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A Virology Quality Assurance Program to Assess Inter-lab Reproducibility of HIV-1 Reservoir Assays

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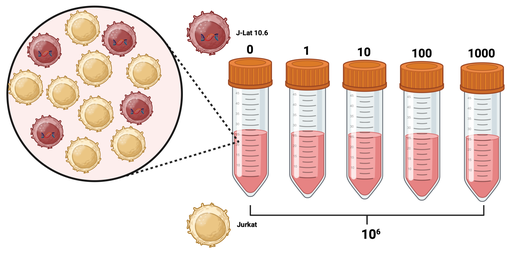
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BACKGROUND

- In recent years there have been significant advances in the development of assays to quantify and characterize the HIV-1 reservoir.
- As these assays are incorporated into HIV-1 cure clinical trials, ensuring the reliability and reproducibility of assay results across different laboratories becomes crucial.
- This study aimed to evaluate the inter-laboratory performance of assays measuring intact proviruses (AMIP) as part of the National Institute of Allergy and Infectious Disease (NIAID) Virology Quality Assurance (VQA) Program at Duke (contract # 75N93019C00015).
- **The long-term goal** of this pilot study is to aid in the development of a proficiency testing program to support clinical trials sites assessing the impact of therapeutic interventions against the HIV-1 reservoir.

METHODS

- We conducted a multi-site study involving eight laboratories routinely performing AMIP.
- A panel of standardized samples comprising serial dilutions of HIV-1 infected cell lines with known proviral DNA levels were distributed blinded to the participating sites for phase I and unblinded for the phase I-b repeat.



- Each of the participating labs received 2 aliquots of each dilution and was asked to perform the assay twice on each aliquot following their standard operating procedures.
- Results were collected and analyzed to establish the degree of concordance among laboratories and to identify discrepancies and potential sources of variability in assay outcomes.

Virology quality assurance programs are needed to harmonize inter-laboratory performances of assays to measure the HIV-1 reservoir

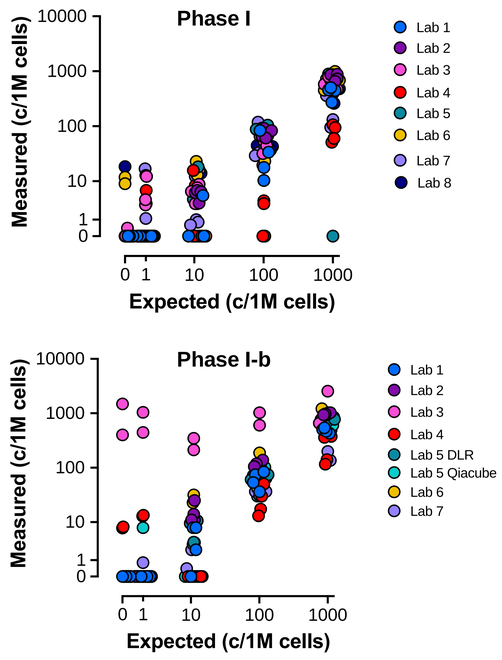


Figure 1. Frequency of intact proviruses. Shown are the frequencies of intact proviruses per million cells reported by each of the participating laboratories versus the expected copy number for phase I (top panel) and phase I-b (bottom panel).

Sample ID	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5		Lab 6		Lab7		Lab8		Expected values
01.01A	0	0	0	0	0.4	0	0	0	0	0	10.8	7.99	0	0	17.4	0	0
01.07A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
01.08A	0	0	0	0	2.7	3.6	0	0	0	0	0	0	0	15.8	0	0	1
01.05A	0	0	0	0	11.2	2.9	5.8	0	0	0	11.8	0	1.1	0	0	0	1
01.02A	0	4.5	5.2	5.8	2.9	7.8	0	0	17.2	0	15.2	0	1	11.2	2.9	5.1	10
01.04A	0	0	3	5.4	6.6	5.5	14.7	7.3	3.7	0	12.0	21.9	0.8	0.6	13	0	10
01.06A	9.3	32.7	65	90	30.9	53	0	0	91.1	103.5	65.7	65.0	18.5	28.1	41.7	36	100
01.10A	16.8	82.6	82	60	80.3	43.5	3.4	2.9	87.2	40.5	92.5	22.1	32.4	117.3	38.2	43.7	100
01.03A	446	456.7	860	875	579.1	469.3	105.3	93.5	738.1	0	688.0	720.0	360	760.6	510	259.8	1,000
01.09A	502	272.1	725	654	673.8	796.7	50	58.9	863.2	890.9	988.9	451.2	130.8	94.5	479.2	390.8	1,000

