Next Generation Sequencing for Determination of HIV Resistance Genotype

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Disclosure

• No conflicts of interest to disclose
Objectives

• Describe current Human Immunodeficiency Virus (HIV) testing algorithm and status of genotyping in Florida

• List steps in implementation of next generation sequencing (NGS) for HIV genotyping

• Discuss challenges and solutions with NGS implementation in the Public Health Laboratory (PHL)
HIV Testing Algorithm at the Florida State Public Health Laboratory

- The Florida Department of Health (Florida Health) Bureau of Public Health Laboratories (BPHL) performs the 2014 Centers for Disease Control and Prevention/Association of Public Health Laboratories’ HIV Diagnostic Algorithm, for identification of acute and established HIV-1 infection

- BPHL performs clinical management testing including HIV-1 viral load, CD4 testing, and genotyping

- Florida Health HIV program supports a “Test & Treat” approach with the goal of early HIV diagnosis and timely patient care
  → Improve health outcomes
  → Reduce transmission
HIV Testing Algorithm at the Florida State Public Health Laboratory

Test & Treat Model

Blood is drawn on patient for HIV testing. Sample is sent to BPHL.

Ab/Ag EIA positive, Geenius HIV-1 positive. HIV-1 infection is diagnosed. Treatment initiated.

Viral load and CD4 testing performed. Genotype and resistance profile determined.
HIV Testing in the PHL
HIV Testing in the PHL
HIV Genotyping and Resistance Determination by DNA Sequencing

- Current method: ViroSeq HIV-1 Genotyping System v2.0, Abbott
  - Sequences the entire HIV-1 protease gene and two-thirds of the reverse transcriptase (RT) gene in seven Sanger sequencing reactions
  - ABI Prism® 3130 Genetic Analyzer
  - Testing applicable to:
    - HIV-1 infected individual at time of initial presentation and diagnosis before initial drug therapy
    - HIV-1 infected individual at drug therapy failure, i.e. increase viral load, prior to drug therapy change
Application of NGS for HIV Genotyping

• NGS has recently become accessible technology for the clinical and public health laboratory
  • The whole genome of a microorganism may be sequenced in a matter of hours or amplicon sequencing can be performed to target specific regions of the genome

• NGS is performed through the following basic steps:
  • DNA sequencing to produce multiple random sequence fragments, called ‘reads’
  • Sequence reads cleaned to ensure quality
  • Reads assembled into contiguous pieces, ‘contigs’ that can be aligned with a known sequence, ‘reference strain’
  • Analysis of aligned sequence is performed using software programs, ‘pipeline’
Amplicon-based whole genome sequencing for HIV

HIV RNA extracted from specimen

cDNA made, DNA amplicons generated by nested PCR

“Massively parallel” sequencing

Sequence data generated

SmartGene Bioinformatics
Amplicon-based NGS was performed on an Illumina® MiSeq or iSeq platform with Nextera XT reagents.

Targeted sequencing of the cDNA amplicon containing the protease (PI), reverse transcriptase (RT), and integrase (INSTI) genes was performed on HIV-positive specimens with a viral load of 1,000 RNA copies/ml or greater.

Sequencing data was analyzed using the SmartGene IDNS® 5 curated pipeline that includes Stanford University’s Genotypic Resistance Interpretation Algorithm, with an interpretation cut-off of 5%.

Tzou et al. JCM 2018 May 25;56(6)
HIV Genotyping and Resistance Determination by NGS

• Proposed method: Targeted, amplicon-based NGS using a laboratory-developed assay
  • Sequences protease (PI), reverse transcriptase (RT), and integrase (INSTI) genes
  • Illumina MiSeq or iSeq, Nextera XT reagents
  • SmartGene pipeline analysis
  • Testing applicable to:
    - HIV-1 infected individual at time of initial presentation and diagnosis before initial drug therapy
    - HIV-1 infected individual at drug therapy failure
## NGS - Drug Resistance Report

**Patient:** HIV_3  
**Sample:** HIV_3  
**Mid/Barcode:** n.a.  
**Subtype:** CRF02

### Drug resistance algorithm: Stanford HIVDB (8.5.0)

#### Nucleoside Reverse Transcriptase Inhibitors (NRTI)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mutations list</th>
<th>Range</th>
<th>Color</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3TC</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>ABC</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>AZT</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>D4T</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>CDD</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>FTC</td>
<td></td>
<td>1</td>
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<td>Susceptible</td>
</tr>
<tr>
<td>TDF</td>
<td></td>
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<td>S</td>
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</table>

#### Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

<table>
<thead>
<tr>
<th>Drug</th>
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<th>Interpretation</th>
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<tr>
<td>ENF</td>
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<tr>
<td>ETR</td>
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<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>NNVP</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>RPV</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
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</tbody>
</table>

