, D30N, J84C\**, J84A\**, N88D\*, N88S\**\}


\{L76S\**\}

**LEXIVA® (fosamprenavir, FPV)**

\{I84V, I84C\**, J84A\**, J47A\**, J54M\**, J54L\**, I50V\**\}

\{V82F\**, J47V\**\}


\{N88S\**, I50L\**\}

**Mutation Notations Key:**

**Red Bold Curly Bracket**

Presence of this mutation alone confers viral resistance

**Blue Bold-Italic 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:**

* 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:**

