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New HIV-1 SuperLow Assay For Viral Load Monitoring

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Introduction

As new antiretroviral therapies (ART) such as Integrase inhibitors become standard of care, accurate ultra low viral load monitoring will become increasingly important. Equally important will be clinical monitoring of ART potency using assays that detect below the current FDA approved assay cutoffs.[1] Here we describe a new HIV-1 SuperLow Assay for detection of HIV-1 RNA at ultra low levels, developed using a modified protocol of a CE marked commercial kit.

We validated the HIV-1 SuperLow Assay for real-time HIV-1 quantitation for hit rate, precision/accuracy, and reportable range utilizing commercial controls. Retrospective analysis of plasma specimens (previously <50 c/mL) was conducted to assess the performance of this assay for the detection of ultra low HIV-1 RNA levels.

Method

- Viral subtype B RNA in HIV-1 negative human plasma and panel members from the 2011 Human Immunodeficiency Virus RNA EQA Programme (available from Quality Control for Molecular Diagnostics (QCMD)) were extracted on bioMerieux's (Durham, NC) NucliSens® easyMAG® platform.
- Extracts were analyzed using bioMONTR's proprietary HIV-1 SuperLow Assay described here-in which utilizes components of bioMerieux's commercially available (RUO) EasyQ® HIV-1 v2.0.
- Testing on the HIV-1 SuperLow Assay was performed using a 2.0 mL sample input, with the exception of QCMD panel samples which were 1.0 mL each. The acceptable maximum allowable standard deviation (SD) criteria of ± 0.50 $\log_{10}$ c/mL was established based on criteria by the HHS Panel on Antiretroviral Guidelines.[2]
- For determination of Precision and the Limit of Detection (LOD), dilutions of Virology Quality Assurance (VQA) viral standards were made in HIV-1 negative human plasma yielding dilutions of approximately 3, 6, 12, 24, 48, 72, and 96 c/mL. At least 27 replicates of each concentration were tested using a single lot of extraction and amplification reagents. Probit analysis to determine the 95% hit rate using Percent Detected (PD) values at each dilution. Excel 2007 (Microsoft) function NORMSINV (z) was used to translate PD values to probit values.
- For testing analytical measurement range, Virology Quality Assurance (VQA) stock material at 10^7 log was diluted 1:10 serially 5X in normal HIV-1 negative human plasma to yield dilutions of 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000 and tested in a single run.

Results (cont'd)

The LOD was determined by testing replicates of normal human plasma spiked with a range of HIV-1 RNA from 3 to 96 c/mL. Probit analysis (See Figure 1) revealed that the concentration of HIV-1 RNA detected with 95% probably is 15 c/mL (1.18 log c/mL) and with 70% probably is 7 c/mL (0.85 log c/mL).

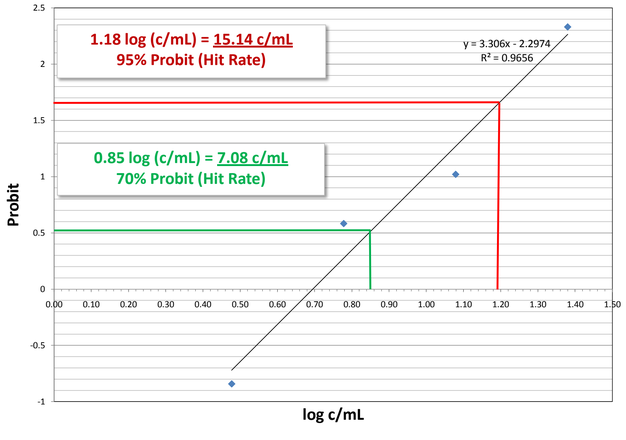
Precision test results are summarized in Table 1. At HIV-1 concentrations below 48 c/mL, the precision was slightly above the expected variation of the commercial assay (+/- 0.30 log) based on published literature.[3] Negative Controls consistently had results of "<Lower Detection Limit".

Retrospective analysis of 251 plasma specimens previously determined to be <50 c/mL with an FDA approved commercially available assay was conducted. 37% (n=92) were quantitated yielding results between 3-400 c/mL. 63% (n=158) reported results of "<Lower Detection Limit (or <2 c/mL). 1 sample yielded an invalid result. Results are presented in Figure 2.

