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ORIGINAL RESEARCH

Higher Genotyping Performance of the Applied Biosystems TaqPath Seq HIV-1 Genotyping Kit Against ViroSeq HIV-1 Genotyping Kit in HIV *Protease*, *Reverse Transcriptase*, and *Integrase* Regions

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Purpose: We assessed the performance of Applied Biosystems TaqPath Seq HIV-1 Genotyping Kit (CE-IVD) (TaqPath Kit) against the ViroSeq HIV-1 Genotyping Assays in genotyping HIV *protease* (PR), *reverse transcriptase* (RT), and *integrase* (INI) regions.

Methods: The study included 43 HIV-1 plasma specimens: 20 from people living with HIV-1C and 23 well-characterized HIV-positive Virology Quality Assurance (VQA) samples with PR-, RT-, and INI mutations. VQA samples included HIV-1 subtypes A, B, C, D, F, G, CRFO2_AG and URF. HIV-1 RNA extracted from all specimens was tested with both genotyping assays. Known HIV drug resistance mutations (DRMs) were identified using the Stanford HIV drug resistance database. Sequencing success rates, nucleotide identity, and DRMs from the two commercial assays were compared.

Results: Of 43 samples, TaqPath Kit amplified 93.0% (40) for HIV PR/RT and 97.7% (42) for HIV INI regions, compared to ViroSeq Assays, which amplified 69.8% (30) and 72.1% (31) for PR/RT and INI, respectively. The TaqPath Kit successfully sequenced 90.0% (36/40) PR/RT and 97.6% (41/42) INI amplicons, while ViroSeq Assays sequenced 53.3% (16/30) PR/RT and 87.1% (27/31) INI amplicons. The mean nucleotide similarity was 98.8% (SD ± 1.30), 99.6% (SD ± 1.32) and 99.2% (SD ± 0.72) for paired RT, PR and INI sequences, respectively. The TaqPath Kit detected 97.2% (35/36) of DRMs identified by the ViroSeq Assays and an additional 83 mutations and polymorphisms in samples that failed genotyping with the ViroSeq Assays. Among these, 85.5% (71/83) were confirmed by the reference sequence, including 39 major DRMs.

Conclusion: The TaqPath Kit demonstrated higher genotyping performance compared to the ViroSeq assays. The TaqPath Kit was able to detect DRMs in the PR, RT and INI regions of various HIV-1 subtypes, offering a critical tool to identify and monitor HIV drug resistance to new and existing antiretroviral drugs targeting these regions.

Keywords: HIV drug resistance, genotyping assays, sequencing outcome, nucleotide identity scores

Introduction

The Joint United Nations programme on HIV/AIDS (UNAIDS) launched the "Treat All" strategy in September 2015. The main purpose of "Treat All" strategy was to initiate all people living with HIV (PLWH) on antiretroviral therapy (ART), irrespective of disease stage or CD4+ T-cell count.[1] In 2014, UNAIDS and partners set targets to diagnose 90%

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of PLWH, initiate ART in 90% of those diagnosed with HIV, and achieve and sustain viral suppression among 90% of those on ART by 2020 to eliminate AIDS epidemic by 2030.[2] With global progress made toward the 2020 targets, UNAIDS increased the 90–90–90 targets to 95–95–95 targets.[2] Despite these major advances including the development of new antiretroviral (ARV) drugs and ARV therapy (ART) treatment guidelines,[3–5] most PLWH continue to face challenges such as poor ART adherence, poor ART penetration, drug to drug interactions and temporary alterations in absorption.[6–9] Such challenges contribute to continued HIV-1 virologic non-suppression which drives HIV transmission and may lead to the development of HIV drug resistance mutations (DRMs). Development of DRMs is associated with decreased ARV efficacy, increased risk of progressing to virologic failure, increased risk of transmission of resistant strains and limited ARV options.[10,11]

Sanger-based population sequencing assays are among the most utilized in resource-limited settings for HIV drug resistance testing (DRT) and surveillance. HIV DRT has predominantly focused on HIV *reverse transcriptase* (RT) and *protease* (PR) regions due to the use of non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) based ART regimens, which were the first FDA approved ARV drug classes. A first generation integrase strand transfer inhibitor (INSTI)-raltegravir was introduced in 2007[12] and subsequently the World Health Organization (WHO) recommended dolutegravir (DTG) as part of the preferred first and second line ART for all population groups in 2018.[5] This use of INSTI-based ART regimen necessitates extending the capacity of HIV DRT to cover the HIV *integrase* (INI) region. Available HIV genotyping sanger-based assays genotype HIV PR/RT regions separately from HIV INI region. The Applied Biosystems™ TaqPath™ Seq HIV-1 Genotyping Kit (Thermo Fisher Scientific) (TaqPath Kit) claims to detect genomic mutations in the PR/RT [RT], and INI regions of the *pol* gene in HIV-1 viral RNA extracted from EDTA plasma and dried blood spots, as an aid in the monitoring of treatment efficacy of people living with HIV (PLWH). This study aims to demonstrate the performance of TaqPath Kit to genotype HIV DRMs for PR/RT and INI using plasma samples and compare its performance to the ViroSeq HIV-1 Integrase Genotyping Kit (Celera Corporation©) and ViroSeq HIV-1 Genotyping System v2.0 (Celera Corporation©) (ViroSeq Assays). The ViroSeq Assays, which genotype PR/RT and INI regions (separate kits), were widely used in resource-limited settings despite being validated primarily for HIV-1 subtype B. In 2021, the manufacturer discontinued the Sanger-based, FDA-cleared ViroSeq HIV-1 Genotyping System and research-use-only ViroSeq HIV-1 Integrase Genotyping Kit. In 2023, Thermo Fisher Scientific (Waltham, MA) introduced the TaqPath Kit, which also uses Sanger sequencing, carries the CE IVDD mark and is available for in vitro diagnostic use in countries recognizing this designation. Before discontinuation, ViroSeq Assays were the gold standard for evaluating new HIV genotyping assays covering similar HIV regions and were therefore used as the comparator assays in this study.

Methods

Sample Size and Population

This is a cross-sectional study utilizing 43 plasma samples with known HIV-1 subtypes. These samples included 20 de-identified clinical samples from PLWH who were previously enrolled in Botswana Combination Prevention Project (BCPP) (2013–2018) and 23 analytical samples for HIV-1 drug resistance from Virology Quality Assurance (VQA) programs (Supplementary Table 1a shows the details of VQA panels). The analytical samples included various HIV subtypes while all clinical samples were HIV-1C subtype confimed with REGA HIV-1 subtyping tool, ver. 3 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/) and COMET (https://comet.lih.lu/) (Figure 1).

