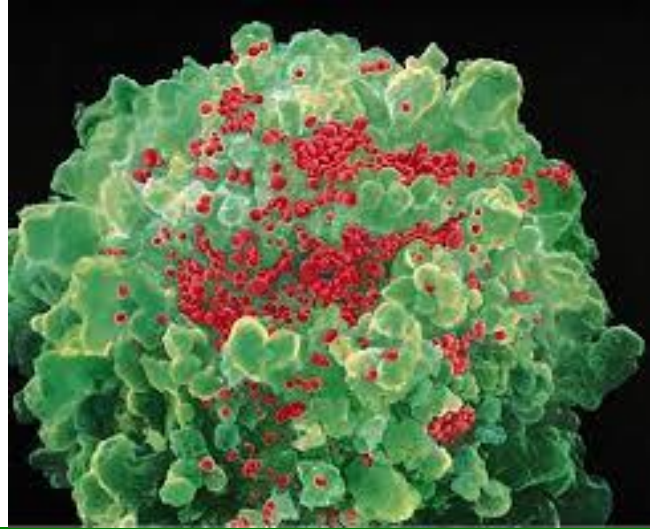

New Assays for HIVDR:

Implications for Point-of-Care Testing

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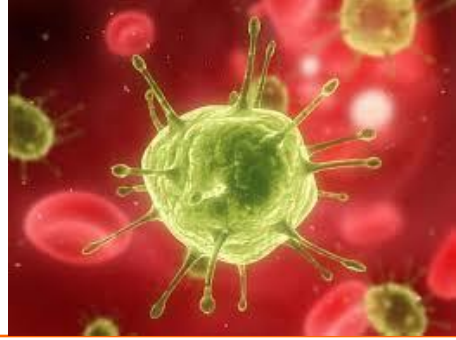
Outline

