A Simple Alternative for HIV Drug Resistance Genotyping: Laboratory Evaluation of the PANDAA qDx HIVDR RTI assay

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Technology Background

Validation of PANDAA qDx HIVDR RTI: a simple and scalable real-time PCR-based HIV drug resistance genotyping kit for the management of NNRTI-based ART failure.
M Denaro[1], H Ghandour[1], JE Long[1], N Renzette[1], D Raiser[1], and Iain J MacLeod[1,2]
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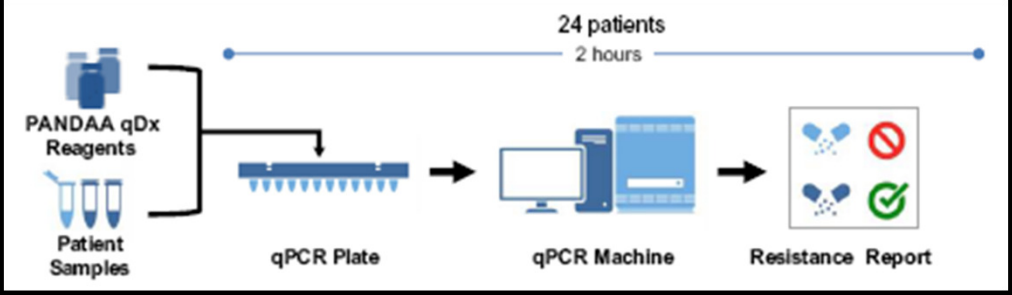
- 6 mutations frequently linked to drug resistance[1]
  - NRTI: K65R, M184V
  - NNRTI: K103N, V106M, Y181C, G190A
- Characterisation of 27 nucleotides surrounding DRMs shows secondary genotypic variability [2]
- Hybridisation–based, point mutation assays must overcome this variability

PANDAA: Pan-degenerate amplification and adaptation[3]
- Focused genotyping approach (patented)
- Compensates for high intra- and inter-patient variability
  → removes effects of secondary polymorphisms
  → enables qPCR
- Applicable to a variety of infectious diseases



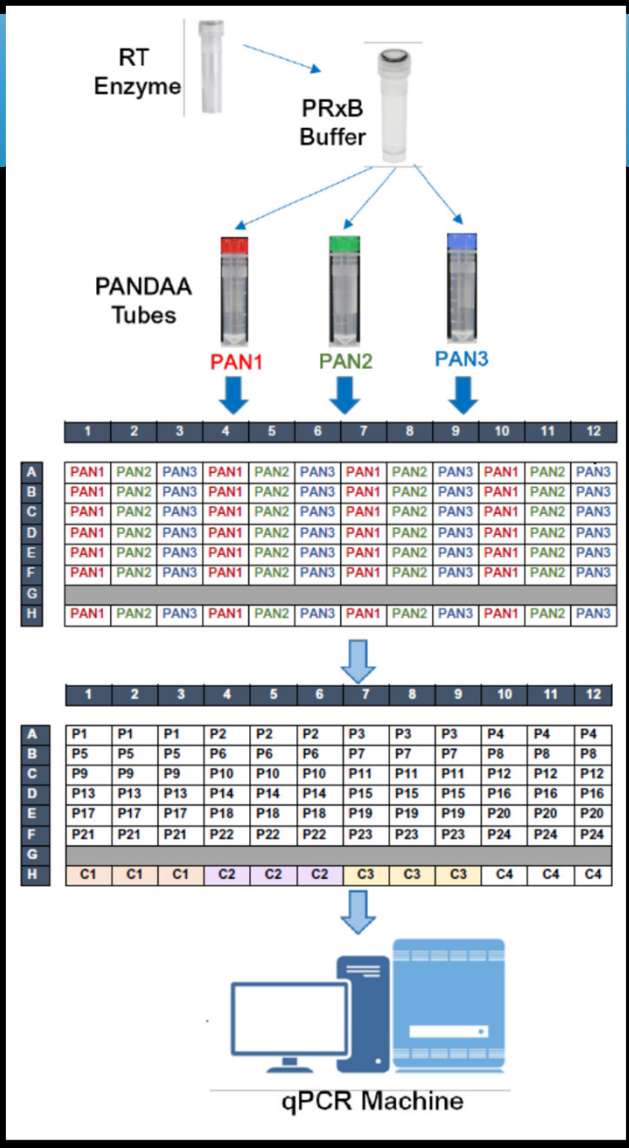
PANDAA qDx HIVDR RTI

- 96-well plate-based assay
  24 patients/plate
- 3 * triplex reactions per patient
  - Well 1: VQ, K65R, Y181C
  - Well 2: VQ, K103N/S, G190A
  - Well 3: VQ, V106M, M184V/I
- Viral Quantifier:
  - Internal Control
  - Quantifies total viral NA present
  - Determines relative abundance of each DRM
- Plate controls
  - Positive: synthetic RNA for DRMs 50%, 10%, and 0% (WT)
  - Negative: Human genomic DNA
- Sanger equivalent sensitivity[3]
  - LOD = 42 (47-122) copies/reaction (synthetic RNA)[3]