Nucleic Acid Purification

Viral RNA was manually extracted from 140µL plasma samples using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Extracted product from each specimen was divided into three aliquots and stored at ≤ −80°C to enable proper preservation of the RNA. All RNA aliquots were thawed at room temperature prior to testing and were tested according to the kits' instructions for use.

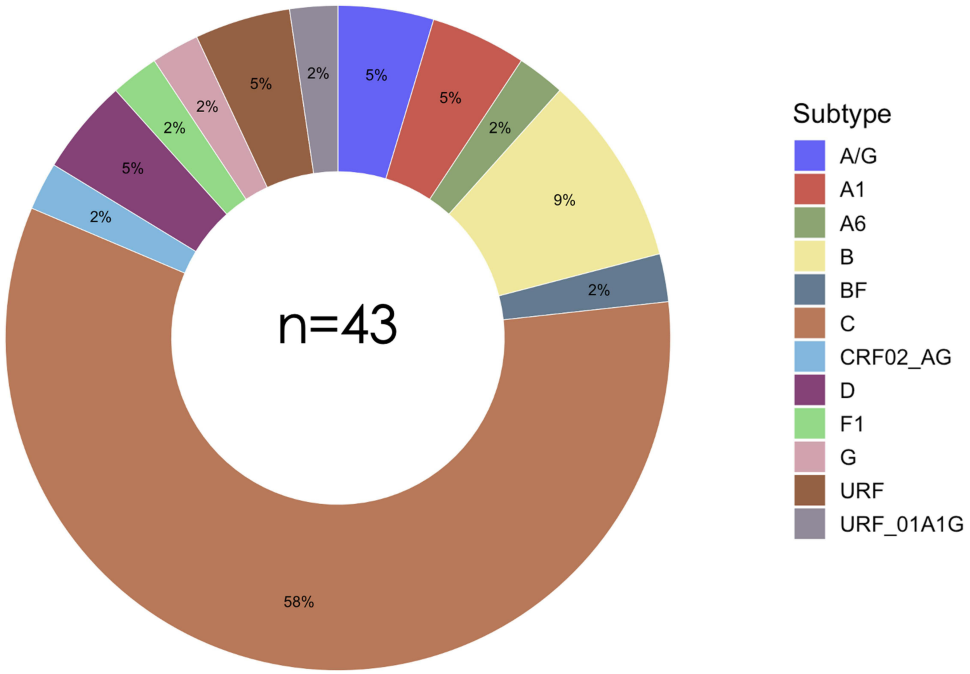

Figure 1 Proportions of different HIV-1 subtypes included in the study. The figure shows that 58% of the samples were from the HIV-1C subtype.

PCR Amplification and Sequencing

ViroSeq Assays

Genotyping using ViroSeq Assays for PR/RT and INI was performed per the manufacturer's instructions for ViroSeq®HIV-1 Genotyping System v2.0 and ViroSeq™ HIV-1 Integrase Genotyping Kit (Catalog numbers shown in Supplementary Table 1b). From the manually extracted RNA, a one-step RT-PCR reaction was performed utilizing 10µL of the extracted RNA for each genotyping kit for HIV PR codons 1–99 and RT codons 1–355 and for INI codons 1–288 regions.

TaqPath Kit

Genotyping using TaqPath Kit was performed per the manufacturer's instructions for TaqPath™ Seq HIV-1 Genotyping kit, Publication Number MAN0026326 Revision B.0 (Catalog number shown in Supplementary Table 1b). From the manually extracted RNA, 10µL of the sample was denatured at 65°C for 10 minutes before RT-PCR was performed which was followed by nested PCR. TaqPath kit covers PR codons 6–99, RT codons 1–251, and INI codons 1–288.

For both kits, the PCR positivity of each sample (PR/RT and INI regions) was visualized by gel electrophoresis. Successfully amplified samples were purified and subjected to the kit-specific cycle sequencing and the sequencing clean up. Cleaned up sequencing reactions were loaded into the ABI 3130xl or ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City USA) following the manufacturer instructions. Failed samples were re-extracted, PCR amplification and sequencing were repeated once according to ViroSeq Assays and TaqPath Kit manufacturer's instructions.

Sequence Analysis for ViroSeq Assays

The quality and read length of sequences obtained from the ABI 3130xl Genetic Analyzer were assessed using Sequencer Version 5.0[13] by manually trimming the beginning and end of each sequence to remove ambiguous nucleotides. Mixed bases or ambiguous nucleotides were confirmed with the sequences covering the same position in the contig. The same software was used to assemble multiple reads of each sequence into a single contig (consensus sequence). Generated sequences were aligned using AliView v1.26[14] with HIV-1 reference strain sequence (HXB2). Known HIV DR mutations were identified using the Stanford HIV drug resistance database (https://hivdb.stanford.edu/hivdb/by-sequences/).

Sequence Analysis for TaqPath Kit

Raw sequences from ABI 3500xl Genetic Analyzer were analyzed using Exatype (https://sanger.exatype.com/) for both HIV PR/RT and INI regions generated. Exatype assembled multiple reads into contigs and manual review and editing were performed to confirm mixed bases. Sequences that passed the quality control on Exatype were deemed successful. The fasta sequences generated from Exatype were exported and analyzed on Stanford HIV drug resistance database to confirm the mutations and identify polymorphisms.

Phylogenetic Analysis

All HIV PR/RT and INI sequences generated by both assays were aligned and assessed using AliView v1.26.[14] Reference-based multiple sequence alignment (MSA) with HIV-1 reference strain sequence (HXB2) was constructed using muscle v3.8.31 implemented in AliView v1.26. MSA was utilised in a phylogenetic tree based on maximum-likelihood (ML) with 1000 bootstrap replicates[15,16] to determine the relatedness in sequences that were successfully generated by two assays and their relationship with reference sequences.

TaqPath Kit Validation Analysis

Amplification Sensitivity

Amplification sensitivity was defined as the percentage of PCR positivity amongst specimens with a specific viral load (VL) range. The acceptance criterion on samples with VL:1000–5000 copies/mL was ≥90% and for VL >5000 copies/mL was ≥95%.

