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Automated High-Throughput Quantification of Low-Level HIV-1 Plasma Viremia

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Introduction

- Low-level plasma HIV-1 viremia (below the limit of detection of commercial assays) persists in the majority of HIV-1 positive individuals despite long-term clinically-effective ART.
- Clearance of HIV-1 viremia remains a critical goal of HIV cure research.
- The lower limit of detection (LLOD) for FDA-cleared plasma HIV-1 RNA assays is 6-30 cp/mL, depending on subtype,
- Single copy HIV-1 RNA assays (SCA) have greater sensitivity but low throughput limits their capacity to monitor the effects on interventions in clinical trials.
- Bakkour, et al. have reported the use of multiple replicates and Poisson statistics to infer HIV-1 RNA concentration below the commercial LLOD for the Hologic Panther automated platform (CROI 2019).

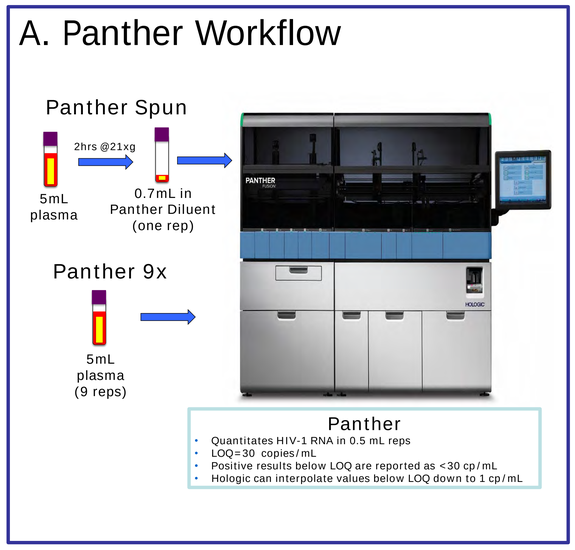
Objectives

- To evaluate the sensitivity for detection and quantitation of low-level viremia using two high-throughput methods on the Hologic Panther platform in comparison with a manual SCA targeting integrase (iSCA v2) with a 95% LOD of 1 copy/ml (Tosiano, et al. J Clin Micro 2019).

Methods

- **Panther 9x**: 9 x 0.5mL reps (5.0 mL total) of Virology Quality Assurance (VQA; Rush) standards were loaded on the Hologic Panther in a single tube and run using the HIV-1 Aptima test (Fig 1A). Statistical inference based on binary outcome (+/-) was used to quantify HIV-1 RNA below the reported LOD (12 copies/ml) when all 9 reps were not above the LOD (Fig 1B). Results were compared to the iSCA v2 assay (Tosiano, et al. J Clin Micro 2019).
- **Panther Spun**: 5.0 mL of VQA standards was concentrated to 0.7 mL by centrifugation and loaded on the Hologic Panther and run using the HIV-1 Aptima test (Fig 1A). The concentration present in the initial 5 mL was calculated from the reported Panther result (Fig 1B). Results were compared to the iSCA2.0 assay (Tosiano, et al. J Clin Micro 2019).
- **iSCA 2.0:** 5 mL VQA standard was centrifuged and total nucleic acid extracted from the pellet. Reverse transcription and real time quantitative PCR were performed, and concentration inferred from a within-run standard curve (Fig 1C).
- **VQA Standards Tested**: 20, 5, 2.5, 1.25, 0.625 cp/mL
- For each method, each concentration was run in 5 replicates per run and each concentration was run in 5 independent runs (total reps = 25 per concentration).
- Mean and standard deviation were calculated for all 25 reps.
- Percent positive assays were calculated for all 25 reps.
- Measurement of HIV-1 RNA in 5 mL of donor plasma was compared using Panther 9x and iSCA v2.

