

Use of Plasma, Dried Plasma, and Dried Blood with Automated Sample Preparation and Detection for TRUGENE® HIV-1 Integrase Genotyping Assay

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Abstract

Introduction: The use of dried blood spots (DBS) for genotypic determination of HIV-1 has been proposed as an alternative to plasma in resource-limited settings.^{1,2} We report the development of automated sample preparation and amplification methods suitable for genotyping and viral load testing using the VERSANT® kPCR Molecular System.* Protease and reverse transcriptase genotyping from DBS was previously reported using the TRUGENE HIV-1 Assay.³ In this work, we compare plasma, dried plasma, and DBS genotypic analysis accuracy utilizing a prototype TRUGENE CLIP® module for the TRUGENE HIV-1 Integrase Assay.[†]

Methods: Nucleic acid extractions were performed on the VERSANT kPCR SP (Sample Preparation). DBS (50 microliter) on filter paper were prepared from normal whole blood samples spiked with HIV-1 virus. Plasma HIV-1 samples (500 microliter), at equivalent concentrations to those used for DBS, were used directly and after dried plasma preparation and recovery with the SampleTanker™ Dried Transportation Kit (Research Think Tank, Alpharetta, GA, US). Development of RT-PCR and CLIP sequencing for HIV-1 integrase was previously described.⁴ The OpenGene® DNA Sequencing System was used to generate genotyping results. Unedited sequencing data were used to determine differences in accuracy of software-based codon determinations.

Results: Software-generated data, with no operator editing, were 90%–100% consistent with the reference HIV-1 integrase region sequence. Genotyping accuracy was equivalent for plasma, dried plasma, and DBS samples with viral loads ranging from 10³ to 10⁵ RNA copies/mL. Some qualitative differences, including variability in sequencing peak intensities, were noted between methods.

Conclusions: Highly efficient and reproducible universal sample preparation protocols for plasma and blood, and amplification and detection methods for the VERSANT kPCR Molecular System and OpenGene DNA Sequencing systems, were developed for use with the TRUGENE HIV-1 Integrase Assay. No significant differences in genotyping accuracy were observed between plasma, dried plasma, and DBS sample types.

* System is CE marked; not commercially available in US.
† Assay in development.

Materials and Methods

Dried blood samples were prepared by spotting 50 µL aliquots on filter paper and drying overnight at room temperature. Dried plasma preparation and recovery were performed with 1 mL sample volume according to the manufacturer's instructions for the SampleTanker Dried Transportation Kit (Research Think Tank, Alpharetta, GA, US). Frozen plasma was processed using 500 µL sample input. Automated nucleic acid extractions were performed on the VERSANT kPCR SP (Sample Preparation) platform. Dilutions of HIV-1 (8E5) were prepared in normal human plasma (NHP) and whole blood.

Genotyping accuracy was analyzed for three sample types: dried blood spots, dried plasma tubes and frozen plasma. The sample panels were prepared from HIV-1-spiked NHP and whole blood from 10³ to 10⁵ RNA copies/mL.

Table 1. Sample panels used for genotyping accuracy analysis.

Sample Type	Sample ID	Log ₁₀ (copies/mL)
Dried blood spots	DBS3	5.0
	DBS4	4.0
	DBS5	2.7
Dried plasma tube	DPT3	4.9
	DPT4	3.9
	DPT5	2.9
Frozen plasma	HIV3	4.9
	HIV4	3.9
	HIV5	2.9

Development of RT-PCR and CLIP sequencing for HIV-1 integrase was previously described.⁴ In this study, the first of four overlapping CLIP modules covering the complete integrase region was utilized to generate a read length of approximately 350–400 bp. The OpenGene DNA Sequencing System was used for data collection and analysis of genotyping results. Software-generated data, with no operator editing, was compared to the reference sequence. Genotyping accuracy was evaluated on the basis of bidirectional coverage of the first 90 codons of integrase.

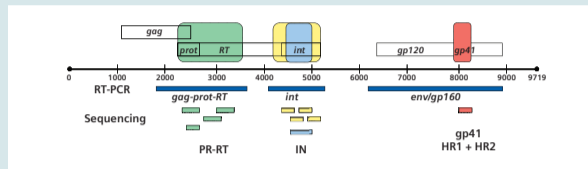


Figure 1. Sequencing region map for TRUGENE-based genotyping modules.

Results

Average codon accuracy was equivalent within 90% confidence intervals for plasma (94%–100%), dried plasma (96%–100%), and DBS (88%–100%). At the lowest concentration, successful amplification was not achieved for 3/3 replicates. However, all sample types yielded equivalent genotyping accuracy for input concentrations as low as 10³ RNA copies/mL. An additional, lower concentration sample (10² RNA copies/mL) was not amplified from DBS, although both frozen and dried plasma methods produced sufficient template for genotyping.

Qualitative differences in sequencing data were noted between sample types at the lowest concentrations. The lower input volume from DBS (50 µL) contained 10- to 20-fold fewer virus particles than plasma (0.5 mL) and dried plasma (1 mL) sample types. This resulted in lower sequencing peak intensities for DBS with respect to frozen plasma (~25% lower) and dried plasma (~50% lower). The difference was not discernable for higher-concentration samples.

