Sensitive Resistance Testing

John Mellors, MD

MTN Regional Meeting 2015

Lab Breakout Session

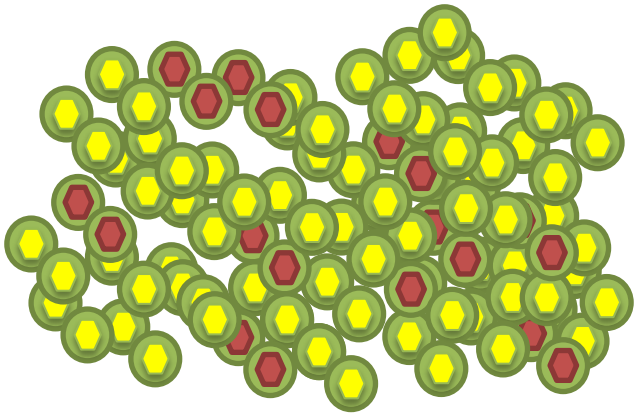
Outline

- Standard vs sensitive resistance testing
- Why does sensitive resistance testing matter?
- Resistance testing at the Virology Core
- Comparison of resistance tests
  - Standard (IHG) vs Sensitive (ASPCR and NGS)
- Sensitive resistance testing in VOICE and ASPIRE

Standard vs. Sensitive Resistance Testing

Standard

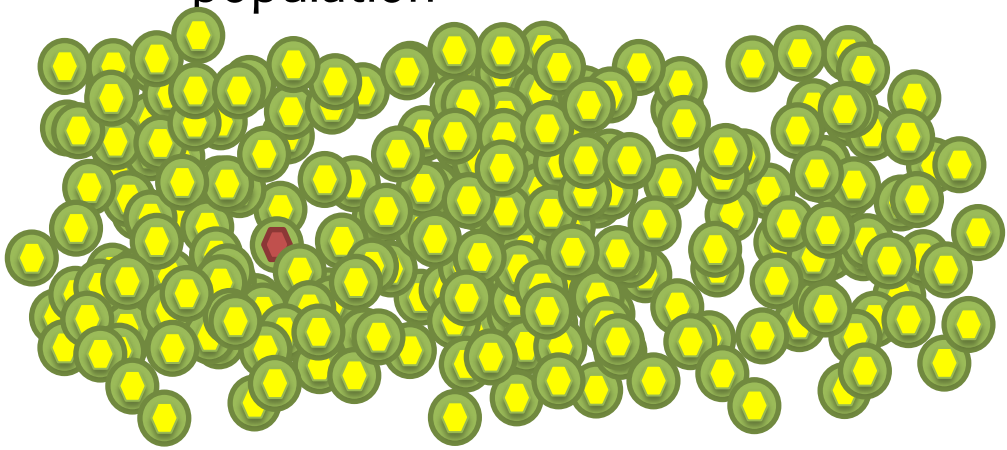
- Can detect drug resistance at a limit 20% of a patient's HIV virus population



  - Results available in 1 week
  - Can be used for clinical care
  - Moderate cost per sample