Figure 1



Results

Figure 2: Results for Each Sample by Assay

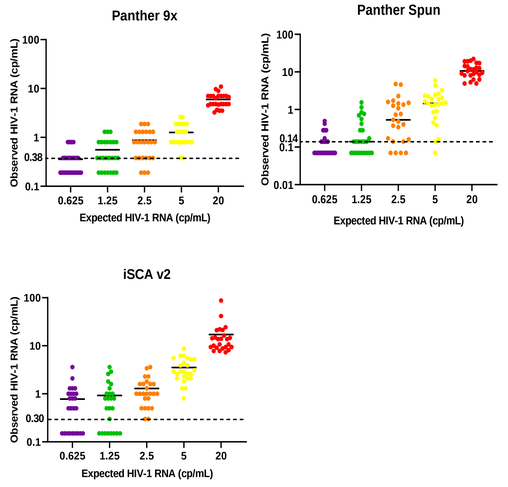


Table 1: Summary of Quantification and %CV

Method	Expected Concentration (cps/mL)	Mean Observed Concentration (cps/mL ± SD)	%CV	Mean %CV
Panther 9x	20.0	6.08 ± 1.93	31.7	33.6
	5.0	1.27 ± 0.63	49.6	
	2.5	0.87 ± 0.31	35.6	
	1.2	0.55 ± 0.16	29.1	
	0.6	0.36 ± 0.08	22.2	
Panther Spun	20.0	11.70 ± 2.56	21.9	35.8
	5.0	1.70 ± 0.58	34.1	
	2.5	1.04 ± 0.57	54.8	
	1.2	0.33 ± 0.13	39.4	
	0.6	0.14 ± 0.04	28.6	
iSCA v2	20.0	17.12 ± 16.40	95.8	68.4
	5.0	3.52 ± 1.92	54.5	
	2.5	1.30 ± 0.90	69.2	
	1.2	1.0 ± 0.60	60	
	0.6	0.80 ± 0.50	62.5	

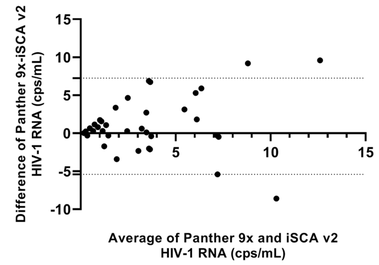
Table 2: % Detection and LOD by Assay

Method	Expected Conc. (cps/mL)	No. Detected	Total Tests	Percent Detected	LOD_{95} (95% CI) (cps/mL)	LOD_{50} (Estimated) (cps/mL)
Panther 9x	20.0	25	25	100	3.0 (2.1, 3.8)	<0.625
	5.0	25	25	100		
	2.5	22	25	88.0		
	1.2	18	25	72.0		
	0.6	13	25	52.0		
	0	0	25	0		
Panther Spun	20.0	25	25	100.0	3.9 (2.8, 5)	0.625-1.25
	5.0	24	25	96.0		
	2.5	21	25	84.0		
	1.2	16	25	64.0		
	0.6	11	25	44.0		
	0	0	25	0		
iSCA v2	20.0	25	25	100	2.3 (1.6, 3)	<0.625
	5.0	25	25	100		
	2.5	25	25	100		
	1.2	17	25	68.0		
	0.6	16	25	64.0		
	0	0	25	0		

Figure 3: Clinical Sample Results (Panther 9X vs. iSCA v2)

Donor	Panther (cps/mL)	iSCA v2 (cps/mL)
1	1.88	<0.3
2	7.03	3.9
3	1.88	0.3
4	2.59	4.6[1]
5	4.81	2.1
6	7.03	<0.3[2]
7	7.03	0.3
8	<0.38	0.5
9	<0.38	0.5
10	<0.38	<0.3
11	2.59	4.7
12	7.03	7.3
13	6.0	14.6
14	4.81	<0.3
15	9.3	3.4
16	7.03	7.5
17	2.59	2.3
18	13.4	4.2
19	3.52	3.4
20	<0.38	<0.3
21	<0.38	<0.3
22	1.30	1.0
23	0.80	<0.3
24	0.38	<0.3
25	1.30	<0.3
26	0.80	<0.3
27	0.38	<0.3
28	4.5	9.9
29	0.38	2.1
30	<0.38	<0.3
31	69	34.6
32	3.52	3.9
33	7.03	5.2
34	0.38	<0.3
35	<0.38	3.6
36	1.30	1.6
37	1.88	4.2
38	8.7	3.4
39	0.38	<0.3
40	1.88	0.8
41	3.52	2.9
42	1.30	<0.3
43	<0.38	<0.3
44	<0.38	<0.3
45	1.30	0.5[2]
46	160.4	41.9
47	1.88	0.8
48	17.4	7.8
49	3.52	<0.3
50	0.80	0.5

1 4.2 mL tested
2 Internal control failure



Conclusions

- Using VQA standards the sensitivity of Panther 9x is comparable to iSCA v2, though quantitation by iSCA v2 is consistently higher than by Panther 9x.
- Panther spun is the least sensitive of the three methods compared.
- Using clinical samples, Panther 9x quantified HIV-1 RNA comparably to iSCA v2, with the Panther 9x estimates slightly higher; Panther 9x is more sensitive (82% of samples detected compared to 62% with iSCA v2).
- The weekly estimated throughput for Panther 9x is 5-10 times that of iSCA v2.
- Multiple replicate testing on Hologic Panther platform using HIV-1 Aptima test is a potentially useful tool for high-throughput monitoring of viremia in clinical trials.
- When <5 mL of plasma is available, iSCA v2 is an important alternative assay.
- Further comparisons of the Panther 9x and iSCA v2 are in progress with blinded panels.
- Assessment of Panther 9x for specimen types other than plasma is needed.

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