Accuracy

The nucleotide sequences were compared in samples successfully sequenced using both TaqPath Kit and ViroSeq Assays for both HIV PR/RT and INI regions. Nucleotide similarity between the paired sequences was assessed using the EMBOSS pairwise alignment tool and NCBI blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandPAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seqandLINK_LOC=blasttabandLAST_PAGE=tblastnandBLAST_INIT=blast2seq). The acceptance criterion was ≥90% of sequence pairs at nucleotide and DRMs positions with at least 98% similarity. In cases where discordant mutations were identified between paired samples generated by both the TaqPath Kit and ViroSeq Assays, available results from reference-provided sequences were used to determine the true call.

Precision

A total of three samples were tested using the TaqPath Kit in five replicates in one run for both HIV PR/RT and INI regions.

Reproducibility

Reproducibility with the TaqPath Kit was assessed by testing five replicates of three different specimens with high viral load, representing three HIV subtypes with varying resistance patterns and all tested in different batches. Testing was performed by 2 operators using two 3500xl genetic analyzers on different dates with different Kit LOT numbers which resulted in 5 runs.

For precision and reproducibility, nucleotide sequence identity was determined using the EMBOSS program for pairwise alignment tool and NCBI blastn tool. The degree of concordance of detected mutations within replicates was determined and the acceptance criterion was ≥90% (9 of 10) of pairwise comparisons be at least 98% identical (with non-matching mixtures counted as a difference).

Statistical Analysis

Descriptive statistics was used to summarize variables where categorical variables were reported as percentages, while continuous variables were reported as medians with first and third quartiles (Q1, Q3). The medians of viral load measurements of successfully genotyped against unsuccessfully genotyped samples were compared using rank sum test. Proportions of successfully sequenced samples by viral load groups and different HIV subtypes were compared using a comparison of proportions test. P-values ≤0.05 were considered statistically significant. Data analysis was performed using STATA version 16 and Figure 2 was visualized using R-packages.

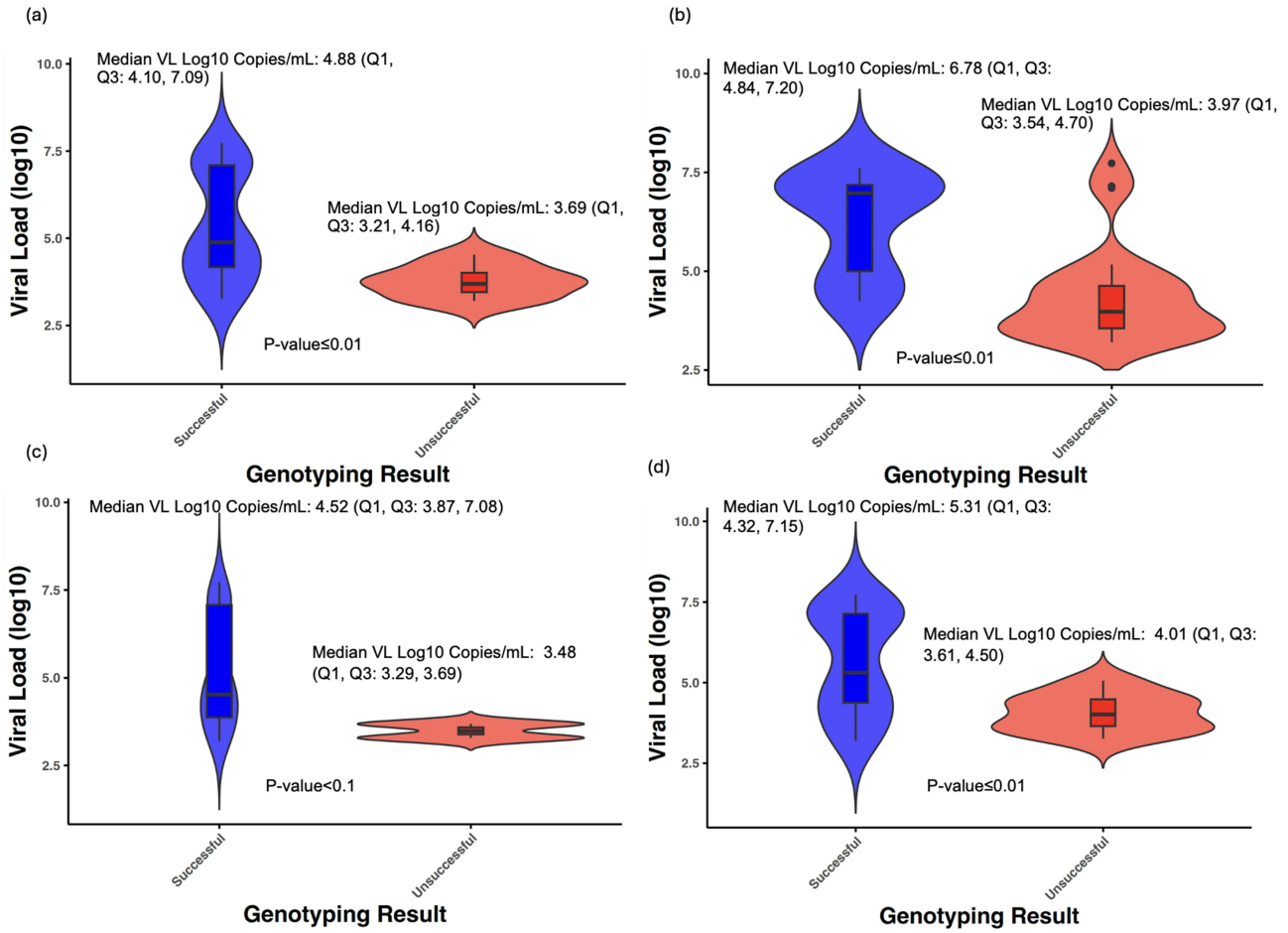

Figure 2 Association between viral load measurements and genotyping outcomes. (**a**) TaqPath Kit for HIV PR/RT. (**b**) ViroSeq Assays for HIV PR/RT. (**c**) TaqPath Kit for HIV INI. (**d**) ViroSeq Assays for HIV INI. This figure illustrates that higher viral load measurements were associated with successful sequencing outcomes, except for the TaqPath Kit in the HIV INI region, where viral load did not impact sequencing success.