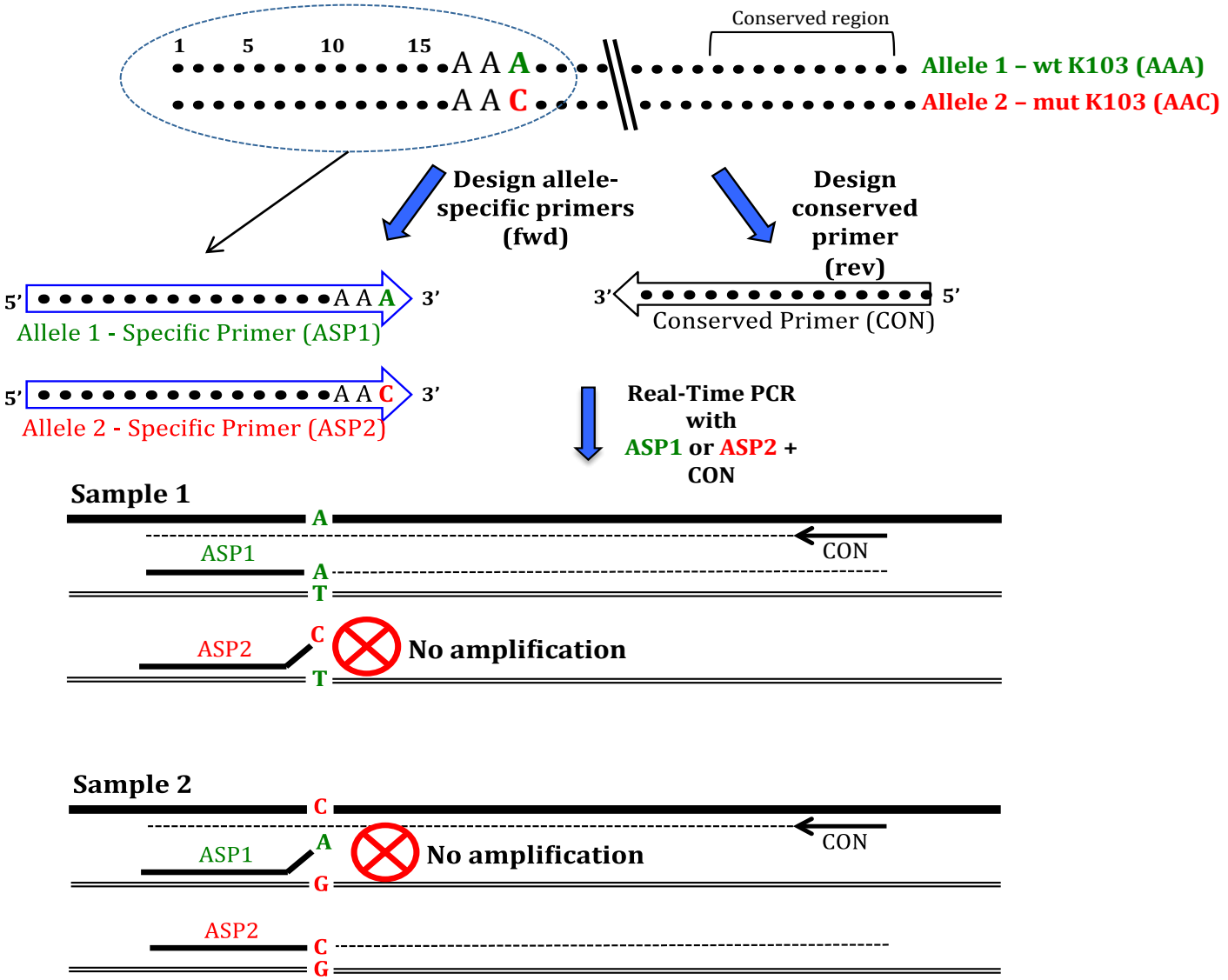


- Point-mutation assays
  - Allele-specific PCR
  - One-step ligation
  - PAANDA
  - Oligonucleotide ligation assay (OLA)

- How do we develop a point of care assay

Allele-specific PCR: Schema

- Developed & implemented at YRG-CARE, Chennai, India



Allele-specific PCR

- **Strengths**
  - Established assay
  - Implemented in a resource limited setting
  - Detects targeted mutations
  - Detects minor variants
  - Low cost
  - Moderate labor
  - No interpretation software/algorithm

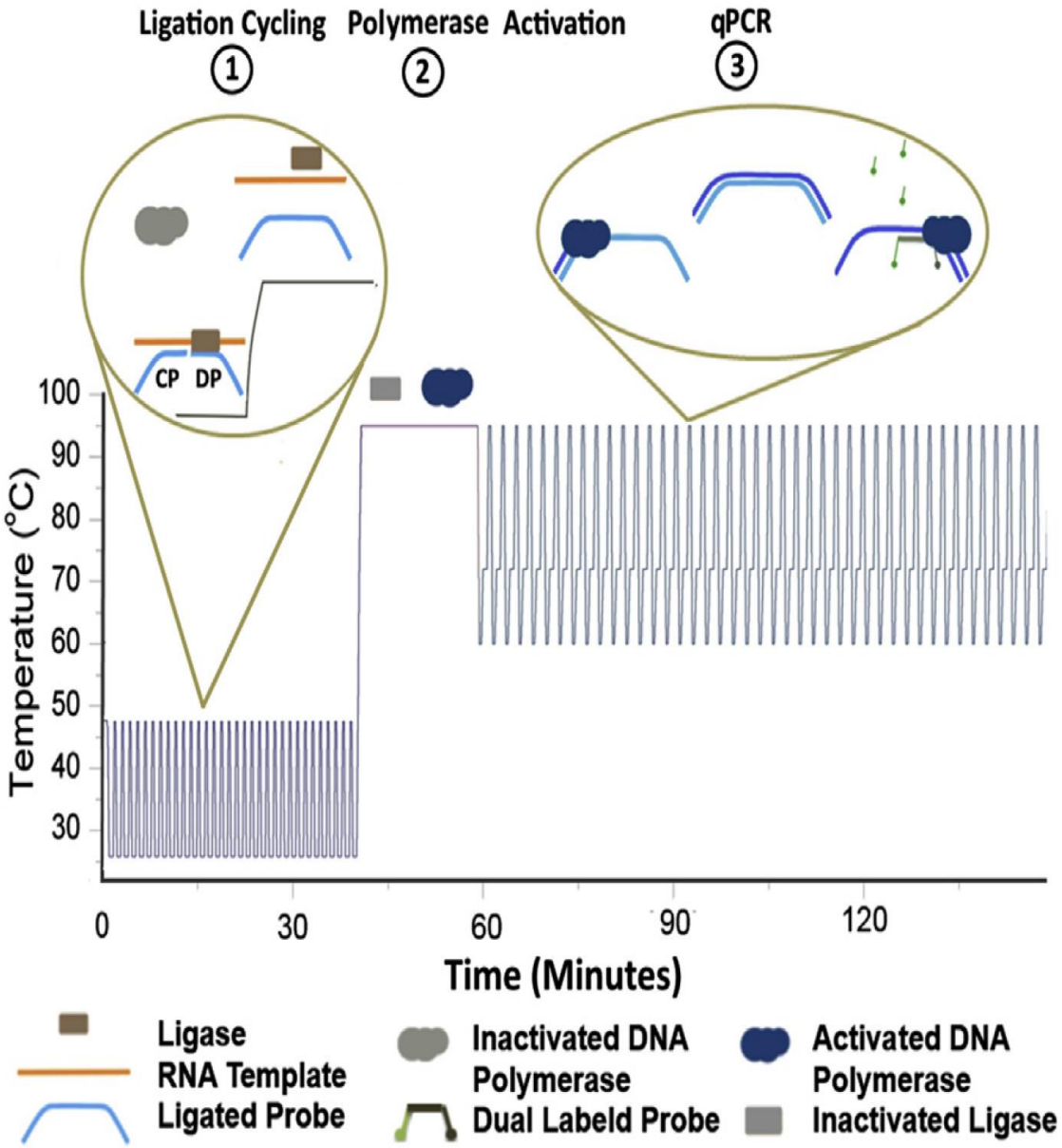
- Weaknesses
  - HIV diversity lessens sensitivity
  - Minor frequency variants can be false positives
  - Few codons
  - Requires laboratory; real-time thermocycler ~$20K
  - Not point-of-care

