A Novel Ambient Storage and Transport Device for Utilization in Infectious Disease Testing: ViveST™

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Anita M. McClernon[1], Andrew B. Freeman[1], Richard J. Carroll[2] and Daniel R. McClernon[1]
[1]bioMONTR® Labs, Research Triangle Park, NC, [2]ViveBio LLC, Alpharetta, GA

Introduction

HCV viral load and HIV-1 drug resistance monitoring are key tools for accessing response to antiviral therapy and are critical tests for patient management. Transportation of frozen plasma has tremendous logistic and cost limitations which limit global access to these tests. Herein we describe the performance of a transformational ambient storage and transport device, ViveST™, for use with viral load and drug resistance assays.

Methods

- For proof of concept, plasma from an HCV infected patient (genotype 1a, IL23B genotype CT) was analyzed prospectively prior to and during therapy (PEG/Interferon, Ribavirin and Telaprevir). Baseline, week 2, week 4, week 12, and week 24 time points were analyzed using Roche COBAS TaqMan assay (frozen plasma only) and Abbott RealTime HCV assay (frozen and plasma processed ViveST).

- To assess ViveST performance for HCV viral load testing, HCV infectious plasma (1 mL) was loaded onto ViveST, dried and stored at ambient temperature. Samples were recovered with 1 mL recovery buffer and analyzed using the Abbott RealTime HCV Assay (Abbott Molecular, Des Plaines, IL). For inter- and intra- assay precision, specimens with varying viral loads (low, mid, high) were analyzed in triplicate on 3 separate runs (n = 27 total). To assess analytical measurement range, a high titer sample was diluted (7 levels) and each level was tested in triplicate (n = 21 total). Four levels (n=23 each) HCV infectious plasma were tested to determine the Limit of Detection.

- Comparative HIV-1 genotypic analysis was performed on duplicate 1mL aliquots of ten (10) paired HIV-1 plasma samples (frozen vs. processed through ViveST) with viral loads ranging from 3.58 to 5.17 LOG c/mL. To assess reproducibility, of the ten paired samples, replicates (neat, 1:2, and 1:4 dilutions) of two samples and replicates (neat and 1:4 dilution) of one sample were analyzed. Frozen plasma pairs were extracted via ETOH (manual extraction per ViroSeq FDA approved package insert). Fresh plasma pairs were loaded onto ViveST, dried and stored at ambient temperature. Samples were recovered with 1 mL recovery buffer and extracted via paramagnetic silica particles using NucliSENS® easyMAG® platform (bioMérieux, Inc., Durham, NC). All extracted RNA was analyzed using the FDA approved ViroSeq HIV-1 Genotyping System v2.0 (Abbott Molecular, Des Plaines, IL). Sequence analysis was performed using an ABI 3100 Genetic Analyzer. HIV-1 sequence concordance was analyzed via bioMONTR's proprietary bioConT sequence analysis tool.

Results

Frozen and ViveST processed plasma demonstrated similar >6 LOG reduction in HCV viral load from baseline to week 12. Patient stopped therapy at week 20 due to psychological factors. Subsequent viral load rebound was detected at Week 24 with frozen plasma and ViveST processed plasma (Roche and Abbott RT). Results are provided in Figure 1.

When a nominal concentration of 37.5 IU/mL (1.57 LOG IU/mL) of HCV infectious plasma was loaded on ViveST, stored for 7 days and analyzed, 91% of the samples (21 of 23) were detected using the Abbott RealTime HCV Assay. For the recovered samples, the average calculated viral load was 5 IU/mL (0.61 LOG IU/mL). The range was 1 IU/mL – 10 IU/mL (0.14 - 1.00 LOG IU/mL). Two of the recovered samples were not detected (See Table 1).

Precision results are summarized in Table 2. HCV Infectious samples processed through ViveST yield reproducible results with a standard deviation of <0.10 LOG IU/mL (intra-assay) and <0.07 LOG IU/mL (inter-assay). The 95% CI were <±0.11 (intra-assay) and <±0.04 (inter-assay).