#### Protease Inhibitors (PI)

<table>
<thead>
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<th>Drug</th>
<th>Mutations list</th>
<th>Range</th>
<th>Color</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV</td>
<td>46V (8.0%)</td>
<td>2</td>
<td>S</td>
<td>Potential Low-Level Resistance</td>
</tr>
<tr>
<td>DRV</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>TPV</td>
<td>46V (8.0%)</td>
<td>2</td>
<td>S</td>
<td>Potential Low-Level Resistance</td>
</tr>
<tr>
<td>IDV</td>
<td>46V (9.0%)</td>
<td>2</td>
<td>S</td>
<td>Potential Low-Level Resistance</td>
</tr>
<tr>
<td>LPV</td>
<td>46V (9.0%)</td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>NFV</td>
<td>46V (9.0%)</td>
<td>3</td>
<td>I</td>
<td>Low-Level Resistance</td>
</tr>
<tr>
<td>SQV</td>
<td>46V (9.0%)</td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>TPV</td>
<td>46V (9.0%)</td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
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</table>

#### Integrase Strand Transfer Inhibitors (INSTI)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mutations list</th>
<th>Range</th>
<th>Color</th>
<th>Interpretation</th>
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</thead>
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<tr>
<td>BC</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>DTG</td>
<td></td>
<td>1</td>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>EVG</td>
<td>157Q (99.9%)</td>
<td>2</td>
<td>S</td>
<td>Potential Low-Level Resistance</td>
</tr>
<tr>
<td>RAL</td>
<td>157Q (99.9%)</td>
<td>2</td>
<td>S</td>
<td>Potential Low-Level Resistance</td>
</tr>
</tbody>
</table>

### Extra parameters

**Pipeline:** HIV1-PR+RT+IN (2.0.5_HIV1_v1.4)  
**Drug resistance algorithm:** Stanford HIVDB (8.5.0)  
**Noise filter [%]:** 0.5  
**Interpretation cut-off [%]:** 5.0  
**Min. coverage [if reads]:** 50

**Number of reads:** 5468

**Mutations HIV1-PR (%):** 41K (97.9), 45R (8.9), 46V (9.0), 63T (98.4), 77I (96.4), 89M (98.1), 93R (96.4)

**Mutations HIV1-RT (%):**
- 23**R** (15.9), 35T (100.0), 35A (99.2), 403 (99.2), 43R (99.6), 60I (98.9), 68G (97.9), 162A (98.7), 173A (98.4), 174E (98.8), 178 (98.7), 203Q (97.7), 207E (98.0), 211K (97.8), 214L (97.4), 245E (97.8), 277R (96.4), 286A (95.6), 292 (97.7), 293V (98.3), 294T (98.5), 326V (98.0), 335D (96.7), 350R (96.2), 360K (97.3), 357K (98.6), 359S (95.2), 366R (95.5), 369R (94.9), 371V (94.8), 376V (94.6), 376S (94.2), 396R (95.5), 403R (92.6), 430D (94.3), 468P (96.7), 471E (97.3), 480I (97.8), 483Y (97.2), 481P (18.0), 491S (81.0), 519N (87.9), 524K (87.8), 527G (87.9), 529N (88.7), 5345 (89.4), 554S (94.9)

**Mutations HIV1-IN (%):**
- 14R (97.1), 31I (97.0), 101I (98.4), 112V (90.2), 123I (97.1), 124A (97.1), 125A (100.0), 134N (97.1), 136T (97.2), 157Q (98.9), 160I (97.8), 201I (98.6), 203M (97.2), 208L (100.0), 225A (6.2), 234I (100.0)

**Clade finders**
- HIV1-PR  
  (AC=B.AY331296, score=443.0, match length=279, %similarity=94.86)  
- HIV1-RT  
  (AC=CRF02_AG_JF320297, score=2440.0, match length=1677, %similarity=93.62)  
- HIV1-IN  
  (AC=CRF02_AG_JF320297, score=856.0, match length=166, %similarity=94.67)
**ViroSeq® HIV-1 Antiretroviral Drug Resistance Report**

