Evaluation of a dilution protocol using the Aptima HIV-1 RNA Quant assay on the Panther system with blood collected in EDTA-microtainer tubes

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Background

- FDA-approved viral load (VL) assays use only venipuncture-derived plasma collected in clinical settings
- Dried blood spots (DBS) have been evaluated as an alternative collection method; however, the limit of quantification (LOQ) of DBS VL assays is not optimal to evaluate viral failure at 2.3 $\log_{10}$ copies/ml ($\log_{10}$)
- Fingerstick whole blood (FSB) collected in an EDTA-microtainer tube (MCT) in non-clinical settings including home self-collection could be an alternative method to obtain plasma and help achieve the goals of the new initiative

Objective

We evaluated the performance of a 1:7 dilution VL protocol with the Hologic Aptima HIV-1 RNA Quant assay using previously characterized plasma controls and plasma derived from FSB collected in MCTs during the Engagement sub-study[1]

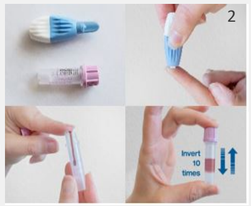
HIV Samples and Analysis

- Standard and 1:7 dilution VL protocols were evaluated by testing 0.7 ml of undiluted and 1:7 diluted plasma in:
  - » Eight commercial controls (SeraCare and Acrometrix)
  - » One control diluted to 2.95, 2.76, 2.56, 2.48 $\log_{10}$
  - » 47 clinical samples from the Engagement sub-study
- Analysis performed to compare protocols:
  - » LOQ in control samples
  - » Correlation and agreement of all quantified results using linear regression (R^2) and Bland-Altman

Methods

Sample Collection → **Sample Processing** → **Viral Load Testing**

Example of self-fingerstick blood collection:
Fill MCT to a minimum volume of 200 µl. Invert 10 times and label tube.



Ship MCT as clinical specimen to CDC at ambient temperature (24-48 hours).

In CDC Laboratory, invert and centrifuge the MCT for 3 minutes at 3,000 rpm to separate a minimum of 100 µl of plasma. Store at -80°C until testing.

Place 100 µl of thawed plasma into a specimen aliquot tube (SAT) containing 600 µl of Hologic specimen diluent.
Invert SAT and place directly on the Panther instrument for testing.
To calculate the viral load, RNA copies/ml are multiplied by 7.

Results

Table 1: Results from prepared dilutions and commercial controls

Standard Mean VL ($\log_{10}$)	1:7 Dilution VL Results			
	n <1.47 $\log_{10}$	n (%) x $\log_{10}$	Mean VL ($\log_{10}$)	SD ($\log_{10}$)
2.21	3/3	0/3 (0%)	<1.47	N/A
2.48	7/18	11/18 (61%)	2.48	0.11
2.56	5/15	10/15 (67%)	2.52	0.12
2.76	0/15	15/15 (100%)	2.79	0.18
2.95	0/10	10/10 (100%)	2.83	0.12

x $\log_{10}$: quantified samples; SD: standard deviation; <1.47 $\log_{10}$: detected but not quantified; TND: target not detected

Table 2: Overall results from clinical samples

Standard VL	1:7 Dilution VL		
	TND	< 1.47 $\log_{10}$	x $\log_{10}$
TND	9	0	0
< 1.47 $\log_{10}$	4	3	0
x $\log_{10}$	1	6	24

Fig 1: Correlation of standard and dilution VL protocols

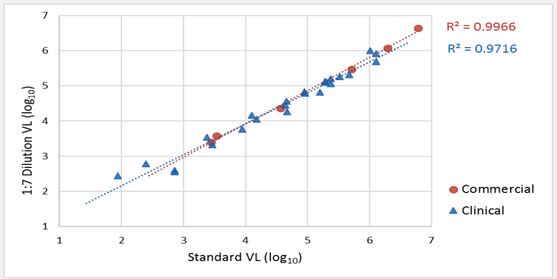
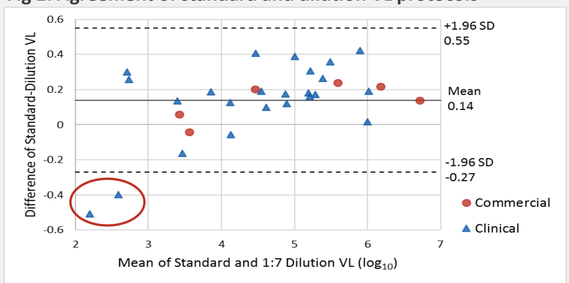


Fig 2: Agreement of standard and dilution VL protocols



[1]Emory University Engagement sub-study: FSB specimens were collected into MCTs by trained staff from individuals participating in the Engagement study. Remnant venipuncture-derived plasma specimens from the same participants were also collected and used for the comparison.
[2]Photos obtained from https://ayassbioscience.com/specimen-collection-instructions/

Results Summary

Limit of Quantification

- With the dilution protocol, 61% of control samples were quantified at 2.48 $\log_{10}$ and lower VLs can be quantified
- The correlation between protocols was high, although the agreement between values at low HIV-1 RNA concentrations was lower (red circle in Fig. 2)
- Two clinical samples with standard VL of 1.94 and 2.39 $\log_{10}$ gave higher values with the dilution protocol, 2.45 and 2.79 $\log_{10}$, respectively
- Six samples with standard VL values ranging from 1.80 – 2.46 $\log_{10}$ were detected <1.47 $\log_{10}$ in the dilution VL, and one sample with standard VL 2.66 $\log_{10}$ was TND in the dilution protocol

Limitations

- Quantities received from MCTs were insufficient to run in duplicate to confirm results
- Dilution errors may have accounted for not reaching the expected LOQ (2.32 $\log_{10}$) when applying the dilution factor

Conclusions

- The concordance between the standard and 1:7 dilution VL protocols was high, using both commercial and clinical HIV samples
- Although the LOQ was not close to the viral failure cutoff of 2.3 $\log_{10}$, FSB collection in MCT offers an alternative to venipuncture plasma collection for VL testing with better sensitivity than DBS protocols
- MCT collection could facilitate surveillance for virologic suppression outside of clinical healthcare settings to optimize treatment methods and further prevent HIV transmission

Affiliates / Partners

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