Results

Baseline Demographics

The median HIV-1 RNA load (viral load [VL]) for the 20 clinical samples was 3.79 [Q1, Q3 (3.37, 4.39)] $\log_{10}$ copies/mL and 6.68 [Q1, Q3 (4.52, 7.17)] $\log_{10}$ copies/mL for the 23 analytical VQA plasma samples.

Amplification and Genotyping Success Among TaqPath Kit and ViroSeq Assays for HIV PR/RT and HIV INI Regions

Among 43 samples that were genotyped, TaqPath Kit successfully amplified 40 (93.0%) and 42 (97.7%) for HIV PR/RT and HIV INI, respectively. The amplification success was 30 (69.8%) and 31 (72.1%) for HIV PR/RT and HIV INI, respectively, using ViroSeq Assays. A total of 36/40 (90.0%) of HIV PR/RT amplicons and 41/42 (97.6%) HIV INI amplicons were successfully sequenced by TaqPath Kit, while 16/30 (53.3%) HIV PR/RT amplicons and 27/31 (87.1%) were successfully sequenced by ViroSeq Assays. Of samples repeated using the TaqPath Kit, 7/10 samples were successfully amplified for HIV PR/RT region and 4/5 successfully amplified for HIV INI region. Among those successfully amplified on the second attempt, 3/7 and all 4 were successfully sequenced for HIV PR/RT and HIV INI, respectively (all these demonstrated in supplementary Figure 1). TaqPath Kit performed statistically higher in amplification of both HIV PR/RT (p-value < 0.01) and HIV INI (p-value < 0.01), and sequencing of HIV PR/RT (p-value < 0.01) compared to ViroSeq Assays (Figure 3). The sequencing results were further stratified by viral load groups of 1000–5000 copies/mL and >5000 copies/mL (Table 1) and by HIV

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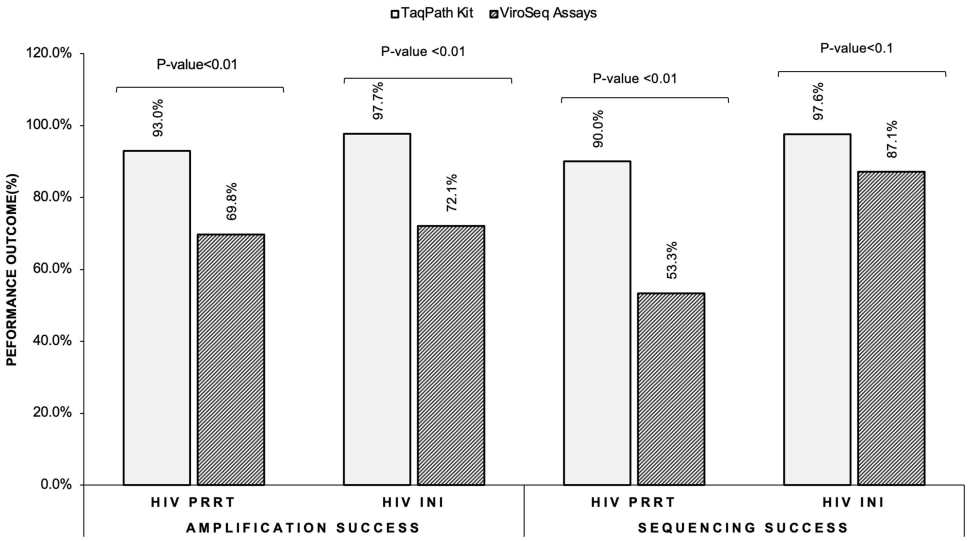

Figure 3 Comparison of amplification and genotyping success between the TaqPath Kit and ViroSeq Assays for HIV PR/RT and HIV INI regions. The TaqPath Kit demonstrated significantly better amplification success for both the HIV protease/reverse transcriptase and integrase regions, while sequencing success was higher only in the HIV protease/reverse transcriptase region.

subtype (Figure 4). Sequenced samples showed statistically higher viral load compared to those that failed genotyping (TaqPath Kit for HIV PR/RT: p-value < 0.01, ViroSeq Assays for HIV PR/RT: p-value < 0.01 and ViroSeq Assays for HIV INI: p-value < 0.01) except in TaqPath Kit for HIV INI (p-value < 0.1) (Figure 2).

Sequence Identity and Concordance of Detecting HIV-1 Drug Resistance Mutations Between the Two Assays

A total of 14 PR, 16 RT, and 27 INI paired sequences were obtained from all samples using both TaqPath Kit and ViroSeq Assays. The mean nucleotide similarity was 98.8% (SD ± 1.30) for paired RT sequences, 99.6% (SD ± 1.32) for paired PR sequences and 99.2% (SD ± 0.72) for paired INI sequences. For sequence pairs in the INI region, the evaluation parameter was met as 100% of paired sequences were at-least 98% identical. In HIV PR and RT regions, 79% (11/14) and 81% (13/16) of sequence pairs, respectively, had a nucleotide similarity of 98% or greater. In 3 samples that failed to reach the 98% target value, nucleotide similarities scores were 96.12%, 96.24% and 97.43%. The maximum likelihood phylogenetic tree was

Table 1 Amplification and Sequencing Success Rates by Viral Load

Specimen type	Target region	Viral load (copies/mL)	Amplification Sensitivity*		Sequencing Success Rate (where the Denominator is the Number of Samples Successfully Amplified)		
			TaqPath Kit	ViroSeq	TaqPath Kit	ViroSeq Assays	
Clinical samples	PR/RT	1000–5000	90% (9/10)	30% (3/10)	78% (7/9)	0% (0/3)	
		>5000	80%[#] (8/10)	80% (8/10)	100% (8/8)	50% (4/8)	
	INI	1000–5000	90% (9/10)	60% (6/10)	89% (8/9)	83.3% (5/6)	
		>5000	100% (10/10)	70% (7/10)	100% (10/10)	86% (6/7)	
VQA samples	PR/RT	1000–5000	100% (2/2)	50% (1/2)	50% (1/2)	0% (0/1)	
		>5000	100% (21/21)	86% (18/21)	95% (20/21)	**PR**	**RT**
						56% (10/18)	67% (12/18)
	INI	1000–5000	100% (2/2)	0% (0/2)	100% (2/2)	0% (0/0)	
		>5000	100% (21/21)	86% (18/21)	100% (21/21)	89% (16/18)	

Notes: *Expected amplification rate was defined as ≥90% of specimens assayed with TaqPath Kit replicates positive with a detectable viral load of between 1000 and 5000 copies/mL, and ≥95% with viral load over 5000 copies/mL. [#] This group failed to meet the expected amplification rate.

