Poster # 13

Observation of Inconsistent HIV-1 Viral Load Results from Roche COBAS®AmpliPrep/COBAS®TaqMan® and Hologic Aptima HIV-1 Quant Assays

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BACKGROUND

The Hologic Aptima HIV-1 Quant Assay designed for the Panther system (Panther) has a relatively large specimen capacity, lower cost, and requires less hands-on and on-board testing time compared to the Roche COBAS®AmpliPrep/ COBAS®TaqMan® HIV-1 Test (COBAS). The NYC Public Health Laboratory (PHL) performed HIV-1 viral load testing on both instruments at different times and observed discrepant results.

METHODS

The NYC PHL tested 945 patient specimens from June 2016 to December 2018. All specimens had been previously tested HIV-positive by the Alere Determine™ HIV-1/2 Ag/Ab Combo or the INSTI® HIV-1/HIV-2 Rapid Antibody Test. In compliance with the HIV-1 Diagnostic Testing Algorithm (1) the same samples were tested at PHL by the Bio-Rad EVOLIS™/HIV 4th Generation Combo Ab/Ag EIA (4th Gen Combo) assay and also by the ViroSeq HIV-1 Genotyping System Abbott (ViroSeq) for antiretroviral drug resistance and by the COBAS (COBAS) or Panther (Panther) instrument (see below) for HIV-1 Viral Load.

COBAS® Ampliprep/ COBAS® TaqMan®/Roche

VS

Panther System/ HOLOGIC

RESULTS

256 specimens from presumptively new HIV cases or known HIV-positive but treatment-naïve patients were tested on the COBAS (June 1, 2016, to April 4, 2017); and 691 specimens from a similar cohort were tested on the Panther (April 5, 2017, to December 31, 2018). The comparison of these two assays is presented in **Table 1**. All 19 specimens with undetectable viral loads on the COBAS were also "Non-reactive" when tested by the 4th Gen Combo assay, indicating that false positive results were obtained from the rapid screening test. Of 70 specimens with undetectable viral loads on the Panther, 50 were also "Non-reactive". However, 20 of 70 specimens (28.6%) were "Reactive" with the HIV 4th Gen Combo assay.

Table 1. Capacity of Cobas® AmpliPrep/Cobas® TaqMen® HIV-1 Test, v. 2.0 and Hologic/Aptima HIV-1 Quant assay.

	Cobas® AmpliPrep/Cobas® TaqMen® HIV-1 Test, version 2.0 (2)	Hologic/Aptima HIV-1 Quant assay (3)
Detection range	20 -10,000,000 copies/mL	30 -10,000,000 copies/mL
Molecular Techniques	Real-Time PCR, fluorescent detection	Transcription Mediated Amplification (TMA), fluorescent detection
Target	*Gag* and LTR	*Pol* and LTR
Testing process	1. Extraction of HIV-1 RNA 2. Reverse transcription of the target RNA to complementary DNA (cDNA) 3. PCR amplification of target cDNA 4. Detection of dual-labeled oligonucleotide detection probe specific to the target	1. Extraction of RNA 2. Target capture 3. TMA target amplification 4. Detection of the amplicon by the fluorescent labeled probe
Hands-on time	40-60 min sample, reagent and instrument priming	30-40 min samples and reagents preparation
On-board	5 hour 30 min (semi-automated)	2 hours 40 min (automated)
Report	30-40 min	15-20 min
Total	6 hours 40 min - 7 hours 10 min	3 hours 25 min – 3 hours 40 min
Number of tests per kit	48/ 3 controls per test	100/ 6 controls per test
Cost per test	$97.74	$50.00
Specimen type	EDTA plasma	EDTA plasma
Specimen volume	1,040 mL	750 mL
Sharing instrument	No	Yes
Reporting to STARLIMS	Manual data entry	Connected to STARLIMS
Tested specimens	254	691
HIV rapid screening test false positive	19 (7.4%)	50 (7.2%)
Case of HIV viral load negative, serology positive	0	20

To address the inconsistent results from the HIV-1 Viral Load assay (Panther System) and the 4th Gen Combo, additional tests were performed (**Table 2**). 9 of 20 samples were retrospectively tested on the COBAS (the kit was unavailable for 11), and 7 of 9 (77.8%) showed a viral load of <20 cp/mL to 510 cp/mL. All 20 specimens were processed for HIV-1 genotyping. Although 13 of 20 showed no PCR product and the assay was not completed, 7 (35%) were successfully genotyped by the HIV-1 ViroSeq assay. Out of these 20 specimens, 15 available specimens were also tested with HIV-1 qualitative assay (Aptima® HIV-1 Qualitative Assay, Hologic). Seven were reactive (positive), while eight were non-reactive (negative) (data not shown).

RESULTS (cont'd)

Table 2. Summary of Testing Results.

#	PANTHER VL, (cp/ml)	HIV 4th Gen Combo	COBAS VL, (cp/ml)	Protease & Reverse Transcriptase Genotyping	Subtype
1	Target not detected	Positive	Target not detected	No PCR product	n/a
2	Target not detected	Positive	n/a	No PCR product	n/a
3	Target not detected	Positive	<20	No resistance	CRF01_AG
4	Target not detected	Positive	120	No PCR product	n/a
5	Target not detected	Positive	510	No resistance	B
6	Target not detected	Positive	315	No resistance	B
7	Target not detected	Positive	91	No resistance	B
8	Target not detected	Positive	Target not detected	No PCR product	n/a
9	Target not detected	n/a	78	No PCR product	n/a
10	Target not detected	Positive	215	No resistance	B
11	Target not detected	Positive	n/a	No PCR product	n/a
12	Target not detected	Positive	n/a	No PCR product	n/a
13	Target not detected	Positive	n/a	No resistance	B
14	Target not detected	Positive	n/a	No PCR product	n/a
15	Target not detected	Positive	n/a	No resistance	B
16	Target not detected	Positive	n/a	No PCR product	n/a
17	Target not detected	Positive	n/a	No PCR product	n/a
18	Target not detected	Positive	n/a	No PCR product	n/a
19	Target not detected	Positive	n/a	No PCR product	n/a
20	Target not detected	Positive	n/a	No PCR product	n/a

CONCLUSIONS

The less costly Aptima HIV-1 Quant Assay did not detect low viral loads in some specimens, which were detected by a similar assay on the COBAS system. Some of these specimens were successfully sequenced by ViroSeq genotyping assay. The discrepancy in viral load detection may result from unrecognized differences in the sensitivities of the COBAS and Panther assays.

REFERENCES

1. HIV Testing, Reporting and Confidentiality in New York State 2017-18 Update: Fact Sheet and Frequently Asked Questions New York State Department of Health AIDS Institute
2. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0. Package insert HIV-1.
3. Aptima® HIV-1 Quant Assay. HIV-1 QUANT DX package insert.