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PILOT VALIDATION OF TRUGENE™ HIV-1 GENOTYPING FROM WHOLE BLOOD EXTRACTIONS

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BACKGROUND

Standard resistance testing for Human Immunodeficiency Virus type 1 (HIV-1) is routinely used for monitoring HIV infection and subsequent treatment strategies[1]. Resistance genotyping strongly correlates with response to antiviral therapy[1,2]. Herein, we describe a pilot validation study for the use of the TRUGENE™ HIV-1 Genotyping Assay (Bayer Healthcare) for the monitoring of Resistance Associated Mutations (RAM) from whole blood collection.

This study evaluates and directly compares real-time plasma and whole blood for suitability in resistance genotyping diagnostics. This study also compares the utility of two extraction platforms across two sample types for obtaining nucleic acids suitable for sequencing.

METHODS

Ten treated HIV-1 infected patients with viral loads ranging from 4,000 to 200,000 copies/mL were selected for genotypic analysis. Plasma specimens were tested for viral load using the Roche Amplicor HIV-1 Monitor™ version 1.5 Standard methodologies. Blood kits containing four VACUTAINER™ EDTA (4 mL each) blood collection tubes were drawn and inverted several times after collection to mix preservatives completely. Plasma was isolated from three of the four blood collection tubes following the VACUTAINER EDTA product insert methodology. One of the blood collection tubes remained unprocessed to represent the whole blood specimen. Matched whole blood and plasma samples were extracted in real-time.

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. For whole blood total nucleic acid extractions, 100 µL was used for NucliSens MiniMAG System and 200 µL for the QIAamp DNA Blood Kit. To normalize the nucleic acid concentration for each extraction methodology, an elution volume equivalent to the input extraction volume was used.

The extracted plasma and whole blood total nucleic acid (viral RNA and DNA) were genotyped using the TRUGENE HIV-1 Genotyping Assay without modification. *It is important to consider that when genotyping HIV-1 from whole blood, both RNA and DNA (proviral and episomal) viral species are simultaneously amplified and sequenced* [3,4].

The plasma virus derived sequences were used as the *reference* for comparing the resultant whole blood derived genotypes. Peripheral Blood Mononuclear Cells (PBMC) HIV-1 genotypes were not included in this statistical analysis. Combined sequences derived from whole blood total nucleic acid were directly compared using the automated MuTanker™ Comparator software (Research Think Tank, Inc).

RESULTS

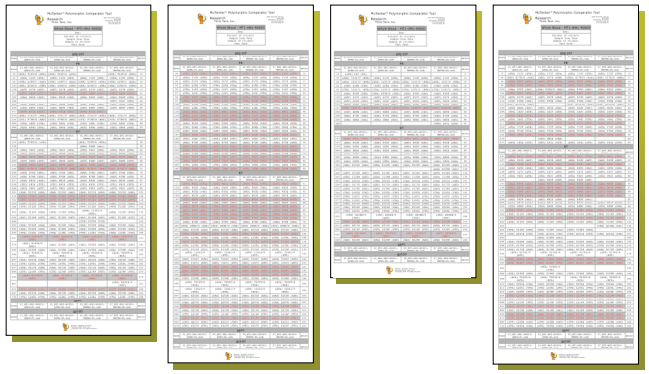
TRUGENE HIV-1 sequences were obtained from 100% (10 of 10) of the plasma specimens using the and the NucliSens MiniMAG Extraction System. For the matched plasma samples extracted using the QIAamp Viral RNA Mini Kit, the success rate was 90% (9 of10). Genotyping results, using the TRUGENE HIV-1 Genotyping Assay, were obtained for 100% (10 of 10) of the whole blood and plasma samples using both the NucliSens MiniMAG Extraction System and QIAamp DNA Blood Mini kit.

Table 1: Percent Similarity Score for Plasma vs. Whole Blood.