Allele-specific PCR: Role in clinical care and design for widespread implementation in resource-limited settings

- Operational in Chennai, India
- Using assay optimized for locally circulating HIV variants
- Used for drug resistance surveillance & individual care
- Has advanced training of laboratory personnel and education of local care givers in a resource limited settings

Ligation on RNA Amplification: Schema

Zhang et al. J Mol Diag 2015



Ligation on RNA Amplification

- Strengths
  - Tolerates many polymorphisms around mutation
  - Fewer false positives compared to ASPCR
  - Work directly on RNA
  - Low cost
  - Minimal labor
  - No interpretation software/algorithm
  - Detects minor variants

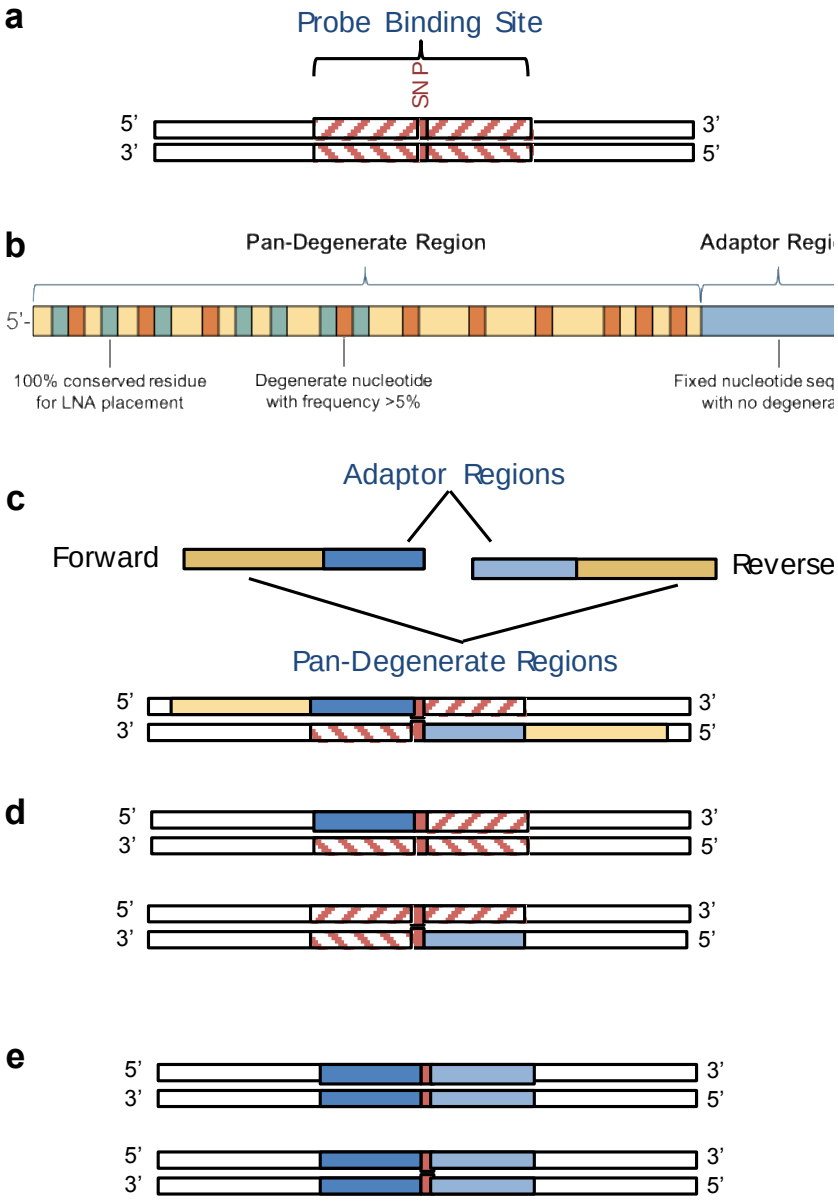
- Weaknesses
  - In development
  - Improved by optimizing to regional variants
  - Requires laboratory; real-time thermocycler ~$20K
  - Not point-of-care