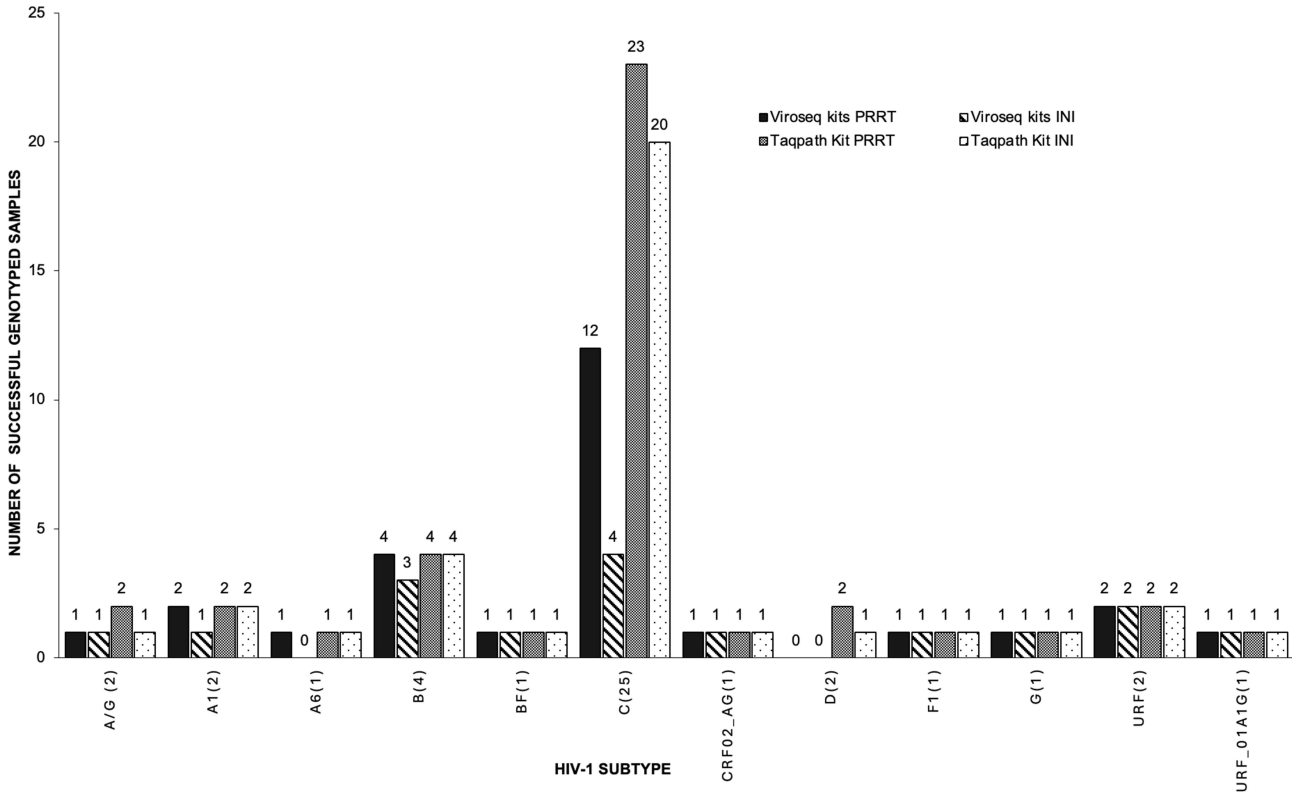

Figure 4 Sequencing success for each kit across different HIV-1 subtypes. The TaqPath Kit performed well across various HIV subtypes, while the ViroSeq Kit showed reduced performance in non-HIV-1B subtypes. The number in brackets shows the total number of samples per subtype HIV.

constructed from HIV PR/RT (Figure 5a) and HIV INI (Figure 5b) sequences generated using TaqPath Kit, ViroSeq Assays and reference sequences. All sequences generated from the same individuals by different assays clustered together.

HIV DRMs were identified in the PR, RT or INI regions of 31 samples. In 2 paired PR sequences with DRMs, both assays identified 5 mutations. Among 13 paired RT sequences with DRMs, the ViroSeq Assay identified 27 mutations while the TaqPath Kit identified 30 mutations. Of 4 paired INI sequences with DRMs, both ViroSeq Assay and TaqPath Kit reported 4 mutations (Table 2). A total of 4 discordant mutations in the RT region were reported in two samples. In one sample, ViroSeq Assays reported K65E, while TaqPath Kit detected K65R in agreement with reference sequence. In a second sample where reference sequence was not available, TaqPath Kit detected mutations E40F, M41L, M184V, L210W and T215Y while ViroSeq Assays detected E40F and M41L. The TaqPath Kit detected 39 mutations in all paired samples and agreed with 97.2% (35/36) of mutations identified with the ViroSeq Assays as shown in Table 1. Additional mutations were detected by the TaqPath Kit in samples that failed sequencing with the ViroSeq Assays including 28 PR DRMs in 9 samples, 50 RT DRMs in 12 samples and 5 INI DRMs in 4 samples. Of the 83 additional mutations, 85.5% (71) mutations agreed with the reference sequences where 39 were major HIV DRMs (Supplementary Table 2).

Precision and Reproducibility for TaqPath Kit in HIV PR/RT and INI Regions

The mean nucleotide similarity of the 10 pairwise nucleotide sequence comparison for each of the three samples tested within the precision test was 99.9% (95% CI:99.8–99.9) and 99.8 (95% CI: 99.7–99.8) for HIV PR/RT and HIV INI, respectively. Likewise, mean nucleotide similarity was 99.8% (95% CI: 99.8–99.9) and 100 (95% CI:99.9–100) for HIV PR/RT and HIV INI regions, respectively, in the reproducibility test.