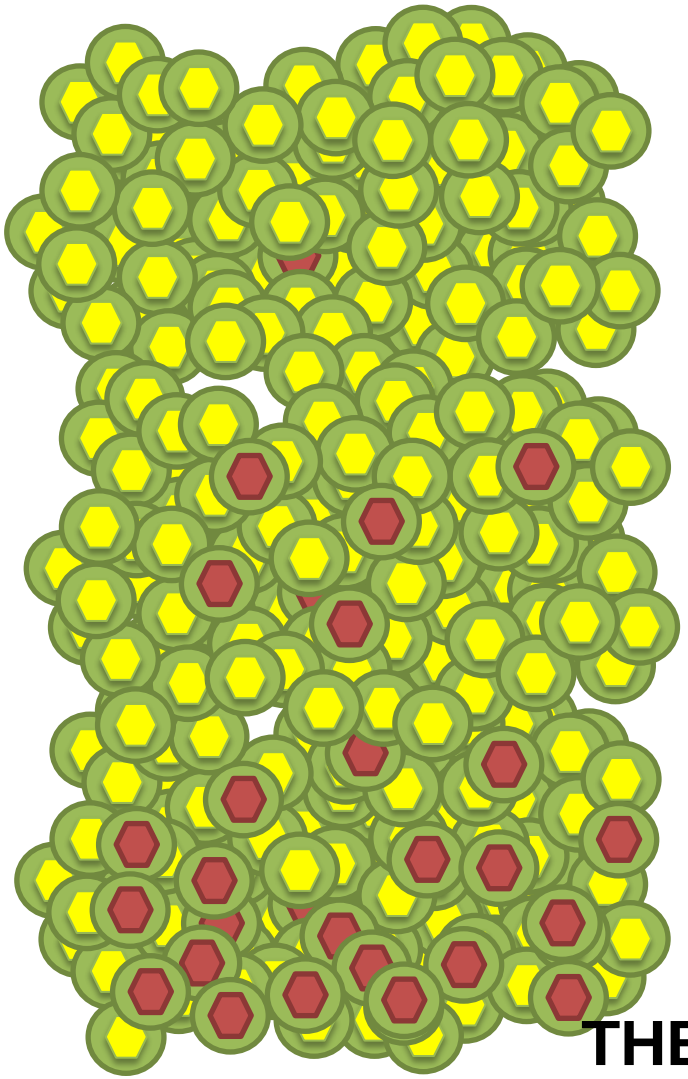
Sensitive

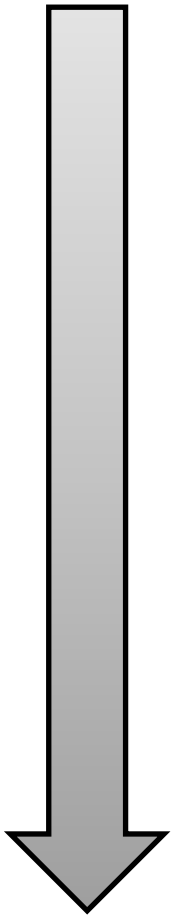
- Can detect drug resistance at a 0.1% of a patient's HIV virus population



  - Laborious technique
  - Research use only
  - High cost per sample

Why is sensitive resistance testing important?





Time in the presence of drug pressure

THERAPY FAILURE

Low Frequency Resistance in VOICE & ASPIRE

VOICE	ASPIRE
No data on low frequency NRTI resistance.	Nevirapine (NVP)-resistant mutant frequencies >1% are significantly associated with increased risk of NVP-containing ART failure (A5208/Octane).
Will seroconverters from tenofovir gel or oral TDF/FTC arms have low frequency resistance? Will it affect future first line treatment with Truvada?	**Will low frequency NNRTI resistance affect efficacy of dapivirine ring? Will if affect future PMTCT or first line treatment with NVP or efavirenz?**

Resistance Testing at Virology Core

Receive samples

- Plasma samples received from sites
- QC on all shipments
- Log samples and assign testing

Endpoint confirmation

- EIA
- WB

Standard resistance testing

- Standard resistance testing (IHG) on all HIV positive samples with VL>200
- Send results to SCHARP and to sites

Sensitive resistance testing

- Sensitive resistance testing (ASPCR or NGS) on all samples with successful IHG
- Send results to SCHARP

Standard vs Sensitive Tests

IHG In-House Genotyping	ASPCR Allele-Specific PCR	NGS Next-Generation Sequencing
"Population" sequence Get one sequence that is the "consensus" for all viruses in that sample Can have mixed bases	Codon specific testing Provides frequency of wild-type vs mutant codon	Similar to IHG, except that "consensus" is not given as output. Individual sequences are generated for all HIV molecules amplified in each sample Can accurately quantitate mixtures at low frequency (0.1%)

ASPCR

- Targets specific codons of interest
- Real-time PCR assay identifies % of viral templates with a specific codon