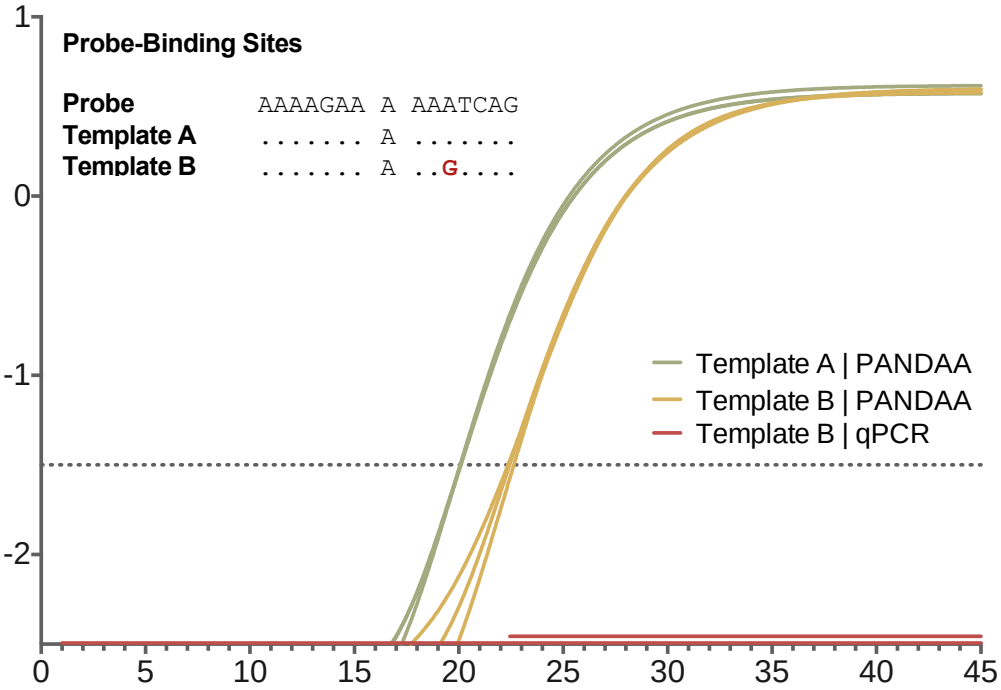
Ligation on RNA Amplification:

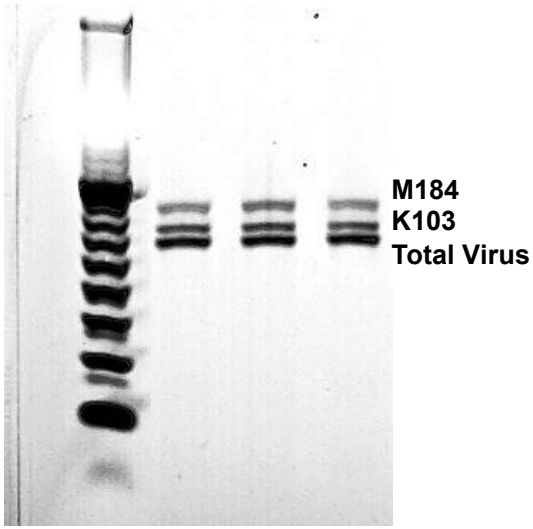
Role in clinical care and design for widespread implementation in resource-limited settings

- Will likely offer laboratory based assay with improved sensitivity and specificity compared to ASPCR