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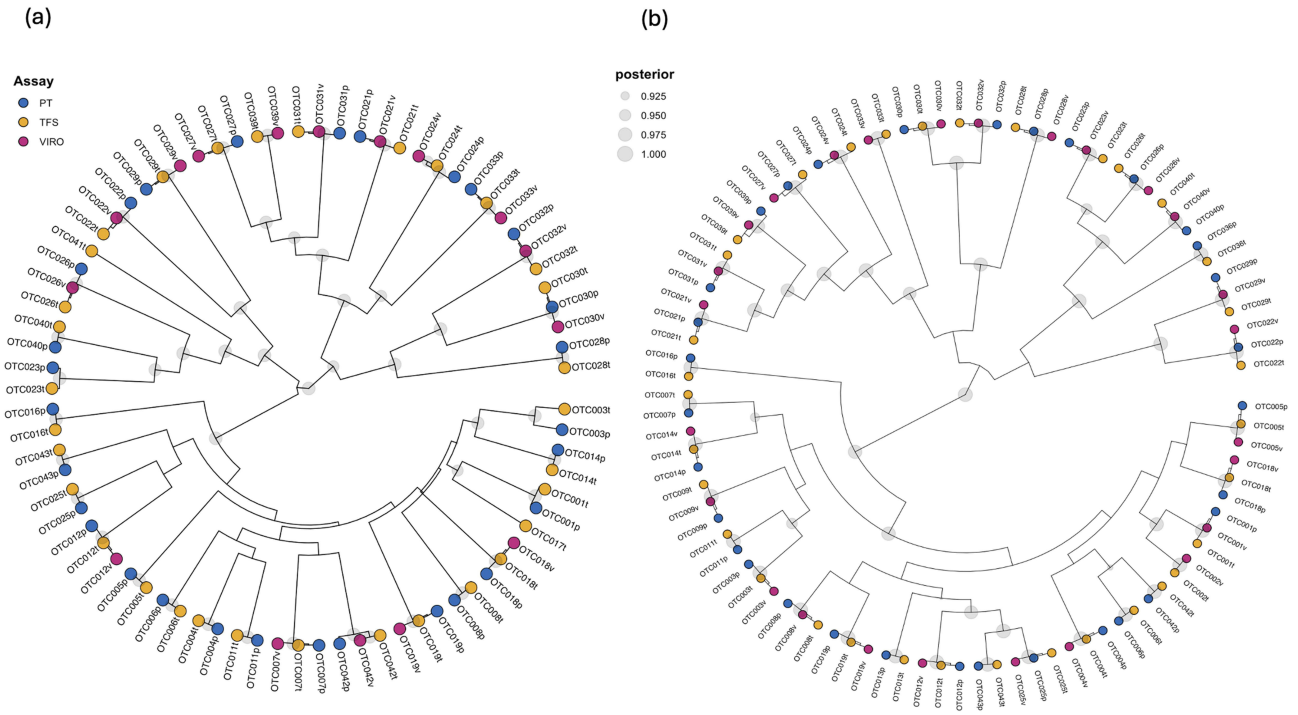

Figure 5 Molecular phylogenetic analysis using the maximum likelihood method of sequences generated by the TaqPath Kit (TFS) and ViroSeq Assays (VIRO), with reference sequences (PT) included where available. (**a**) Phylogenetic tree of HIV PR/RT sequences from both assays. (**b**) Phylogenetic tree of HIV INI sequences from both assays. The figure shows clustering of the same samples generated by both assays alongside their reference sequences.

Discussion

This study evaluated the performance of the TaqPath Seq HIV-1 Genotyping Kit in genotyping both the HIV-1 PR/RT and INI regions and compared its performance against the ViroSeq Assays. Our findings revealed a high level of nucleotide sequence concordance between the two assays, not only at the nucleotide level but also with drug resistance

Table 2 Comparison of HIV-1 Drug Resistance Mutations Detected Using the TaqPath Kit and ViroSeq Assay in Paired Samples

Sample ID	Genotyped Region	Expected Mutations from Reference Sequence	Detected Mutations	
			ViroSeq Assays	TaqPath Kit
OTC022	Protease	M46I, I54V, L76V, V82A	M46I, I54V, L76V, V82A	M46I, I54V, L76V, V82A
OTC023	Protease	L90M	L90M	L90M
OTC007	Reverse Transcriptase	M184V, K103N P225H*	M184V, K103N P225H*	M184V, K103N, P225H*
OTC021	Reverse Transcriptase	M184V, K103N	M184V, K103N	M184V, K103N
OTC022	Reverse Transcriptase	K103S	K103S	K103S
OTC023	Reverse Transcriptase	T215C*, Y188L	T215C*, Y188L	T215C*, Y188L
OTC024	Reverse Transcriptase	E138A, V179E*	E138A, V179E*	E138A, V179E*
OTC026	Reverse Transcriptase	D67N*, K219Q, K103N	D67N*, K219Q, K103N	D67N*, K219Q, K103N

(*Continued*)

Table 2 (Continued).

Sample ID	Genotyped Region	Expected Mutations from Reference Sequence	Detected Mutations	
			ViroSeq Assays	TaqPath Kit
OTC027	Reverse Transcriptase	E138A	E138A	E138A
OTC029	Reverse Transcriptase	K103N	K103N	K103N
OTC030	Reverse Transcriptase	K65R, Y115F, M184V, K101E, Y181C, G190S	**K65E**, Y115F, M184V, K101E, Y181C, G190S	K65R, Y115F, M184V, K101E, Y181C, G190S,
OTC031	Reverse Transcriptase	K103N, Y188L	K103N, Y188L	K103N, Y188L
OTC033	Reverse Transcriptase	V179E*	V179E*	V179E*
OTC037	Reverse Transcriptase	Not available	E40F*, M41L[#]	E40F*, M41L, **M184V, L210W, T215Y**
OTC039	Reverse Transcriptase	V179I*	V179I*	V179I*
OTC005	Integrase	Q95K*	Q95K*	Q95K*
OTC031	Integrase	E157Q*	E157Q*	E157Q*
OTC037	Integrase	**None**	D232N*	D232N*
OTC040	Integrase	D232N*	D232N*	D232N*

Notes: *Indicate non-major mutations including minor, accessory, or other mutations as defined in the Stanford HIV DR database; [#]Sequence quality for this sample at HIV codon positions 1–5 in PR and 172–560 in RT regions was low. This led to a shorter sequence that excluded 17 drug-resistance positions: RT 179, 181, 184, 188, 190, 210, 215, 219, 221, 225, 227, 230, 234, 236, 238, 318, 348; Discordant mutations are bolded.

mutations. These results indicate that the TaqPath kit is a reliable tool to identify and monitor HIV drug resistance mutations across all major ART drug classes. Precision and reproducibility of the TaqPath Kit surpassed 99% for both the HIV PR/RT and INI regions, further supporting its accuracy and consistency in genotyping HIV-1.