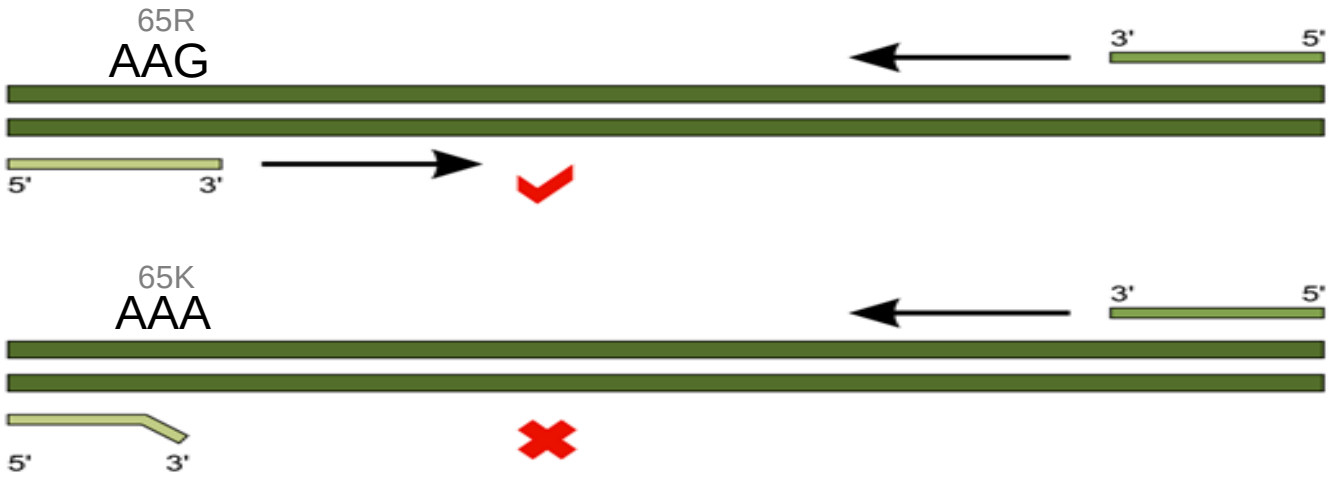


Image modified from:
http://www.tankonyvtar.hu/en/tartalom/tamop425/0011_1A_Molelkularis_diagnoszitka_en_book/ch14.html

ASPCR Method

Step 1

- Extract HIV-1 RNA
- Convert RNA to cDNA

Step 2

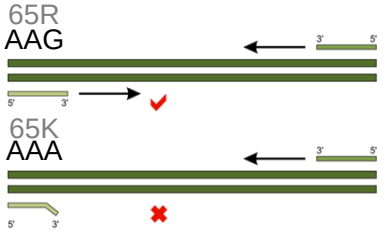
- Create large pool of templates through PCR amplification of patient HIV-1cDNA

Step 3

- Use ASPCR codon specific primers to determine presence of wild-type and mutant codons
- All samples are run with both primer sets

Output

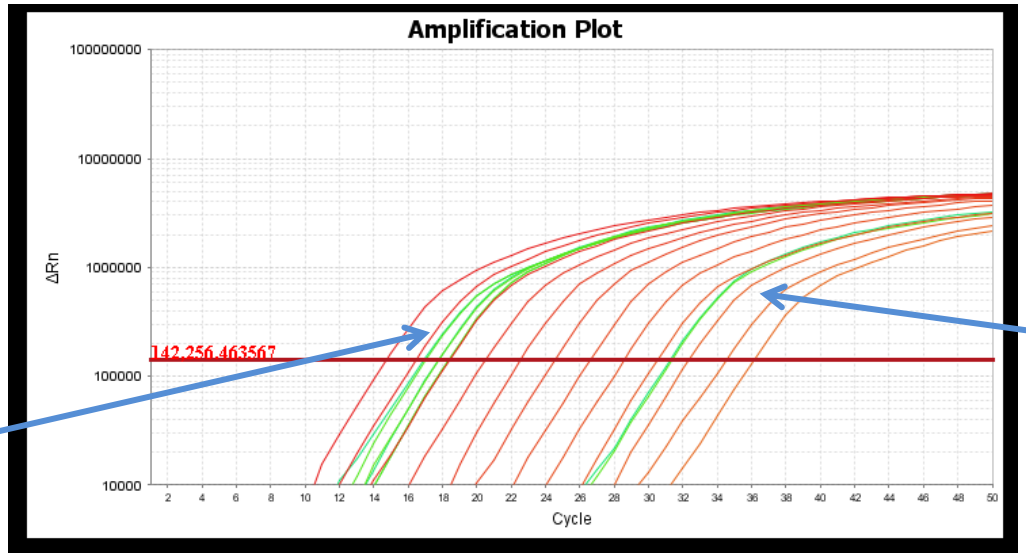
- Presence and frequency of wild-type vs mutant codon is generated based on standard curves of wild-type and mutant mixtures