Sample ID	Lab 1		Lab 2		Lab 3		Lab 4		Lab 5 (DLR)		Lab 5 (QIAmp)		Lab 6		Lab7		Expected values
01.01A	0	0	0	0	1485.4	7.18	0	0	0	0	0	0	0	0	6.8	0.0	0
01.07A	0	0	0	0	399.1	0	0	0	0	0	0	9.0	0	0	0.0	0.0	0
01.08A	0	0	0	0	443.3	0	12.37	0	0	0	0	11.9	0	0	0.8	0.0	1
01.05A	0	0	0	0	1041.5	0	0	0	0	6.9	0	0	0	0	0.0	0.0	1
01.02A	6.9	6.9	10	13	344.6	0	0	8.5	2.9	0	0	30.6	0	3.2	0.4		10
01.04A	2.1	0	24	10	211.6	0	0	9.6	3.3	0	0	21.8	0	0.0	2.1		10
01.06A	73.3	82.6	117	137	603.6	16.36	12.03	73.2	62.8	42.9	63.8	59.0	186.6	35.3	35.6		100
01.10A	52.5	35.2	117	104	1022.6	28.85	49.09	73.4	59.6	29.1	101.0	40.9	115.9	29.7	71.2		100
01.03A	428.3	503.1	939	1026	656.1	139.56	114.72	716.2	760.6	490.9	576.4	767.4	1213.2	133.7	134.8		1,000
01.09A	465.7	538.6	975	938	2544.4	374.84	359.40	850.4	775.7	601.0	691.3	1024.6	792.6	197.8	396.2		1,000

Fold difference	≦2	2>≦3	>3 or positive in negative sample or 2x higher than expected value	<2 above expected values

Figure 2. Heatmaps of observed differences in assay results among participating labs. Shown are the frequencies of intact proviruses per million cells reported by each of the participating laboratories and the fold difference between the reported results and the expected values for phase I (top panel) and phase I-b (bottom panel).

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Cell Viability (%)	67 (59-86)	62.9 (51-91)	99.11 (98.5-99.6)	74 (50.8-93.8)	68.8 (63-82)	64 (53-90)	65 (55-74)
TOTAL Avg DNA amount (µg)	35	33	11.4	12	22.6 Qiacube 24.2 DLR	21.2	16.4
ASSAY LOD (copies/10^6 cells)	15.9	>3	N/A	N/A	10	N/A	Using PROBIT at C95, LOD (Intact) = 399.7039
# replicates for HIV	4	4	11-18	Up to 8	4	4	9-23
# replicates for RPP30	4	4	2	3	2	1	4
DNA amount for HIV (ng)	750	700	310-600	500	**Variable** 416-2028 Qiacube 1605-7425 DLR	500	750
DNA amount for RPP30 (ng)	7.5	7	7	5	**Variable** 2.6-18.65 Qiacube 5.3- 24.75 DLR	25	7.5 (15 for 3 samples)
Shearing Index (Avg)	0.74	0.38	0.44	0.39	0.49 (DLR) 0.36 (Qiacube)	0.36	0.42
Instrument	Bio-Rad Automatic droplet generator	Bio-Rad Automatic droplet generator	Bio-Rad **Manual droplet generator**	Bio-Rad Automatic droplet generator	QIAcuity ddPCR	QIAcuity ddPCR	Bio-Rad **Manual droplet generator**
Frozen primer/probe mix?	Yes	Yes	Yes	No	Yes	Yes	Yes

Table 1. Overview of experimental conditions. Shown are the experimental conditions employed by each participating laboratory to perform AMIP. Three labs with lower assay performance reported lower DNA recovery compared to the other labs (highlighted in red).

CONCLUSIONS

- We observed significant variability in AMIP results across laboratories, with several labs exhibiting discrepancies for both experimental and biological replicates. For some laboratories, the reported results differed from the expected values by 3 to 10-fold.
- Factors such as sample handling, assay reagents, and differences in analytical protocols were identified as potential contributors to this variability during phase I.
- Most of the labs were able to improve their results for the phase I-b repeat, with the number of intact proviruses detected closer to the expected values and by showing more consistent results for biological and experimental replicates.
- However, results for the laboratories with lower assay performance continued to show a significant underestimation of intact proviral copies, inconsistent results for biological and experimental replicates and the reported values differed significantly from the expected ones.
- The establishment of a virology quality assurance program is essential in harmonizing inter-laboratory performances of assays to measure the size and expression of the HIV-1 reservoir, thereby strengthening the utility of these assays in clinical and research applications.
- Further investigations will focus on rigorous inter-laboratory training and the development of standardized protocols to minimize variability and improve assay performance and data comparability.
- Participating labs will remain unidentifiable until they consent to release, as performance evaluations can be sensitive.

ACKNOWLEDGEMENTS

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