1. Rhee et al, 2015, PLoSOne 10(12)
2. Clutter et al (2016) Viruses 8(48)
3. Denaro et al (2019) IAS Conference

Methodology

- Each well contains FAM, NED and VIC(VQ) labelled probes
- TAT (post-RNA extraction):
  - Preparation: 30 minutes
  - Amplification and detection: 90 minutes
  - Analysis (automated): 15 minutes
- Reaction volume: 20µl RNA
  - 15µl RNA and 5µl PANDAA mastermix
- Study population:
  - 202 PLWH with virological failure
    - Previously analysed by Sanger Sequencing
  - Retrospective specimens
  - Median VL: log 4.6 log_{10}cp/mL
    - Input VL: log 4,3 log_{10}cp/mL*



**Based on available volume for RNA extraction: Where possible 500µl plasma was used for RNA extraction with samples >10000 cp/mL and 1000µl for ≤10000 cp/mL*

Results

- Specimens tested: n=202*
- Specimens detected: n=161
- Specimens not quantified: n=38
  - No VQ signal across wells
- Specimens excluded: n=3
  - Low volume specimen

	Median	25% Percentile	75% Percentile
Viral Load			**p<0.0001**
No VQ Detected	8 380	4 373	28 675
VQ Detected	65 400	20 600	312 000
Copies / Extraction**			**p<0.0001**
No VQ Detected	7 190	3 025	12 865
VQ Detected	32 450	9 806	147 500
Days Since VL			**p=0,861**
No VQ Detected	0	0	7
VQ Detected	0	0	8
Days in Transit			**p<0,0001**
No VQ Detected	2	0	1
VQ Detected	1	0	2

Overall RTI DR

PANDAA \ Sanger	DRM	WT
DRM	152	0
WT	0	9

NRTI DR

PANDAA \ Sanger	DRM	WT
DRM	138	1
WT	4	18

NNRTI DR

PANDAA \ Sanger	DRM	WT
DRM	131	2
WT	10	18

NRTI - FP

M184IV (n=1)

NRTI - FN

M184IV (n=2)

K65R, M184IV (n=2)

NNRTI - FP

K103N (n=2)

K103S (n=2)

V106M (n=1)

G190A (n=2)

K103S, V106M (n=2)

V106M, G190A (n=1)

NNRTI - FN

K103N (n=1)

Y181C (n=1)

- No particular mutation linked to false positives/negatives
- Chromatograms will be examined (ethics pending)

- Specimens with no VQ had a lower median VL and fewer copies/extraction.
- Specimen integrity may have been affected by freeze-thaw cycles (unknown).

**Duplicates excluded*

***Not all specimens had the preferred volume of plasma available for extraction*

Results

- Variable sensitivity (75-96%)
- Consistent specificity (>90%).
- Using the presence of any mutation as an indicator of RTI HIV DR, sensitivity and specificity were 100%

- Assay and analysis software were user-friendly
- TAT was ~2hrs from RNA input
  - <3,5 hours total TAT
- All software interfaced seamlessly with QS3

Drug Resistance Call per Sample by PANDAA qDx HIVDR RTI vs Sanger Sequencing

	Sensitivity % (n)		95% CI	Specificity % (n)		95% CI	PPV	NPV
Acquired RTI DR	100.0	(152/152)	97.6 - 100.0	100.0	(9/9)	66.4 - 100.0	100.0	100.0
Acquired NRTI DR	97.2	(138/142)	92.9 - 99.2	94.7	(18/19)	68.3 - 98.8	99.3	81.8
K65R	82.7	(43/52)	69.7 - 91.8	97.2	(106/109)	92.2 - 99.4	93.5	92.2
M184VI	95.0	(133/140)	90.0 - 98.0	95.2	(20/21)	76.2 - 99.9	99.3	74.1
Acquired NNRTI DR	92.9	(131/141)	87.3 - 96.5	90.0	(18/20)	68.3 - 98.8	98.5	64.3
K103NS	96.3	(79/82)	89.7 - 99.2	96.2	(76/79)	89.3 - 99.2	96.3	96.2
V106M	79.4	(54/68)	67.9 - 88.3	100.0	(93/93)	96.1 - 100.0	100.0	86.9
Y181C	88.9	(16/18)	65.3 - 98.6	98.6	(141/143)	95.0 - 99.8	88.9	98.6
G190A	75.7	(28/37)	58.8 - 88.2	100.0	(124/124)	97.1 - 100.0	93.2	94.4

Conclusions

- PANDAA qDx HIVDR RTI may be used to expand simple HIVDR genotyping to RLS (centralised)
- High specificity indicates potential use as a qualitative proxy for adherence and for patient triage for complete genotyping

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Disclosure
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