VALIDATION OF VIROSEQ HIV- 1 GENOTYPING SYSTEM V2.0 USING AUTOMATED EXTRACTION: EASYMAG®

AMP 2013
ID11

Anita McClernon, Andrew Freeman and Daniel McClernon
bioMONTR® Labs, Research Triangle Park, NC

Introduction

HIV-1 resistance testing is standard of care for HIV-1 patient management. The FDA approved ViroSeq® HIV-1 Genotyping System utilizes a manual ETOH sample extraction and gel electrophoresis for amplicon quantitation. Herein we describe the performance of the ViroSeq HIV-1 assay when used with the NucliSens® easyMAG® for automated nucleic acid extraction and NanoDrop 2000 spectrophotometer for amplicon quantitation.

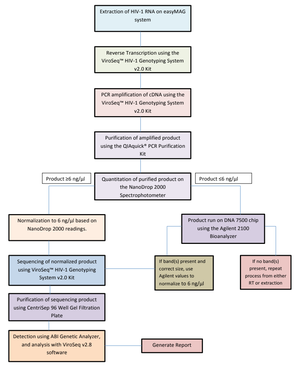
We validated bioMONTR's modified protocol using the ViroSeq HIV-1 Genotyping System for intra- and inter-assay precision, accuracy, specificity, analytical sensitivity (detection of mutations), assay sensitivity (limit of detection) and subtype analysis.

Methods

- For purposes of this validation, all analysis was performed using the ViroSeq® HIV-1 Genotyping System v2.0 (Abbott Molecular, Des Plaines, IL) with the following modifications (see Figure 1):
  - Plasma samples (0.5 or 1.0 mL) were extracted using the NucliSens® easyMAG® (bioMérieux Inc., Durham NC),
  - PCR purification was performed using QIAquick® PCR Purification Kit (QIAGEN Sciences, Maryland).
  - Prior to cycle sequencing, PCR products were normalized based on concentrations obtained using a NanoDrop 2000 Spectrophotometer (ThermoScientific).
- PCR amplicons from a subset of the validation samples were analyzed for correct size using the Agilent 2100 Bioanalyzer and the Agilent DNA 7500 Kit (Agilent Technologies, Germany).
- To assess precision, HIV-1 plasma was diluted to ~4.5 LOG c/mL (2 sets, 5 replicates each) and assayed on different days by different operators. Results were compared at the NT level for concordance (within run and between runs).
- To demonstrate accuracy, 13 samples (range ~3.3 to ~6 LOG c/mL) were analyzed and duplicate aliquots were tested by an independent commercial laboratory. Results from both laboratories were compared at the NT level and drug resistance mutations.
- To determine if co-infecting organisms affect amplification, HIV-1 plasma (~4.5 LOG c/mL) was spiked with HCV and HBV. Aliquots (neat and spiked) were analyzed concurrently.
- Analytical sensitivity was performed by mixing non-congruent HIV-1 sequences to create wild type/mutant ratios (85/15, 75/25, 50/50). Majority species/mixture was recorded for each sample.
- Assay sensitivity was assessed using HIV-1 samples diluted to 300 c/mL.
- A commercially available HIV RNA (Group M) Genotype Performance Panel (SeraCare Cat. No. PRD201-1.2) was analyzed and results analyzed through HIV BLAST programs.
- HIV-1 sequence concordance was analyzed by bioMONTR's proprietary bioConT tool. An acceptance criteria of ≥95% concordance was applied for all analysis.

Methods (cont'd)

Figure 1. ViroSeq HIV-1 assay workflow diagram.



Results

- Intra-Precision concordance was >99.3% (data not shown) and inter-assay concordance was >99.2% (Table 1) when compared individually, and as a group. Positive controls for each validation assay were also compared and showed 100% concordance at the nucleotide level (data not shown).
- 12/13 paired samples analyzed at bioMONTR and an independent commercial lab were >98.7% concordant at the nucleotide level. One sample did not produce amplified product. For 10/12 (83%) there was 100% concordance for drug resistance mutations. A genotypic report was not generated for 1/12 (0.08%) due to lack of double stranded coverage. For 1/12 (0.08%), a V75I mutation was identified by the send-out laboratory (based on unidirectional coverage) while no mutations were identified by bioMONTR (data not shown).
- The presence of HCV and HBV had no impact on amplification or sequencing. Spiked samples were >99% concordant at the nucleotide level when compared to the neat sample (data not shown).
- For the Analytical Sensitivity (detection of mutations), double stranded sequence was obtained for all samples across all target locations and species mixture was detected in both directions. Target locations included L10/M46 (PRO) and M41/D67/V75/G190/L210/T215 (RT). All mixtures were detected across all dilutions, with the exception of M41V (RT) for 85/15 wild type/mutant mixture. Representative electropherograms are provided in Figure 2.
- Successful genotypic analysis was performed on samples with HIV-1 viral concentrations of ~300 c/mL and ~500 c/mL. All diluted samples were >99% concordant when compared to the original undiluted sample at ~30,000 c/mL (data not shown).

