Validation of eMAG™ for HIV-1 Low Viremia Testing

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Introduction

Significant interest in long-acting antiretroviral drugs (ARVs) has set the bar for future HIV/AIDS care. As new highly active antiretroviral therapies (HAART) have become standard of care, accurate low viral load monitoring using assays that detect below the FDA approved cutoffs will become increasingly important, especially for eradication studies.

This study is an extension of the previously described validation of the HIV-1 SuperLow assay for Research Use Only (bioMONTR Labs) used for detection of single copy HIV-1 RNA, developed using the bioMerieux NucliSens easyMAG system for RNA isolation and downstream NASBA amplification chemistry. A side-by-side comparison between bioMerieux's easyMAG system with their new eMAG system was conducted for use with the HIV-1 SuperLow Assay.

The new eMAG system utilizes the same chemistry as the easyMAG system but allows higher throughput and improved flexibility with 2 independent sections of 24 reactions each. The system also offers increased process traceability as well as parallel processing of various samples types and volumes.

Methods

Viral subtype B RNA was extracted from Virology Quality Assurance (VQA) stock material using bioMerieux's easyMAG and eMAG systems. The eluates were analyzed with bioMONTR's HIV-1 SuperLow Assay and the proprietary algorithm for quantitation of HIV-1 RNA.

Linearity, reproducibility, and accuracy studies were conducted as outlined below:

- For linearity, stock material at 150,000 c/mL was serially diluted in normal human plasma to yield dilutions of approximately 1,000 c/mL, 500 c/mL, 250 c/mL and 125 c/mL. The samples were extracted in triplicate on each system.
- For reproducibility, stock material was diluted in normal human plasma to generate low titer HIV-1 positive samples at ~100 c/mL which was extracted on the eMAG (n = 22) and the easyMAG (n = 17) systems.
- For accuracy, EQA proficiency panel samples from College of American Pathologists (CAP) were analyzed.

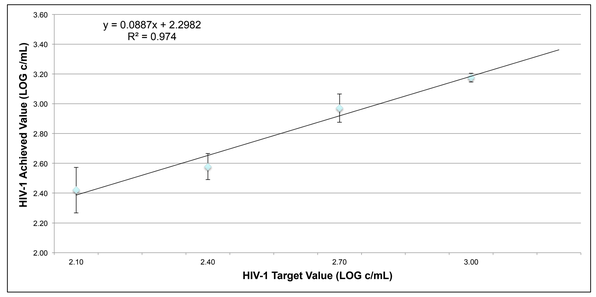
Retrospective analysis of clinical samples previously determined to be <40 c/mL with an FDA approved commercially available assay was conducted.

Results

Linearity study demonstrated a proportional relationship between the dilution factor and number of HIV-1 RNA copies (R^2 = 0.974, See Figure 1).

Replicate samples extracted on the eMAG yielded comparable results to those extracted on the previously validated easyMAG system (data not shown).

Figure 1: VQA Linearity Analysis for eMAG



See Table 1 for results of the reproducibility study. Replicate low titer HIV-1 samples extracted on eMAG (n = 22) yielded results of 1.89 LOG c/mL (SD = 0.27) while replicate samples extracted on easyMAG (n = 17) yielded results of 1.98 LOG c/mL (SD = 0.23).

Table 1: Results of Reproducibility Testing

HIV-1 Viral Load (Target = 2 LOG c/mL)		
	easyMAG (n = 17)	**eMAG (n = 22)**
Average (LOG c/mL)	1.98	1.89
Std Dev	0.23	0.27

Repository CAP EQA samples tested as part of the Accuracy study yield results that were within the expected range (Table 2) when compared to results reported in the 2014 HIV-B Viral Load Participant Summary from the College of American Pathologists (CAP).

Table 2: Results of Accuracy Study

CAP ID	eMAG (LOG c/mL)	CAP results (LOG c/mL)		
		Median	Low Value	High Value
HV2-06	4.32	4.04	3.83	4.22
HV2-07	< LDL	Negative	Negative	Negative
HV2-08	2.43	2.60	2.20	2.87
HV2-09	5.18	5.05	4.91	5.25
HV2-10	4.2	4.04	3.87	4.25

Results (cont'd)

Retrospective analysis of clinical samples previously determined to be <40 c/mL with an FDA approved assay provided quantitative results for 16 of the 25 samples analyzed (65%). The results are outlined in Table 3.

Table 3: HIV-1 SuperLow Results

Sample ID	HIV-1 (c/mL)
1	< LDL
2	7
3	11
4	23
5	10
6	29
7	110
8	9
9	230
10	< LDL
11	5
12	< LDL
13	< LDL
14	< LDL
15	< LDL
16	16
17	< LDL
18	44
19	< LDL
20	9
21	13
22	< LDL
23	6
24	28
25	4

Conclusions

- The eMAG system efficiently and effectively recovers HIV-1 RNA that can be utilized for detection of ultra low levels using our proprietary HIV-1 SuperLow Assay.
- The eMAG system offers the full benefits of automation and flexibility utilizing the gold standard for extraction chemistry.
- Quantification of samples <40 c/mL uisng the eMAG in combination with bioMONTR's single copy HIV-1 SuperLow Assay offers monitoring of low viremia samples which have become important in development of long-acting acting antiretrovirals and with eradication studies.

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