PANDAA: Schema







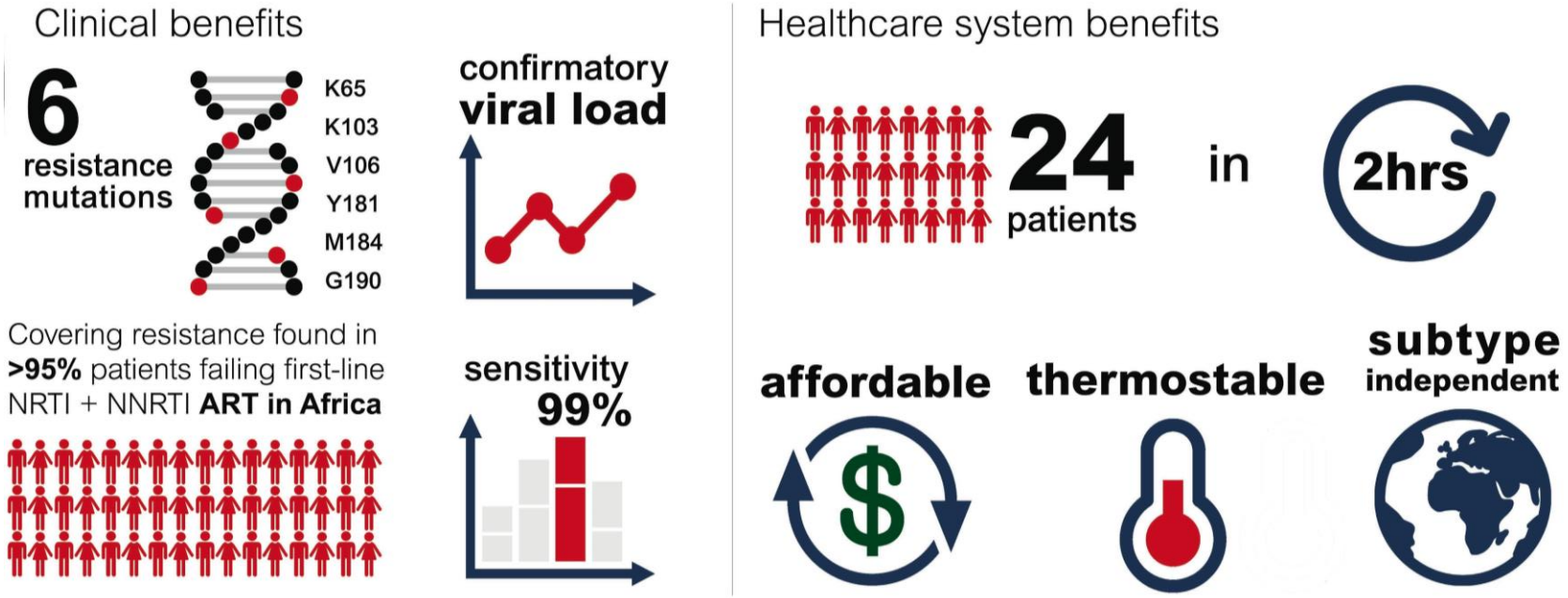
ALDATU BIOSCIENCES

PANDAA

- **Strengths**
  - Tolerates many polymorphisms around mutation
  - Minimal labor
  - Detects minor variants
  - Rapid (~90 minutes to result)
  - Sensitive to 1% of the virus population; highly specific
  - Can provide viral load information ("viral quantifier")
  - HIV subtype-independent
  - Enables "focused genotyping" (pre-defined codons relevant to a given clinical decision (e.g. first-line failures)
  - Relatively inexpensive compared to Sanger
  - High throughput

- **Weaknesses**
  - Does not detect DRM linkage
  - Few codons
  - Requires laboratory; real-time thermocycler ~$20K
  - Not point-of-care

PANDAA in Future Clinical Care



Pros	Cons

PANDAA HIV6 Product (First-line Failures in LMICs)