Results

- All subtype specific samples from the HIV RNA (Group M) Performance Panel amplified and sequenced. The negative sample (PRD201-09) did not amplify. Results from panel members #1- #6 were run through ViroBLAST and were >95% concordant with the top matching results for their subtype. Results from panel member #7 and #8 were run through HIV BLAST and were 91% and 93% concordant to subtype sequences in their group, respectively. Subtype results were 100% concordant with expected panel subtypes (data not shown) with subtypes A/G,B,C,D,A/E,F,G and H being represented.
- PCR amplicons from a subset of the validation samples were analyzed using the Agilent 2100 Bioanalyzer and the Agilent DNA 7500 Kit demonstrated PCR amplicons with an average length of ~1.6 kb in the range of the 1.8 kb amplicon expected according to the ViroSeq package insert. (see Figure 3).

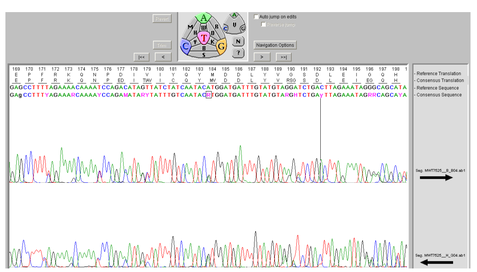
Table 1. Inter-assay precision results

Validation Sample	1	2	3	4	5	9	10	11	12
2	99.7	-	-	-	-	-	-	-	-
3	99.5	99.6	-	-	-	-	-	-	-
4	99.7	100	99.6	-	-	-	-	-	-
5	99.9	99.8	99.4	99.8	-	-	-	-	-
9	99.5	99.4	99.4	99.4	99.4	-	-	-	-
10	99.3	99.2	99.0	99.2	99.2	99.5	-	-	-
11	99.4	99.5	99.3	99.5	99.5	99.6	99.3	-	-
12	99.5	99.6	99.4	99.6	99.6	99.4	99.3	99.8	-
13	99.4	99.5	99.6	99.5	99.4	99.4	99.4	99.6	99.6

Figure 2. ViroSeq HIV-1 Electropherograms of (A) 75/25 wild type/mutant mixture and (B) 25/75 wild type/mutant mixture

A
75 WT/25 M

B
25 WT/75 M



Results (cont'd)

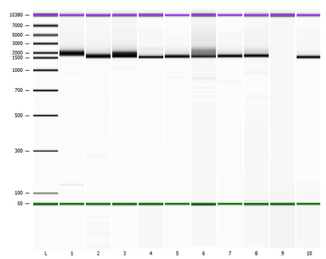


Figure 3. HIV RNA Genotype Performance Panel. Lanes 1-9 represent panel samples PRD201-01 through PRD201-09, respectively. Lane 10 represents the ViroSeq Kit Positive Control. **Band Sizes:** Lane 1(1,972 bp), Lane 2(1,666 bp), Lane 3(1,694 bp), Lane 4(1,577 bp), Lane 5(1,643 bp), Lane 6(1,651 bp), Lane 7(1,692 bp), Lane 8(1,717 bp), Lane 9(negative sample, no amplification) and Lane 10(1,569 bp).

Conclusions

- The easyMAG extraction system and the NanoDrop can be utilized in combination with the ViroSeq HIV-1 Drug Resistance assay.
- bioMONTR's modified protocol using the ViroSeq HIV-1 Genotyping System v2.0 is demonstrated to have improved assay sensitivity (~500 c/mL) compared to the FDA approved protocol (~2,000 c/mL). The modified protocol is also demonstrated to have comparable precision, accuracy and analytical specificity.
- With improved extraction efficiency of bioMONTR Labs modified protocol, we routinely report mixtures down to ~20% minority species (based on dual coverage) as compared to the standard ViroSeq protocol which reports minority species at ~40%.
- The use of the easyMAG automated platform combined with amplicon NanoDrop quantitation improves sensitivity, workflow and turnaround time for HIV-1 drug resistance analysis.

Acknowledgments

The authors would like to thank bioMérieux Inc. for their support during this validation study.

Send correspondence to:
Daniel McClernon
dmcclernon@biomontr.com

bioMONTR® Labs