Diagnostic accuracy of a near point-of-care HIV drug resistance test OLA-Simple: a field validation study in Kenya

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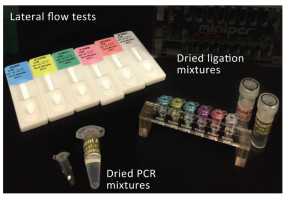
BACKGROUND

- Dolutegravir (DTG) is associated with improved antiretroviral therapy (ART) outcomes. Despite data suggesting that acquired HIV drug resistance (HIVDR) does not undermine efficacy of DTG-ART, **additional studies are needed to further evaluate the effects of extensive pre-existing nucleos(t)ide resistance on DTG-ART across subtypes.**

- In 2019, we reported development and clinical validation of "OLA-Simple," an easy-to-use, point-mutation HIVDR test.[1-3]



- **Ready-to-go dried reagents** - *easy assay set up*
- **Custom-designed lateral flow tests** - *visual readout*
- **In-house software to guide** "*molecular biology novices*"

99.6% specificity
98% sensitivity
Validated in specimens from A, B, C, D, and AE subtypes

No training required
Guided by software, 1st-time users processed specimens with 97% accuracy.

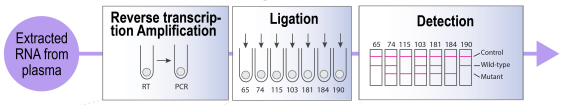
~$850 equipment
<$20 reagents/ sample tested
to detect DR for NRTIs (TDF/3TC) and NNRTIs (EFV/NVP) from DNA/RNA

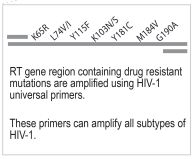
Same-day results
<4-hour total time
<10-min hands-on time

- **This work presents:**
  - Our recent probe development to include **detection of HIVDR mutations against Abacavir**
  - Our **first-ever clinical validation of OLA-Simple** that is performed in laboratories **from Kenya.**

METHODS

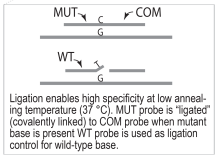
OLA Simple CHEMISTRY



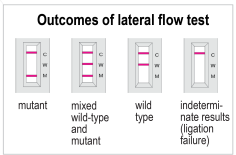


RT gene region containing drug resistant mutations are amplified using HIV-1 universal primers.

These primers can amplify all subtypes of HIV-1.



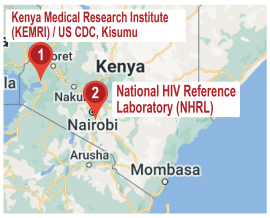
Ligation enables high specificity at low annealing temperature (37 °C). MUT probe is "ligated" (covalently linked) to COM probe when mutant base is present WT probe is used as ligation control for wild-type base.



STUDY DESIGN

Study time frame: April-June 2022.

STUDY SITES



PHASE I (3 days on-site training)

Total of seven lab technicians performed OLA-Simple on two blinded control specimens (negative plasma spiked with DNA).

PHASE II 8-10 weeks of independent testing by local lab technicians

Blind testing at KEMRI/CDC: 87 plasmas collected from children and pregnant/postpartum women

Blind testing at NHRL: 59 archived plasmas

OLA-Simple results for K65R, L74V/I, K103N/S, Y115F, Y181C, M184V, G190A were blindly **classified by software** as mutant, wildtype, or indeterminate (excluded two samples with incorrect image result formats)

Compared to reference assays: Sanger sequencing and laboratory OLA. Laboratory OLA detects ≥2% low-frequency variants [4].

Presented at XXX International Workshop on HIV Drug Resistance and Treatment Strategies

RESULTS AND DISCUSSION

PHASE I | On-site training local researchers using DNA controls

- Three and four technicians were trained at NHRL and KEMRI-CDC, respectively. During the observed training, US trainer observed the run performed by the local researchers. During the unobserved training, technicians operated independently without observation and relied completely on the software guide.

- Genotype classifications of the results obtained both during the observed and unobserved runs had a 100% agreements with the genotypes of the DNA controls.

PHASE II | Local researchers evaluated OLA-Simple on plasma specimens

- RT-PCR for OLA successfully amplified 134 specimens (91.8%) with plasma HIV RNA between 27 - 11013 copies/reaction (median: 317). The ones that failed had 35 - 1940 copies/reactions; thus RT-PCR failure could be due to a combination of factors:
  - Low RNA input (near the limit detection of RT-PCR)
  - PCR inhibitors carried from the extraction step, and/or
  - RNA degradation due to introduction of multiple freeze-thaw cycles of plasmas.

- From successfully amplified specimens, 132 had OLA-Simple image data for analysis of 924 codons which included 275 mutant, 612 wild-type and 37 (4%) indeterminate results (see Table 1).

Table 1. Summary of OLA-Simple results compared to Sanger with discordant results adjudicated by sensitive benchmark (laboratory OLA)

	K65R	L74V/I	K103N/S	Y115F	Y181C	M184V	G190A	Total
True (-)	114	96	50	112	103	35	88	**589**
True (+)	6	25	69	11	21	87	33	**258**
False (+)	2	3	8	0	2	2	6	**23**
False (-)	0	0	4	4	1	4	1	**14**
Indeterminate	10	8	1	5	5	4	4	**37**

Sensitivity
94.7% [95%CI: 91.3-97.1]

Specificity
96.3% [95%CI: 94.5-97.6%]

Indeterminate
4% [95%CI:2.8-5.5]

- OLA-Simple detected 6 low frequency mutant variants missed by Sanger (confirmed by laboratory OLA sensitive to 2% mutant) and misclassified 23 (2.4%) wild-type codons as mutant and 14 (1.6%) mutant codons as wild-type.

- Based on these results, OLA-Simple had **sensitivity of 94.7% and specificity of 96.3%.**

CONCLUSIONS AND NEXT STEPS

- This in-field validation study serves as a significant step towards implementation of OLA-Simple in LMICs.It reveals OLA-Simple's high sensitivity and specificity.

- Our next steps include validation of these probes on prospective samples, and developing OLA probes to detect DTG HIVDR mutations.

ACKNOWLEDGMENTS

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