ASPCR results

- SYBR green based assay
- Frequency is determined by standard of wild-type and mutant mixtures of known %

Std curve
Samples



Higher % mutant

Low % mutant

VOICE ASPCR Results

Mutation	# Detected by Standard Genotyping/ # Seroconverted on Study Product	# Detected by ASPCR/ # Tested	Detection Limit	Range of Mutant Frequency
K65R	0/301	3/276	0.1%	0.5 – 15%
M184V	1/301	2/288	0.1%	0.5% - 98%
M184I	0/301	11/285	0.1%	0.5 – 5.2%
K70E	0/301	0/283	0.3%	-

- Detection of low frequency mutants did not differ across treatment arms or with the detection of tenofovir at any follow-up visit.
- Results presented at CROI 2015 (Panousis, et al.)

Sensitive Resistance Testing: VOICE

- **Resistance selection in VOICE remains LOW.**
- Mutant detection was ***not*** associated with treatment arm or detectable TFV.
  - Low frequency mutants may be transmitted resistance or spontaneously arising mutants of unknown clinical significance.
- Low product use in the VOICE trial could explain the infrequent selection of resistance among seroconverters.

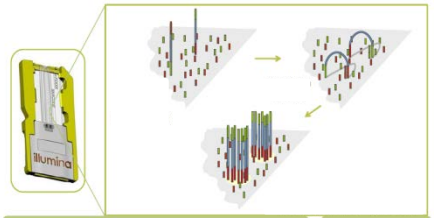
NGS

- Provides sequence for targeted region, not codon specific (aa56-227)
- 100,000 reads per sample
- Samples can be combined and run in a high-throughput format with the use of Sample ID tags that are added during PCR amplification

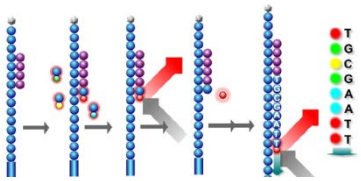
NGS Method

Step 1
- Extract HIV-1 RNA
- Convert RNA to cDNA

Step 2
- Create large pool of templates through PCR amplification of patient HIV-1 cDNA



Step 3
- Use Illumina MiSeq platform to perform sequencing reaction



Output
- Sequence is generated for ALL amplified cDNAs
- Sequence read is the entire length of amplicon
- ***100,000s of reads per sample!***

NGS results

- Output of assay= FASTQ files (like FASTA- string of sequence)

- Strong need for bioinformatics tools to process data
- Bioinformatics tools separate and analyze samples based on sample ID tags; report frequency of mutations in each sample

Next Steps

- Finish developing NGS assay for all subtypes
- Test VOICE samples with NGS to confirm low frequency mutations observed with ASPCR
- Test ASPIRE samples with NGS

Acknowledgements

University of Pittsburgh
Urvi Parikh
Elias Halvas
Constantinos Panousis
Kerri Penrose

MTN
VOICE Team
ASPIRE Team
LC Core
SCHARP

National Cancer Institute
Valerie Boltz
Mary Kearney

Participants!