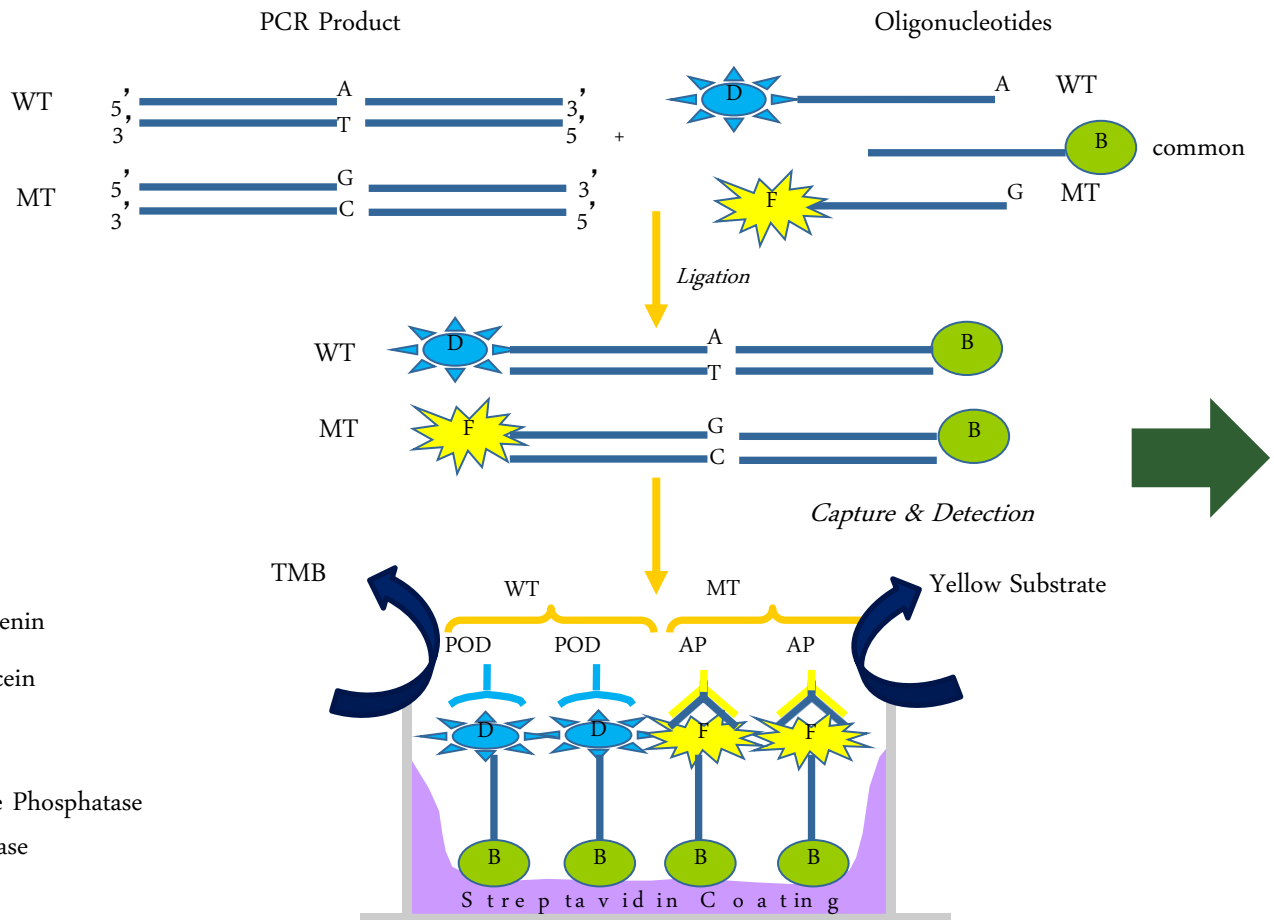
- Plugs in to existing qPCR infrastructure
- Simple ("sample-in, answer-out")
- Lyophilized / thermostable (no cold chain req'd)
- LyoSpheres are customizable - DRM detection reagent sets can be swapped in/out depending on need (e.g. change in standardized drug regimens)
- Automated data analysis, customizable readout for different users / audiences (e.g. researchers vs. clinicians vs. lower-level healthcare workers)

- Dependent on existing qPCR infrastructure
- No integrated sample prep

ALDATU BIOSCIENCES

Oligonucleotide Ligation Assay (OLA)

- Specimen - Blood cells, plasma, whole blood or filter papers
- Steps - PCR or RT-PCR, ligation of discriminatory probes & EIA



