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GENOTYPING OF HIV-1 PROVIRAL DNA FROM PBMC's USING TRUGENE™ HIV-1 GENOTYPING ASSAY AND NucliSens® MiniMAG EXTRACTION

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BACKGROUND

During Human Immunodeficiency Virus type 1 (HIV-1) infection two versions of the virus are found in the circulating blood stream, the proviral DNA and the plasma RNA virus[1,2]. Herein, we describe a modified method for the use of the TRUGENE™ HIV-1 Genotyping Assay (Bayer Healthcare) for monitoring Resistance Associated Mutations (RAM) from the peripheral blood mononuclear cells (PBMC) viral DNA compartment.

This study presents a comparison of HIV-1 viral RNA obtained from plasma and proviral DNA obtained from PBMC's. Furthermore, this study examines the utility of modifications in the TRUGENE HIV-1 Genotyping Assay for genotyping of proviral DNA from total nucleic acid (DNA and RNA) using a magnetic silica-based extraction method.

METHODS

Ten treated HIV-1 infected patients with viral loads ranging from 4,000 to 200,000 copies/mL were selected for genotypic analysis. Blood kits containing three VACUTAINER™ EDTA (4 mL each) and one VACUTAINER™ CPT (8 mL) blood collection tubes were drawn and inverted several times after collection to mix preservatives completely. Blood samples were processed according to the VACUTAINER EDTA or VACUTAINER CPT product insert methodologies. Processed plasma was aliquoted into 2 mL cryo-vials and frozen at -70°C until tested. Collected PBMC's were counted and separated into paired 0.5 mL aliquots containing 1 x 10^6 to 5 x 10^6 cells. Plasma specimens were tested for viral load using the Roche Amplicor HIV-1 Monitor™ version 1.5 Standard methodology.

Paired 1.0 mL plasma specimens were extracted using modified protocols for the NucliSens® MiniMAG System (bioMerieux) and the QIAamp Viral RNA Mini Kit (Qiagen). A standardized elution volume of 60 µL was used for all plasma extractions. The paired cellular aliquots were centrifuged and resuspended in a standardized 200 µL volume of PBS prior to extraction. PMBC specimens were extracted using the NucliSens MiniMAG Extraction System and QIAamp DNA Blood Mini kit. A standardized elution volume of 200µL was used for all PBMC proviral extractions.

The extracted RNA was genotyped using TRUGENE HIV-1 Genotyping Assay without modification. The extracted proviral DNA was genotyped by modifying the TRUGENE HIV-1 Genotyping Assay. This was accomplished by excluding the reverse transcriptase, RNase inhibitor and DTT from the TRUGENE HIV-1 protocol and by eliminating the reverse transcription step during PCR thermal cycling. The standard CLIP™ (Bayer HealthCare) sequencing methodology was unchanged.

The plasma virus derived sequences were used as the reference for comparing the proviral sequences derived from cellular extracted material. Sequences derived from RNA and DNA were directly compared using the automated MuTanker™ Comparator software (Research Think Tank, Inc).

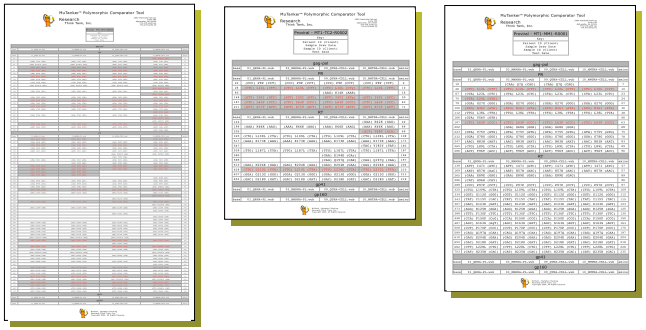
RESULTS

Sequences from RNA were obtained for 100% of the plasma specimens using the TRUGENE HIV-1 Genotyping Assay and the NucliSens MiniMAG Extraction System. For the matched plasma samples extracted using the QIAamp Viral RNA Mini Kit, the success rate was 90%. Genotyping results were obtained for 60% (6 of 10) using the NucliSens MiniMAG Extraction System and 80% (8 of 10) for QIAamp DNA Blood Mini kit using the modified TRUGENE HIV-1 Genotyping Assay for proviral DNA.

Table 1: Summary of plasma and PBMC derived genotype.

SAMPLE	VIRAL LOAD (c/mL)	PBMC COUNT (# cells x 106)	HIV-1 NUCLEIC ACID TYPE	VOLUME EXTRACTED (mL)	QIAamp	MiniMAG
1	31,800	1.89	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
2	24,846	5.20	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
3	28,368	4.65	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
4	41,800	2.50	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
5	72,700	4.10	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
6	99,524	5.20	RNA	1.0	YES	YES
			DNA	0.5	YES	YES
7	182,105	3.67	RNA	1.0	YES	YES
			DNA	0.5	YES	NO
8	61,100	3.66	RNA	1.0	YES	YES
			DNA	0.5	NO	NO
9	3,930	5.16	RNA	1.0	YES	YES
			DNA	0.5	YES	NO
10	4,120	1.12	RNA	1.0	YES	NO
			DNA	0.5	NO	NO

Figure 1: Example of three plasma-proviral MuTanker comparative analysis report



Four of six proviral genotyping failures (2 MiniMag and 2 QIAamp) were observed in the same two patients with viral loads of 4,000 and 61,000.

In some sample comparisons, the number of polymorphisms were different between plasma and cellular compartments, however, the RAM were consistent across both. Overall, the PBMC modified protocol reduced assay time by one hour.

Comparison of the plasma reference sequence to successful PBMC sequences were concordant at ≥97% at the nucleotide level, ≥98% at the amino acid level and 100% in reported RAM. Variation in mixture sequences was observed between plasma derived RNA and PBMC derived total nucleic acids.

CONCLUSIONS

- The modified TRUGENE HIV-1 Genotyping Assay demonstrates the potential utility of the current kit to genotype PBMC derived HIV-1 proviral DNA.
- Accuracy and reproducibility of results for proviral genotyping were comparable to the TRUGENE product insert specifications for plasma HIV-1 analysis.
- RAM concordance between plasma and PBMC derived genotype was 100%.
- Concordance of protease and reverse transcriptase results demonstrate an additional application for the TRUGENE HIV-1 Genotyping Assay to be used in proviral genotyping.
- No correlation could be established between viral load and the success or failure of proviral genotyping using the modified TRUGENE HIV-1 assay.
- Interestingly, proviral genotyping failures (4 of 6) were predominantly localized to two specific patients. This may suggest possible sample integrity issues for these specimens.
- Because of the lack of viral load correlation to PBMC genotypic failure, this method may be useful as an alternative monitoring tool for low level viremic patients.

REFERENCES

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2. Lal RB, Chakrabarti S, Yang C. 2005. Impact of genetic diversity of HIV-1 on diagnostic, antiretroviral therapy & vaccine development. *Indian J Med Res.* Apr. 121:278-318.

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