Panel members from the 2011 Human Immunodeficiency Virus RNA EQA Programme (available from QCMD) were analyzed. Results are presented in Table 2.

Testing diluted samples from 3 to 8.3M c/mL demonstrated a direct proportional relationship between the dilution factor and the number of HIV-1 RNA copies reported by the assay; thus, confirming the manufacturer's reported linear quantitative range of 25 to 7.9M c/mL (data not shown).

Figure 1. Results of the Probit Analysis



Results (cont'd)

Table 1. Precision Test Results

	Target Input (copies/mL)						
	3	6	12	24	48	72	96
N	25	25	26	35	27	27	27
Mean, c/mL	3	6	9	23	52	83	75
Std Dev, c/mL	1	6	7	18	41	56	41
Mean, LOG c/mL	0.33	0.61	0.81	1.23	1.60	1.83	1.81
Std Dev, LOG c/mL	0.28	0.36	0.32	0.34	0.33	0.26	0.25

Figure 2. Retrospective Analysis of Plasma Specimens

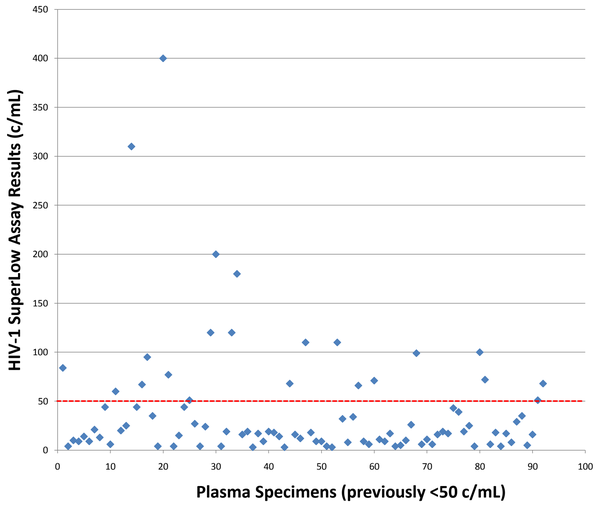


Table 2. Results from QCMD 2011 Human Immunodeficiency Virus RNA EQA Programme.

Panel Code	Sample Content	Mean QCMD Concentration (N = 181)		HIV-1 SuperLow Results (N = 1)	
		c/mL	Log c/mL	c/mL	Log c/mL
HIVRNA11-01	HIV Type C	10,914	4.04	4,600	3.66
HIVRNA11-02	HIV Type C	1,106	3.04	770	2.89
HIVRNA11-03	HIV Type B	2,924	3.47	2,200	3.34
HIVRNA11-04	HIV-1 Neg Plasma	Negative	Negative	<LDL	<LDL
HIVRNA11-05	HIV Type B	3,119	3.49	3,100	3.49
HIVRNA11-06	HIV Type A/G	207,014	5.32	140,000	5.15
HIVRNA11-07	HIV Type B	11,508	4.06	11,000	4.04
HIVRNA11-08	HIV Type B	449	2.65	350	2.54

Conclusions

- The HIV-1 SuperLow Assay demonstrated impressive hit rates: 95% at 15 c/mL and 70% at 7 c/mL.
- The HIV-1 SuperLow Assay has a reportable range of 2 to 10,000,000 c/mL.
- The assay was verified to have acceptable precision and accuracy well within the range considered to be statistically significant for clinical interpretation. As expected, the precision decreases when the concentration of the analyte decreases.
- All results obtained from QCMD Panel Members were as expected and quantitative performance on paired samples were within 0.5 log units of the median. bioMONTR's Quantitative Consensus Panel Score ranked in the 73rd percentile of all datasets (i.e., 27% of all datasets had the same, or better, score).
- The assay produced reportable quantitative results as low as 3 c/mL for samples previously reported as <50 c/mL with an FDA approved commercial assay.
- The HIV-1 SuperLow Assay will be a valuable tool for monitoring HIV-1 viral load and patient response in drug development and clinical trial programs.

References

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