			Plasma to Whole Blood Comparison using NucliSens MiniMAG Extraction System				Plasma to Whole Blood Comparison using QIAamp DNA Blood Mini Kit			
			RAM		Polymorphic Fingerprint		RAM		Polymorphic Fingerprint	
Sample ID	Clade	Viral Load	NA (165 bases)	AA (55 aa)	NA (925 bases)	AA (308 aa)	NA (165 bases)	AA (55 aa)	NA (925 bases)	AA (308 aa)
MT1-JM2-R0002	B	3,930	93.6%	88.2%	95.5%	94.2%	95.8%	90.9%	97.4%	96.4%
MT1-JD1-R0006*	B	4,120	99.1%	97.3%	97.5%	95.0%	NA	NA	NA	NA
MT1-MM1-R0001	B	24,846	99.7%	100.0%	98.5%	100.0%	99.7%	100.0%	99.8%	100.0%
MT1-TC2-R0002	B	28,368	99.7%	100.0%	99.9%	100.0%	99.7%	100.0%	99.9%	100.0%
MT1-OM1-R0001	B	41,800	100.0%	100.0%	99.1%	100.0%	100.0%	100.0%	99.8%	99.7%
MT1-WH1-R0003	B	31,800	100.0%	100.0%	99.7%	100.0%	100.0%	100.0%	99.6%	99.7%
MT1-MB1-R0004	B	69,100	98.2%	100.0%	97.2%	96.8%	99.4%	100.0%	99.8%	100.0%
MT1-PR1-R0002	B	72,700	100.0%	100.0%	99.9%	100.0%	100.0%	100.0%	99.8%	100.0%
MT1-HB1-R0003	B	99,524	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
MT1-EH1-R0002	B	182,105	99.7%	100.0%	99.5%	99.4%	100.0%	100.0%	99.9%	100.0%
Mean Similarity Scores			**99.1%**	**98.4%**	**98.8%**	**98.7%**	**99.4%**	**98.9%**	**99.5%**	**99.5%**

*Mean Percent Similarity is based on 9 of 10 sample comparison for QIAamp DNA Blood Mini Kit

Figure 1: Example MuTanker Comparator Reports.



Whole blood and plasma sequences were obtained first pass without repeating the assay or they were defined as "No Sequence". Stringent criterion for the genotype results were used in this analysis including nucleotide for nucleotide comparisons and heterozygous base-calling. When compared to matched reference plasma sequences, whole blood specimens genotype from the MiniMAG extractions had mean similarity scores of >99% and >98% at the nucleotide and >98.5% and >98.5% amino acid levels for RAM and polymorphic fingerprints, respectively. Also, QIAamp extractions had mean similarity scores of >99%, >98.5%, >99% and >99%. These values were obtained using the TRUGENE HIV-1 Genotyping Kit protocol without modification and the MuTanker Comparator software.

Of particular note, the whole blood genotypes with the lowest similarity scores were of poor genotype quality and were below internal quality standards for reporting. See Samples 9 and 10 of Poster TP47, GENOTYPING OF HIV-1 PROVIRAL DNA FROM PBMC's USING TRUGENE™ HIV-1 GENOTYPING ASSAY AND NucliSens® MiniMAG EXTRACTION.

When comparing the overall whole blood results to plasma, the genotype concordances were >99% at the nucleotide level, >99% at the amino acid level and >98.5% for the reported RAM.

CONCLUSIONS

- This preliminary study highlights the potential validity of whole blood genotyping.
- Whole blood genotyping may allow for a more comprehensive evaluation of HIV-1 resistance in patients undergoing anti-retroviral therapy.
- When compared to plasma, whole blood genotyping may prove to be a more effective monitoring tool when contemplating salvage therapy alternatives, due to the inclusion of the archived proviral mutant species.
- Concordance of protease and reverse transcriptase results among plasma and whole blood demonstrates potential utility in cooperative studies or in routine testing of whole blood collected in remote places in developing countries.

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