Measurement of HCV Viral Load and Viral Genotype from Dried Blood Spots (DBS) and a Dried Ambient Transport Matrix (ViveST) Using the Abbott *m*2000 System

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Introduction

The use of specimen types other than plasma for HCV diagnostic assays may eliminate logistical limitations and improve clinical management of patients on anti-viral therapy in resource-limited settings. This study evaluates the performance of the RealTi*m*e HCV viral load (RTVL) and Abbott RealTi*m*e HCV Genotype II (RTGTII) assays with DBS using alternate extraction protocols available with the Abbott *m*2000 system. This study also compares the performance of ViveST, a dried ambient transport matrix, to plasma for viral load determination with the RTVL assay.

Methods

Samples

- Citrated whole blood from ten HCV positive patients was used to prepare DBS on Whatman 903 paper and Nitrocellulose for evaluation of RTVL and RTGTII assays. For each patient sample 50ul and 75ul card was generated.
- Nineteen HCV positive plasma samples were aliquoted into 4, 1mL volumes. Two aliquots were stored at -80°C and two aliquots were loaded and dried onto ViveST devices overnight. The ViveST samples were reconstituted with molecular grade water. The performance of the ViveST sample collection device for RTVL and RTGTII was evaluated.

Sample Extraction DBS

- Parameters evaluated during protocol optimization included sample type, volume, number of DBS per test, extraction chemistry and extraction protocols.
- Protocols was evaluated with either 1 or 2 spots of 50 and 75ul DBS. 1.5 mL of bulk lysis buffer (Abbott Molecular, Inc) per sample was used to release the sample from DBS. The samples were incubated 45min, the volume from the spots removed by pressing against the side of the tube and the residual volume was transferred to reaction vessel for processing on m2000sp.

Sample Extraction DBS cont

- Suitability of DBS for the RTGTII was assessed using the standard CE marked m2000 protocol.
- RTVL calibrators were processed with initial run to generate a standard curve.
- RTVL controls were included on each run to demonstrate run validity.

Sample Extraction ViveST

- HCV viral load testing was performed on one frozen plasma aliquot and one ViveST recovered aliquot using RTVL assay.
- HCV Genotyping was performed on one frozen plasma aliquot and one ViveST recovered aliquot using RTGTII

Amplification and Detection

- Standard RTVL amplification and detection protocol was used.

- **Analytical Sensitivity (Limit of Detection, LoD**) DBS
  - Replicates of HCV DBS were prepared in HCV-seronegative whole blood at the following concentrations: 1000 IU/ml, 750 IU/ml, 500 IU/ml and 250 IU/ml.

Results

For RTVL, 75 µl Whatman DBS and 50ul Nitrocellulose collection filter (Centro De Gonomas), using the 1mL total nucleic acid (m2000-RNADNA-Plasma-LL-1000-70-v100110) extraction procedure gave the closest correlation to results obtained with plasma (Figure 1). The Whatman DBS mean bias (plasma – DBS) was 0.41 log IU/mL while Nitrocellulose mean bias was 0.2 log IU/mL. Whatman DBS replicates at the lowest viral load tested (250 IU/ml, n=10) were positive (Table 1). HCV viral genotype from DBS was demonstrated using the standard protocols and gave expected results (Table 2). Good correlation was observed between the plasma RTVL and ViveST using the standard protocol with a mean bias (plasma- ViveST) of 0.32 log IU/mL (Table 3, Figure 2). RTGTII results demonstrated 100% concordance (Table 4) between ViveST and frozen plasma with tested genotypes (1,1a,1b, 2, and 3).

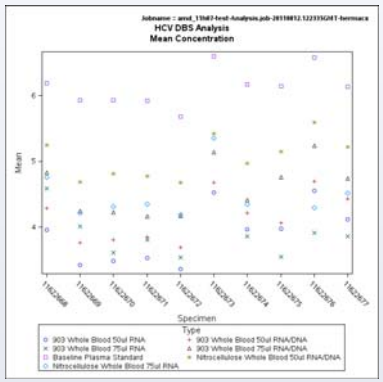

Figure 1. Sample type, volume, number of DBS per test , extraction chemistry and extraction protocols.

	Expected (IU/mL)	(2x75ul) Whatman Detected %
Level 1	7000	100
Level 2	3000	100
Level 3	1000	100
Level 4	500	90
Level 5	250	100

Table 1 Whatman 903 LOD

Type	HCV GT II	HCV GT II	HCV GT II	HCV GT II
Sample	WB 1x75ul	NC 1x75ul	Plasma sample	WB 1x75ul
Extraction	1ml RNADNA	1ml RNADNA	HCV RUO GT	HCV RUO GT
11622668	1a	1a	1a	1a
11622669	1a	1a	1a	1a
11622670	1a	1a	1a	1a
11622671	1a	1a	1a	1a
11622672	1a	1a	1a	1a
11622673	1a	1a	1a	1a
11622674	1a	1a	1a	1a
11622675	1a	1a	1a	1a
11622676	3	NA	3	3
11622677	1a	1a	1a	1a

Table 2 HCV GT II results compared to plasma samples using the standard RTGTII kit.

Results of Comparative Analysis using Abbott's RealTime HCV Testing (Fresh Plasma versus ViveST)	
Fresh Plasma, Mean Viral Load LOG IU/mL (n = 19)	4.64
ViveST, Mean Viral Load LOG IU/mL (n = 19)	4.32
Mean Difference, LOG IU/mL (Fresh vs ViveST)	-0.32
Std Dev, LOG IU/mL	0.15
R^2	0.97

Table 3 **Results of Comparative Analysis using Abbott's RealTime HCV Testing (Fresh Plasma versus ViveST)**

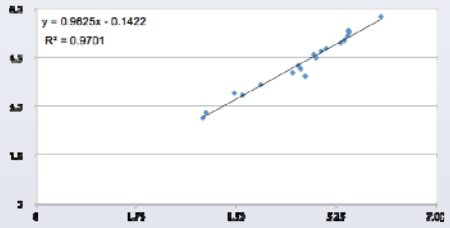

Figure 2. Sample Correlation, HCV Viral Load: Fresh Plasma versus Samples Processed thru ViveST.

Results of Comparative Analysis Using Abbott's RealTime HCV Genotype II RUO Assay		
Sample ID	**Fresh Plasma**	**Processed Through ViveST**
b1169-1	2	2
b1170-2	1, 1a	1, 1a
b1171-3	1, 1a	1, 1a
b1172-4	1, 1a	1, 1a
b1173-5	1, 1a	1, 1a
b1174-6	1, 1a	1, 1a
b1175-7	1, 1a	1, 1a
b1176-8	1, 1a	1, 1a
b1177-9	1, 1a	1, 1a
b1169-10	2	2
b1179-11	3	3
b1180-12	1, 1a	1, 1a
b1184-13	3	3
b1178-14	3	3
b1172-15	1, 1a	1, 1a
b1185-16	1, 1a	1, 1a
b1186-17	1, 1b	1, 1b
b1172-18	1, 1a	1, 1a
b1187-19	1	1

Table 4 HCV genotyping results between plasma recovered from ViveST compared to frozen plasma with HCV genotypes 1, 1a, 1b, 2 and 3.

Conclusion

DBS and ViveST device are suitable specimen type for RTVL and RTGTII on the *m*2000 platform. Future studies of the clinical utility of these protocols are warranted.