Wild type

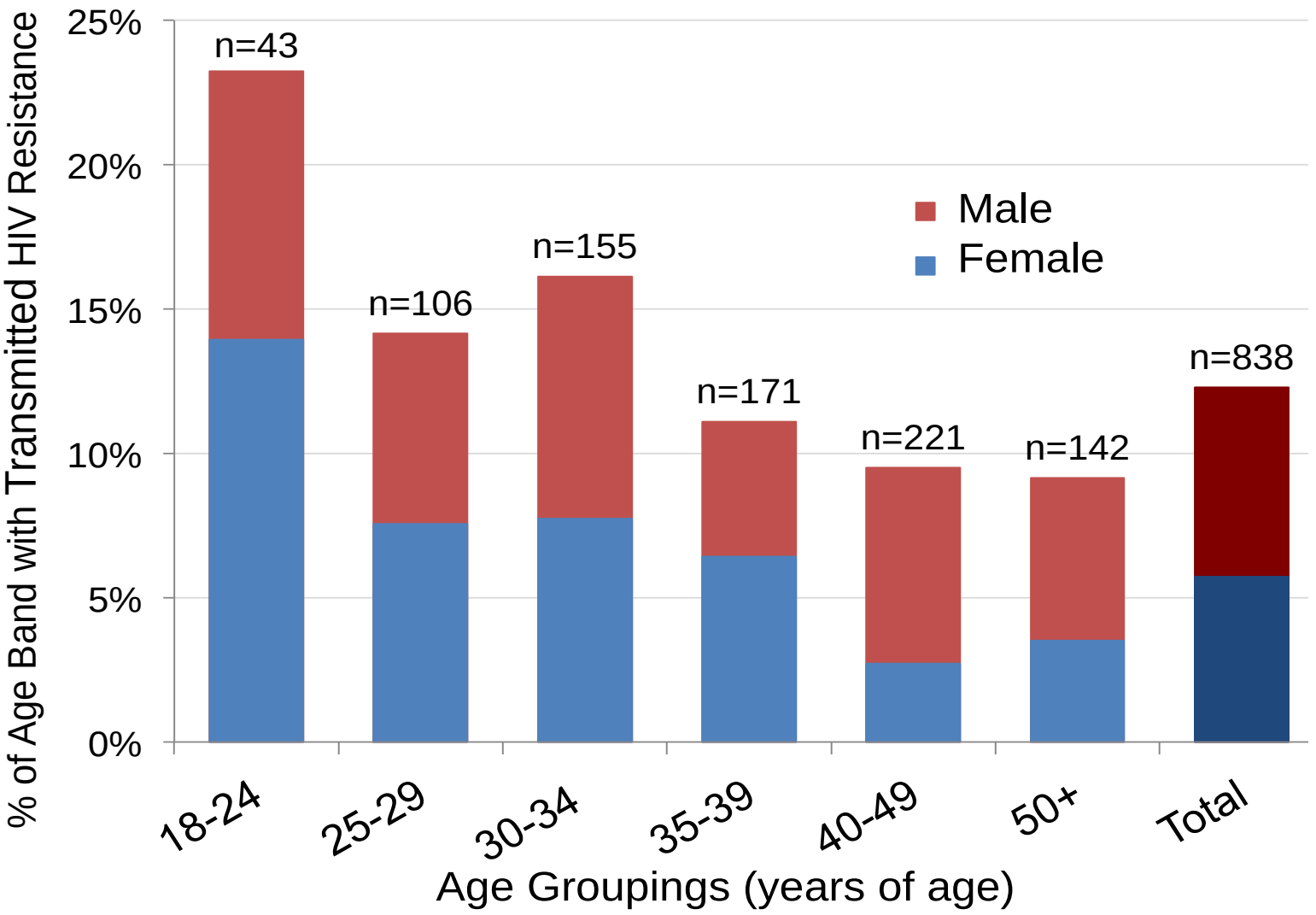
Mutant

OLA

- Strengths
  - Highly sensitive, detects ≥2% mutant frequency
  - Highly specific, ~100% due to ligase requirements
  - Quantifies mutant frequencies
  - Minimal equipment (simple thermocycler; ~$3K)

- Weaknesses
  - Requires technical skill
  - Low-cost only if batch test specimens
  - Turn-around-time ~8 hours
  - Not point-of-care

OLA detected high prevalence of DR among 838 ARV-naïve Kenyan adults qualifying for ART in 2013-2014



Pre-ART OLA & 12-month Virologic Outcome

- 988 enrolled & randomized to pre-ART OLA vs. standard-of-care (SOC)
- OLA codons: K103N, Y181C, M184V & G190A
- If OLA ≥10% mutant Rx Lopinavir/rt-ART; otherwise SOC NNRTI-ART
- 803 with plasma HIV RNA after 12-months ART
- **Intent-to-treat OLA vs. SOC arms:** 34 (8.5%) vs. 39* (9.7%); P= 0.562; underpowered

Virologic failure (VF) at 1-year of ART by study arm & frequency of resistance at enrollment