The WHO recommends a minimum of ≥98% nucleotide sequence similarity among two genotyping assays.[17] In this study, the accuracy was assessed using 43 samples and the mean nucleotide similarity scores were 98.8±1.3% for HIV PR, 99.6% (SD ± 1.32) for HIV RT and 99.2±0.7% for HIV INI from samples successfully genotyped by both kits. Our findings report nucleotide identity scores like previously published HIV genotyping assay comparison studies.[18,19] Both assays detected relevant mutations despite two samples with discordant mutations. Our concordance was 97%, which was statistically similar to 100% reported in a previously optimized genotyping assay that was validated against ViroSeq Assay[20] and 98% that was reported in an inhouse-genotyping assay compared with ViroSeq Assay.[21] The TaqPath Kit detected K65R in agreement with the reference sequence, while ViroSeq Assays detected K65E in the same sample. K65R is known to confer reduced susceptibility to tenofovir and abacavir when present alone, and in combination with other mutations, it also impacts susceptibility to lamivudine and emtricitabine[22] (https://hivdb.stanford.edu/dr-summary/pattern-scores/NRTI/). In contrast, K65E is an extremely rare mutation that has not been extensively studied in terms of its effect on the susceptibility of NRTIs.[23] The differential detection of discordant mutations between the two assays could be attributed to detection of nucleotide mixtures because of subjectivity in base calling or amplification bias by the two assays.[24,25] This is not the first study to report discrepancies of mutations when compared with ViroSeq as one study reported concordance rate of 98.22% to 99.65% with discordant mutations caused by base mixture.[18] In one sample with 5 RT mutations detected by TaqPath Kit compared to 2 RT detected by ViroSeq assay, the reference sequences were not provided for HIV PR/RT region. The discordant mutations in this pair, M184V, L210W, and T215Y, could be due to low sequence coverage in ViroSeq assay. HIV codon positions 1–5 in PR and 172–560 in RT regions with drug-resistance positions: RT 179, 181, 184, 188, 190, 210, 215, 219, 221, 225, 227, 230, 234, 236, 238, 318, 348 were not successfully sequenced. This made it difficult to determine if ViroSeq assay was able to detect mutations that TaqPath Kit reported in this sample. For samples with discordant mutations, deep sequencing can be used as an alternative to confirm the results.

Our data also demonstrate a broader subtype coverage using the TaqPath Kit, which outperformed ViroSeq Assays in genotyping HIV-1 non-B subtype samples. Of 38 non-B subtypes samples tested, TaqPath Kit successfully sequenced the PR/RT region in 81.6% (31) compared to 31.6% (12) using ViroSeq Assays and the INI region in 94.7% (36) compared to 57.9% (22) with ViroSeq assay. Several studies report limitations with ViroSeq assay on non-B subtypes which made up most of the samples.[26,27] The amplification sensitivity for the ViroSeq assay may have been affected due to the use of a common extraction method for both assays, which was not the recommended extraction method. However, it is worth noting that the TaqPath kit employed a different extraction method during the manufacturer's validation, which also differed from the method used in the study. Future studies should consider using the optimal extraction methods recommended by manufacturers to avoid potential biases.

While the overall performance of the TaqPath Kit was satisfactory, it exhibited weaker amplification and sequencing results for the PR/RT region compared to the INI region. Overall, the amplification sensitivity was 93.5% (29/31). However, the TaqPath Kit amplified the PR/RT region in 80% (8/10) of clinical samples with VL higher than 5000 copies/mL falling short of the recommended amplification sensitivity threshold of ≥95% for this VL group as per the WHO recommendation.[17] Additionally, the TaqPath Kit successfully sequenced the HIV PR/RT region in 72% (8/11) of the amplified samples with a VL ranging from 1000 to 5000 copies/mL. The weaker performance observed for the PR/RT region was corroborated by statistically significant lower viral load in samples that did not yield successful HIV PR/RT genotyping results. These findings highlight the need for further optimization of the kit specifically for HIV PR/RT genotyping with clinical samples. Despite lower performance for the PR/RT region, the TaqPath kit was able to identify 71 additional mutations including 39 major DRMs, which would have otherwise been missed in samples that failed to genotype using the ViroSeq Assays.

One strength of the study is utilizing a genotyping kit that covers both HIV PR/RT and HIV INI regions, where most of the studies have previously compared either HIV PR/RT or HIV INI separately.[28–30] However, it is important to acknowledge that the study was limited by the number of samples tested. Due to the small number of samples available for each subtype, except for subtype C, it was not feasible to quantitatively assess the sensitivity of the TaqPath Kit for subtypes that were not validated by the manufacturer. However, this sample size of 43 yielded 116 tests from accuracy, amplification sensitivity, precision and reproducibility tests meeting WHO number of tests for method validation. Therefore, further studies with larger sample sizes are warranted to address this limitation.

Conclusion

The TaqPath Seq HIV-1 Genotyping Kit demonstrated higher amplification and sequencing success rates when compared to ViroSeq HIV-1 Genotyping assays across multiple HIV-1 subtypes and different viral load ranges. The use of this kit is recommended in resource-limited settings for HIV genotyping of mutations associated with NNRTIs, NRTIs, PIs and INSTIs covering the HIV regions targeted by most ARV drugs.

Data Sharing Statement

All relevant data are within the paper, including the figures and tables. HIV-1 sequences and associated clinical data are available on reasonable request through the Botswana Harvard Health Partnership (info@bhp.org.bw).

Ethical Approval

The study was approved by Botswana Health Research and Development Committee (HRDC) (HRDC NUMBER 00976. The BCPP study was approved by the Institutional Review Boards (IRBs) at the US Centers for Disease Control and Prevention and the Botswana Health Research and Development Committee and is registered at ClinicalTrials.gov (NCT01965470). All BCPP participants provided written informed consent for their samples to be used in future studies. The study was conducted according to the principle expressed in the declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

OO and AG are employees of Thermo Fisher Scientific. All other authors declare no competing interest in this work.