Testing diluted samples from 1.3 to 6.6 LOG IU/mL demonstrated a direct proportional relationship between the dilution factor and number of HCV copies reported (R^2 = >0.99). See Figure 2.

Figure 1 Prospective Analysis of HCV Patient During Therapy using Roche COBAS TaqMan (frozen plasma) and Abbott RealTime HCV (frozen plasma and plasma processed through ViveST. Note: Log 0=Target Not Detected

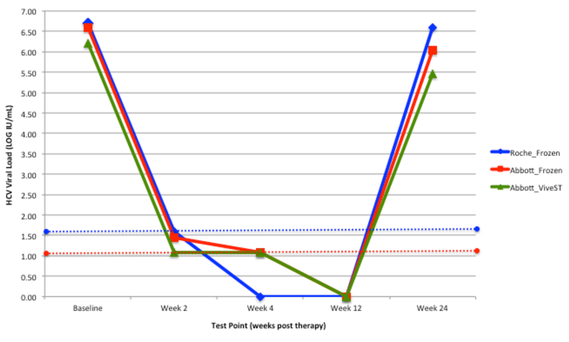


Table 1 Abbott RealTime HCV Limit of Detection (LOD) for Plasma Processed though ViveST Devices

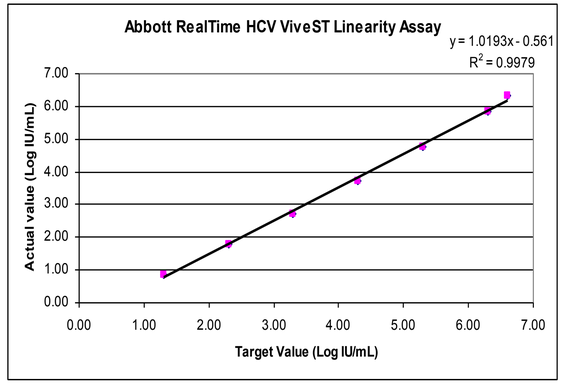
Target Viral Load (IU/mL)	Number tested	Number Detected	Percent Detected (%)	Calculated Mean Viral Load (IU/mL)
300	23	23	100%	40
150	23	23	100%	13
75	23	23	100%	12
37.5	23	21	91%	5

Results (cont'd)

Table 2 ViveST_Abbott RealTime HCV: Intra-assay and Inter-assay Precision

	Intra-assay precision									Inter-assay precision		
Concentration:	Low			Medium			High			Low	Medium	High
Timepoint (Day)	1	2	3	1	2	3	1	2	3			
Replicates (n)	3	3	3	3	3	3	3	3	3	9	9	9
Mean	2.21	2.18	2.22	3.71	3.63	3.63	5.16	5.03	5.10	2.20	3.66	5.10
Standard Deviation	0.08	0.03	0.10	0.04	0.05	0.02	0.01	0.05	0.02	0.07	0.05	0.06
95% Confidence Interval	0.03	0.03	0.11	0.01	0.06	0.02	0.00	0.06	0.02	0.04	0.03	0.04

Figure 2 ViveST_Abbott RealTime HCV Assay: Analytical Measurement Range



HIV-1 drug resistance mutations demonstrated 100% concordance for 10/10 pairs between frozen plasma and ViveST processed plasma samples. Per bioMONTR bioConT sequence analysis tool, there was >99% concordance at the nucleotide level comparing ViveST versus frozen plasma for Protease and Reverse Transcriptase regions (See Table 3). Sequence quality from ViveST processed plasma was comparable to that obtained from frozen plasma (See Figure 3).

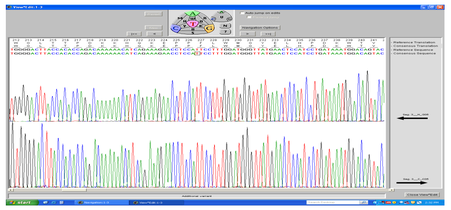
Table 3 ViroSeq HIV-1 Genotyping System v2.0: Comparison of Frozen Plasma versus ViveST Processed Plasma