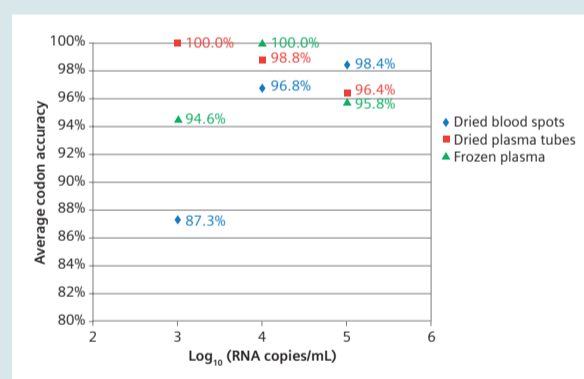


Figure 2. Average codon accuracy, without operator confirmation of automated basecalling, was not significantly different across sample types. Average accuracy for frozen plasma was 97% (90% CI: ±3%), dried plasma 98% (±2%), and dried blood spots 94% (±6%).

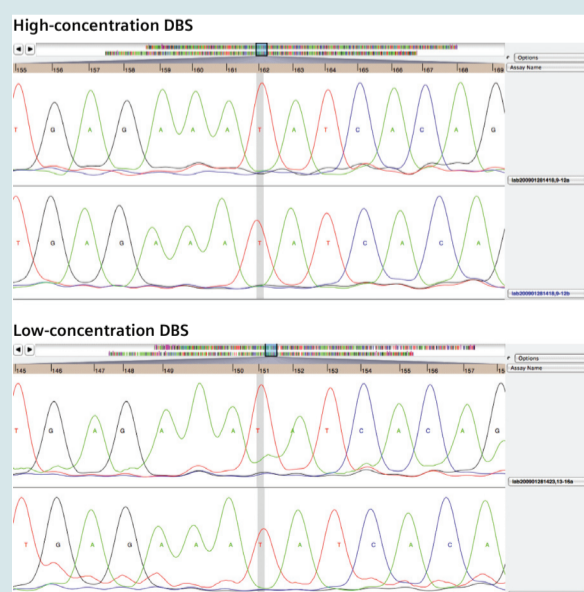


Figure 3. Examples of bidirectional sequence data for DBS samples at high and low concentration (10⁵ and 10³ RNA copies/mL). Lower peak intensities at the lower concentration resulted in lower unedited sequencing accuracy (87.3% vs. 98.4%). However, overall sequence quality was sufficient for operator confirmation of results with 100% accuracy.

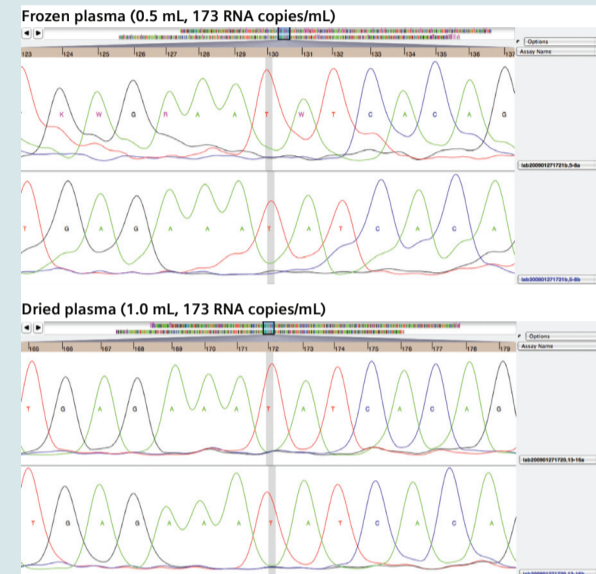


Figure 4. Improvement in sequence quality with the use of the SampleTanker dried plasma preparation was noted at very low input concentration (173 RNA copies/mL). Although increased input volume may contribute to this effect (1 mL dried vs. 0.5 mL frozen), the quality of the dried plasma sequence was comparable to frozen plasma several orders of magnitude higher in concentration. The filtering action or affinity-based capture of interfering substances by the porous capsule used for absorption of plasma before drying is proposed as a possible mechanism.

Conclusions

Universal sample-preparation protocols for plasma and blood were developed on the VERSANT kPCR SP (Sample Preparation) platform. These methods were developed for use with the VERSANT kPCR Molecular System and the OpenGene DNA Sequencing System.

Compatibility with the TRUGENE HIV-1 Integrase Assay was evaluated. No significant differences in average accuracy were observed between plasma, dried plasma, and DBS sample types. Lower sequencing peak intensities were noted for lower-concentration DBS samples. These were attributed to lower input copies of RNA present in the 50 µL spot volume. Larger spot size or processing of multiple spots per genotyping reaction could be attempted to increase sensitivity. Unexpectedly high DNA sequencing quality was noted at the lowest concentration tested with the dried plasma preparation method utilizing the SampleTanker kit.

Additional studies will be performed to evaluate performance of these methods with paired HIV-positive plasma and blood from patients.

References

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2. J Antimicrob Chemother. 2008 Jun;61(6):1217-20.
3. J Clin Virol. 2008 Apr;41(4):283-7.
4. Antiviral Therapy. 2008 Jun;13 Suppl 3:A126.

The VERSANT kPCR Molecular System is CE marked; not commercially available in the US. The TRUGENE HIV-1 Integrase Genotyping Assay is currently in development.

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