References

1. Mens H, Kearney M, Wiegand A, et al. Amplifying and quantifying HIV-1 RNA in HIV infected individuals with viral loads below the limit of detection by standard clinical assays. *J Visualized Exp*. 2011;55:2960.
2. Joint United Nations Programme on HIV/AIDS, Joint United Nations Programme on HIV/Aids. 90-90-90: an ambitious treatment target to help end the AIDS epidemic. 2014.
3. EACS. *European AIDS clinical society guidelines*. 2021.
4. DHHS. Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV, department of human and health services. 2019.
5. World Health Organization. *Updated Recommendations on First-Line and second-Line Antiretroviral Regimens and Post-Exposure Prophylaxis and Recommendations on Early Infant Diagnosis of HIV: Interim Guidance*; 2018.
6. Croome N, Ahluwalia M, Hughes LD, et al. Patient-reported barriers and facilitators to antiretroviral adherence in sub-Saharan Africa. *Aids*. 2017;31(7):995–1007. doi:10.1097/QAD.0000000000001416
7. Wallis CL, Godfrey C, Fitzgibbon JE, et al. Key factors influencing the emergence of human immunodeficiency virus drug resistance in low- and middle-income countries. *J Infect Dis*. 2017;216(suppl_9):S851–s856. doi:10.1093/infdis/jix409
8. Tobin NH, Learn GH, Holte SE, et al. Evidence that low-level viremias during effective highly active antiretroviral therapy result from two processes: expression of archival virus and replication of virus. *J Virol*. 2005;79(15):9625–9634. doi:10.1128/JVI.79.15.9625-9634.2005
9. Chun TW, Justement JS, Murray D, et al. Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication. *Aids*. 2010;24(18):2803–2808. doi:10.1097/QAD.0b013e328340a239
10. Genet A, Mekonnen Z, Yizengaw E, et al. First line antiretroviral treatment failure and associated factors among people living with HIV in northwest Ethiopia. *Afr Health Sci*. 2021;21(1):263–272. doi:10.4314/ahs.v21i1.34
11. Bogoch I, Walmsley S. First-line regimen failure of antiretroviral therapy: a clinical and evidence-based approach. *Curr Opin HIV AIDS*. 2009;4(6):493–498. doi:10.1097/COH.0b013e328331b5e2
12. HIVinfo.NIH.gov, *FDA-Approved HIV Medicines*. 2023.
13. Codes G. *Sequencher® Version 5.4.6 DNA Sequence Analysis Software*. G.C.C;Ann Arbor, MI USA:2017.
14. Larsson A. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*. 2014;30(22):3276–3278. doi:10.1093/bioinformatics/btu531
15. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*. 1993;10(3):512–526. doi:10.1093/oxfordjournals.molbev.a040023

16. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33 (7):1870–1874. doi:10.1093/molbev/msw054
17. Organisation WH. *HIVResNet HIV Drug Resistance Laboratory Operational Framework*. *second edition* ed. Geneva: World Health Organization; 2020.*Licence: CC BY-NC-SA 3.0 IGO*.
18. Zhou Z, Wagar N, DeVos JR, et al. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PLoS One*. 2011;6(11):e28184. doi:10.1371/journal.pone.0028184
19. Magomere EO, Nyangahu DD, Kimoloi S, et al. Performance characteristics of a modified HIV-1 drug resistance genotyping method for use in resource-limited settings. *F1000Res*. 2019;8:1518. doi:10.12688/f1000research.20083.1
20. Manasa J, Danaviah S, Pillay S, et al. An affordable HIV-1 drug resistance monitoring method for resource limited settings. *J Vis Exp*. 2014; 85. doi:10.3791/51242-v
21. Inzaule S, Yang C, Kasembeli A, et al. Field evaluation of a broadly sensitive HIV-1 in-house genotyping assay for use with both plasma and dried blood spot specimens in a resource-limited country. *J Clin Microbiol*. 2013;51(2):529–539. doi:10.1128/JCM.02347-12
22. Melikian George L, Rhee S-Y, Taylor J, et al. Standardized comparison of the relative impacts of HIV-1 Reverse Transcriptase (RT) mutations on nucleoside RT inhibitor susceptibility. *Antimicrob Agents Chemother*. 2012;56(5):2305–2313. doi:10.1128/AAC.05487-11
23. Fourati S, Visseaux B, Armenia D, et al. Identification of a rare mutation at reverse transcriptase Lys65 (K65E) in HIV-1-infected patients failing on nucleos(t)ide reverse transcriptase inhibitors. *J Antimicrob Chemother*. 2013;68(10):2199–2204. doi:10.1093/jac/dkt200
24. Parkin N, Bremer J, Bertagnolio S. Genotyping external quality assurance in the World Health Organization HIV drug resistance laboratory network during 2007-2010. *Clin Infect Dis*. 2012;5(Suppl 4):S266–72. doi:10.1093/cid/cir992
25. Galli RA, Sattha B, Wynhoven B, et al. Sources and magnitude of intralaboratory variability in a sequence-based genotypic assay for human immunodeficiency virus type 1 drug resistance. *J Clin Microbiol*. 2003;41(7):2900–2907. doi:10.1128/JCM.41.7.2900-2907.2003
26. Aghokeng AF, Mpoudi-Ngole E, Chia JE, et al. High failure rate of the ViroSeq HIV-1 genotyping system for drug resistance testing in Cameroon, a country with broad HIV-1 genetic diversity. *J Clin Microbiol*. 2011;49(4):1635–1641. doi:10.1128/JCM.01478-10
27. Beddows S, Galpin S, Kazmi SH, et al. Performance of two commercially available sequence-based HIV-1 genotyping systems for the detection of drug resistance against HIV type 1 group M subtypes. *J Med Virol*. 2003;70(3):337–342. doi:10.1002/jmv.10401
28. Seatla KK, Choga WT, Mogwele M, et al. *Comparison of an in-house* 'home-brew' *and commercial ViroSeq integrase genotyping assays on HIV-1 subtype C samples*. *PLoS One*. 2019;14(11):e0224292. doi:10.1371/journal.pone.0224292
29. Chaturbhuj DN, Deshmukh PS, Hingankar NK, et al. Evaluations of an in-house drug resistance method for HIV-1 drug resistance using ViroSeq™ 2.0 genotyping system as a gold standard. *J Virolog Method*. 2013;189(1):87–92. doi:10.1016/j.jviromet.2013.01.001
30. Praparattanapan J. Comparison of in-house HIV-1 genotypic drug resistant test with commercial HIV-1 genotypic test kit. *Asian Biomed*. 2011;5.