Sample Information					Viral Load		NanoDrop Values	Drug Resistance Mutations			Concordance based on Drug Resistance Mutations	Concordance at the Nucleotide Level
Sample	Level	Replicate	Assay	Dilution Factor	c/mL	LOG c/mL	purified PCR product (ng/ul)	NRTI	NNRTI	PI		
1	1	1	ETOH ViroSeq	1	148,140	5.17	19	No Mutations Identified			100%	99.92%
		4	VivST_easyMAG				14.1	No Mutations Identified				
	2	1	ETOH ViroSeq	1:2	74,080	4.87	32.1	No Mutations Identified			100%	100.00%
		2	ViveST_easyMAG				12.7	No Mutations Identified				
	3	1	ETOH ViroSeq	1:4	37,040	4.57	24.3	No Mutations Identified			100%	99.92%
		2	ViveST_easyMAG				10	No Mutations Identified				
2	1	1	ETOH ViroSeq	1	134,424	5.13	18.3	No Mutations Identified			100%	99.16%
		2	ViveST_easyMAG				1.7	No Mutations Identified				
	2	1	ETOH ViroSeq	1:2	67,212	4.83	48.8	No Mutations Identified			100%	99.62%
		2	ViveST_easyMAG				9.1	No Mutations Identified				
	3	1	ETOH ViroSeq	1:4	33,606	4.53	18.5	No Mutations Identified			100%	99.54%
		2	ViveST_easyMAG				7.3	No Mutations Identified				
3	1	1	ETOH ViroSeq	1	15,176	4.18	11.6	M41L, E44D, D67N, L74I, L74V, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M	100%	99.46%
		2	ViveST_easyMAG				10.9	M41L, E44D, D67N, L74I, L74V, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M		
	3	1	ETOH ViroSeq	1:4	3,794	3.58	15.7	M41L, E44D, D67N, L74I, L74V, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M	100%	99.23%
		2	ViveST_easyMAG				7.8	M41L, E44D, D67N, L74I, L74V, V118I, M184V, L210W, T215Y, K219N	V108I, Y181I	L10I, V32I, M46I, F53L, I54V, Q58E, A71V, V82A, L90M		
4	2	1	ETOH ViroSeq	1:2	28,400	4.45	4.1	M41L, T215Y			100%	99.00%
		2	ViveST_easyMAG				4.1	M41L, T215Y				
5	1	1	ETOH ViroSeq	1	24,336	4.39	7.1	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M	100%	99.54%
		2	ViveST_easyMAG				14.8	M41L, T69N, K70R, M184V, L210W, T215F, K219E	K103N, V108I, Y181C	L10F, V11I, K43T, I54V, A71V, V82A, I84V, L90M		

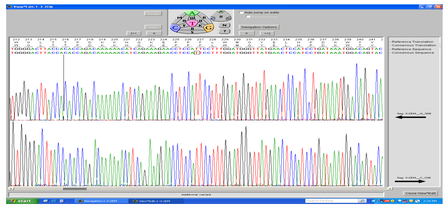
Results (cont'd)

Figure 3 ViroSeq HIV-1 electropherograms of (A) frozen plasma and (B) ViveST processed plasma.

A



B



Conclusions

- ViveST sample transportation and storage device demonstrates utility for transporting plasma obtained from HCV positive samples for Abbott RealTime HCV Assay.
- Plasma samples recovered from ViveST yielded reproducible results with a standard deviation of <0.10 LOG IU/mL (intra-assay) and <0.07 LOG IU/mL (inter-assay). The 95% CI were <±0.11 (intra-assay) and <±0.04 (inter-assay)
- When stored on ViveST, 91% of samples (21 of 23) with a viral load of 37.5 IU/mL were detected using the Abbott RealTime HCV Assay.
- HCV patient specimens processed through ViveST and tested produced viral load profiles similar to frozen plasma.
- Plasma samples stored on ViveST yielded equivalent genotypic data as compared to frozen plasma; confirming ViveST utility for transporting plasma obtained from HIV-1 positive individual for HIV-1 resistance testing.
- ViveST has great potential to offer a global solution for infectious disease testing and reduce costs in both developed and developing countries.

Send correspondence to:
Daniel McClernon
dmcclernon@biomontr.com

bioMONTR® Labs