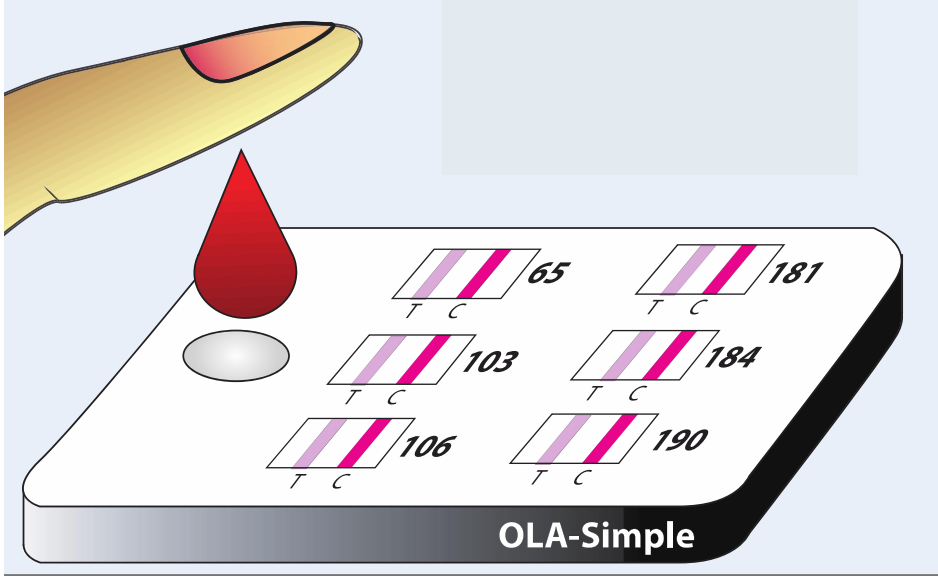
	OLA (n=400)		SOC (n=403)		Chi^2 p-value
OLA results	#Tested	# (%) VF	# Tested	# (%) VF	
Wild-type	351	26 (7.4%)	363	22 (6.1%)	0.472
2-9%	13	3 (23.1%)	14	5 (35.7%)	0.472
≥10%	36	5 (13.9%)	26	12* (46.2%)	0.005

VF at 1-year of NVP- vs. EFV-ART by frequency of resistance at enrollment

	Nevirapine-based-ART		Efavirenz-based-ART		
% mutant	# subjects	# (%) VF	# subjects	# (%) VF	p-value
Wild-type	252	27 (10.7%)	453	20 (4.2%)	0.001
2-9%	8	5 (62.5%)	19	3 (15.8%)	0.015
≥10%	10	6 (60.0%)	21	6 (28.6%)	0.093

POINT: PDR affects VS, even low-frequency

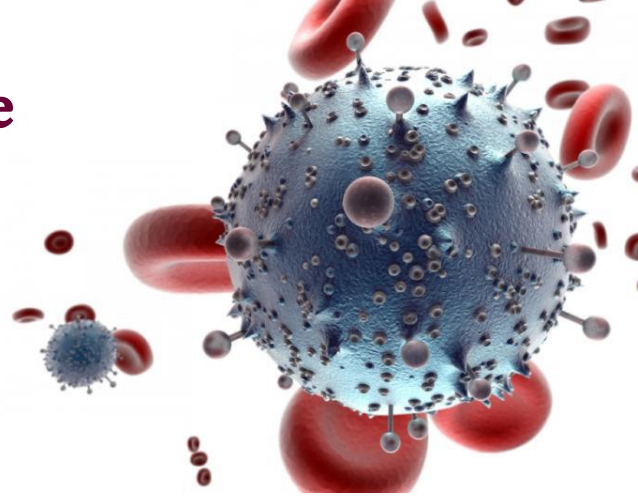
Point-of-Care assay



- **Priorities**
  - Detect DR to 1st-line-ART
  - Detects low-frequency variants
  - Rapid turn-around-time
  - Economical
  - Minimal technical training

- **Remaining Challenges**
  - Input of >300+ viral templates to detect minor variants
  - Rapid nucleic acid extraction
  - Rapid amplification and prevention of amplicon carry-over
  - Reagent stability
  - Need assay to test antiretroviral drugs for each ART combination

Ongoing conversion of OLA to Point-of-Care assay



- Rapid method (i.e., stimuli-responsive regents) to capture adequate amount of nucleic acids
- Isothermal amplification
- Same-pot ligation
- Paper detection
- NEED to know next version of WHO 1st-line-ART

Seattle

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Thank you!

Questions?