### Drug Resistance:

<table>
<thead>
<tr>
<th>NRTI Class</th>
<th>Evidence of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMTRIVA® (emtricitabine, FTC)</td>
<td>None</td>
</tr>
<tr>
<td>EPIVIR® (lamivudine, 3TC)</td>
<td>None</td>
</tr>
<tr>
<td>RETROVIR® (zidovudine, ZDV)</td>
<td>None</td>
</tr>
<tr>
<td>VIDEX® (didanosine, ddI)</td>
<td>None</td>
</tr>
<tr>
<td>VIREAD® (tenofovir, TDF)</td>
<td>None</td>
</tr>
<tr>
<td>ZERIT® (stavudine, d4T)</td>
<td>None</td>
</tr>
<tr>
<td>ZIAGEN® (abacavir, ABC)</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NNRTI Class</th>
<th>Evidence of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDURANT® (rilpivirine, RPV)</td>
<td>None</td>
</tr>
<tr>
<td>INTELENCE® (etravirine, ETR)</td>
<td>None</td>
</tr>
<tr>
<td>SUSTIVA® (efavirenz, EFV)</td>
<td>None</td>
</tr>
<tr>
<td>VIRAMUNE® (nevirapine, NVP)</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PI Class</th>
<th>Evidence of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTIVU® (tipranavir, TPV)</td>
<td>None</td>
</tr>
<tr>
<td>CRMixVIR® (indinavir, IDV)</td>
<td>None</td>
</tr>
<tr>
<td>FORTOVASE® / INVIrase® (saquinavir, SQV)</td>
<td>None</td>
</tr>
<tr>
<td>KALETRA® (lopinavir + ritonavir, LPV)</td>
<td>None</td>
</tr>
<tr>
<td>LEXIVA® (fosamprenavir, FPV)</td>
<td>None</td>
</tr>
<tr>
<td>PREZISTA® (darunavir, DRV)</td>
<td>None</td>
</tr>
<tr>
<td>REYATAZ® (atazanavir, ATV)</td>
<td>None</td>
</tr>
<tr>
<td>VIRACEPT® (nelfinavir, NFV)</td>
<td>None</td>
</tr>
</tbody>
</table>

### 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**

- V31, S37N, R41K, L63T, V77I, L89M, I93L

- **RT**

Practical Considerations for Implementation of NGS for HIV Genotyping at BPHL

• Instrumentation: BPHL Molecular Section has four MiSeq, two iSeq
  • Ancillary equipment may also be required for DNA quantitation, quality measurement and automation

• Infrastructure: IT, networking, data exchange

• Staffing: BPHL Molecular Section has four sequencing staff with experience/training in sequencing and data analysis.
  • BPHL is adding a dedicated HIV sequencing member to the team, with support from the Florida Health HIV Section, Molecular Surveillance Team
  • Two HIV laboratory staff trained in cDNA production
Practical Considerations for Implementation of NGS for HIV Genotyping at BPHL

• Methodology/determining test parameters:
  • Appropriate primers for cDNA amplicon and NGS targets for sequencing
  • Number of copies/ml required to generate sequence
  • Percentage interpretation cut-off

• Test performance and verification:
  • Perform side-by-side comparison of current method (ViroSeq, FDA-approved) and proposed method (amplicon-based NGS, laboratory-developed test) and evaluate test quality e.g. accuracy, timeliness, cost etc.
  • Perform testing on Proficiency Testing samples/known characterized samples

• Discuss implementation with HIV Program:
  • Provision of consensus sequence and new report format
36 specimens with paired ViroSeq and NGS results were analyzed

- 30/36 (83.3%) were concordant with clinically significant mutations common to NRTI, NNRTI and PI resistance
- Substantial differences between the two methods:
  - NGS identified mutations present in a lower percentage of the population sampled (i.e. minority variants)
  - NGS identified INSTI mutations not detected by ViroSeq
  - Presence/absence of mutations due to use of different algorithms for analysis

NGS was performed on 20 specimens by MiSeq and iSeq for comparison

- Sequence data was comparable
- The iSeq will sequence about 1/10 DNA in half the time and therefore fewer samples needed for each run to make it cost-effective
Next Steps for Implementation of NGS in Florida

• NGS is more sensitive than Sanger in the detection of minority variants and can detect rare or uncommon HIV subtypes

• The SmartGene pipeline uses the most up-to-date Stanford algorithm and is a trusted means of data analysis for epidemiological purposes

• Using samples with at least 1,000 RNA copies/ml was sufficient to generate sequence in most cases

• The iSeq is an appropriate platform for performing HIV targeted NGS

• The interpretation cut-off is set at 5% but still requires more analysis
Potential Impact of NGS Genotyping on Florida HIV Program

- Partner information is not always readily available
- Molecular surveillance data can be used to supplement (not replace) partner information
Potential Impact of NGS Genotyping on Florida HIV Program

• BPHL plans to continue to make improvements in its testing algorithm in line with a “Test & Treat” model: this would include performing NGS genotyping on all newly-identified HIV cases diagnosed at BPHL

  → Patient impact: diagnosis and treatment more accessible in a timelier manner

  → Public health impact: essential surveillance data available for use by local, state and federal agencies

  → Laboratory impact: new techniques, more data to analyze
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