ViveST™ FOR HBV TESTING: A TRANSFORMATIONAL AMBIENT STORAGE AND TRANSPORTATION DEVICE

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HEP DART™ 2013

Introduction

With the advent of direct-acting antivirals for HBV treatment, HBV viral load testing will increase dramatically. Monitoring HBV usually requires frozen plasma to be shipped to central laboratories requiring dry ice and special packaging which is expensive and laborious.

In this study, we evaluated the performance of the Abbott RealTi*me* HBV assay (Abbott Molecular Inc., Des Plaines, IL) when using HBV infectious plasma stored on ViveST™, a transformational dried sample storage and transportation device.

Methods

- For all testing described below, HBV infectious plasma (1.0 mL) was loaded onto ViveST (ViveBio LLC, Alpharetta, GA), dried, capped and stored at ambient temperature. Samples were recovered with 1.0 mL molecular grade water and analyzed using the Abbott RealTi*me* HBV assay (0.5 mL protocol).
- To assess inter-assay and intra-assay precision, specimens with varying viral loads (low, mid, high) were analyzed in triplicate on 3 separate runs (n = 27) after storage for 1, 4 and 7 days (RT).
- To assess linearity, a high titer HBV infectious plasma sample was diluted in normal human plasma (7 levels). Each level was loaded onto ViveST in triplicate, stored 7 days (RT), recovered and tested on a single run (n = 21). For comparison purposes, identical aliquots of frozen plasma were thawed and analyzed simultaneously.
- To demonstrate accuracy of the ViveST device, 1 mL aliquots of HBV infectious clinical samples (N = 10) were loaded onto ViveST, dried overnight, capped and stored at ambient temperature for 7 days. Samples were reconstituted from ViveST with 1 mL molecular grade water and analyzed simultaneously with identical 1 mL aliquots of each sample that were stored frozen (7 days @-80ºC) using the Abbott RealTime HBV assay.
- To evaluate stability, HBV infectious plasma samples with varying viral load values (3 levels, 3 replicates each) were analyzed after being stored (RT) on ViveST for 1, 4, 7, 14, 30 and 60 days. As a control, identical aliquots were frozen (3 levels, 3 replicates each), thawed, and analyzed on Day 1 with ViveST recovered samples (N=9 frozen).
- For determination of the LOD/LOQ, HBV infectious plasma was diluted in normal human plasma to yield dilutions of approximately 1.5 to 50 IU/mL. To confirm the HBV DNA concentration, the diluted samples were analyzed and linear regression analysis was performed. 15 replicates of each concentration were then loaded onto ViveST and stored for 7 days (RT). After recovery, samples were tested using a single lot of extraction and amplification reagents. Probit analysis was performed to determine the 95% hit rate using Percent Detected (PD) values at each dilution. Excel 2011 (MAC) function NORMSINV(z) was used to translate PD values to probit values.

Results

Precision results are summarized in Table 1. HBV infectious samples processed through ViveST yielded reproducibility results with a standard deviation <0.13 LOG IU/mL (intra-assay) and <0.10 LOG IU/mL (inter-assay). The 95%CI were <±0.14 (intra-assay) and <±0.06 (inter-assay).

Analysis of diluted samples from ~1 to ~7 LOG IU/mL demonstrated a direct proportional relationship between the dilution factor and the number of HBV copies reported with R^2>0.99 (see Figure 1).

Regression analysis (see Figure 2) on the results of the accuracy study yielded R^2>0.99 and a Pearson Correlation Coefficient (R) >0.99. The average difference between the results in this 10 sample correlation study was 0.10 LOG IU/mL. 100% of the samples provided results within ±0.27 LOG (data not shown).

Table 1. ViveST_Abbott RealTime HBV Precision

ViveST_Abbott RealTime HBV_Intra-assay and Inter-assay Precision												
	Intra-Assay Precision									Inter-Assay Precision		
Concentration	Low			Mid			High			Low	Mid	High
Run #	1	2	3	1	2	3	1	2	3			
Days Stored	1	3	7	1	3	7	1	3	7			
Replicates	3	3	3	3	3	3	3	3	3	9	9	9
Mean (LOG IU/mL)	3.61	3.57	3.66	4.74	4.70	4.83	5.81	5.82	5.84	3.61	4.76	5.82
Std Dev	0.04	0.05	0.06	0.02	0.13	0.10	0.02	0.04	0.03	0.06	0.10	0.03
95% CI	0.04	0.05	0.07	0.02	0.14	0.11	0.03	0.04	0.03	0.04	0.06	0.02

Figure 1. ViveST_Abbott RealTime HBV Analytical Measurement Range

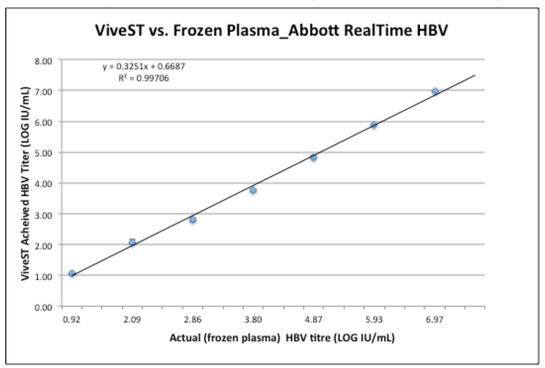
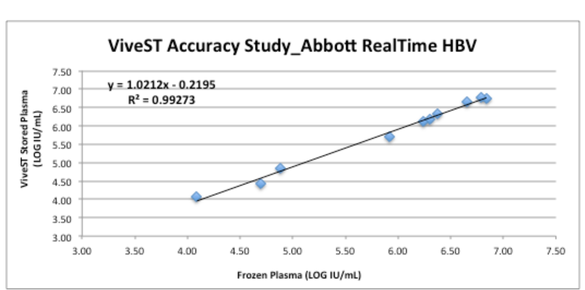


Figure 2. Regression Analysis: ViveST versus Frozen Plasma



Results (cont'd)

For HBV infectious plasma stored on ViveST over a 60 day period (RT) there was no reduction in viral load when compared to frozen plasma (See Table 2 and Figure 3). The Standard Deviation across all levels/all test points ranged from 0.02 to 0.13 (data not shown). A linear fit (R^2>0.99) was retained over the course of the 60 day study as indicated by linear regression analysis across all test points (See Figure 3).

Table 2. Results Summary for the ViveST HBV 60 Day Stability Study

Level	Target HBV titre (LOG IU/mL)	Frozen (LOG IU/mL)	Mean Results (LOG IU/mL): Ambient Storage (Days)					
			1	4	7	14	30	60
1	5.97	5.81	5.81	5.82	5.84	5.91	5.83	5.83
2	4.97	4.71	4.74	4.70	4.78	4.83	4.71	4.77
3	3.97	3.71	3.61	3.57	3.66	3.74	3.71	3.70

Figure 3. Comparison of Target vs Actual titres_ViveST 60 Day Stability Study

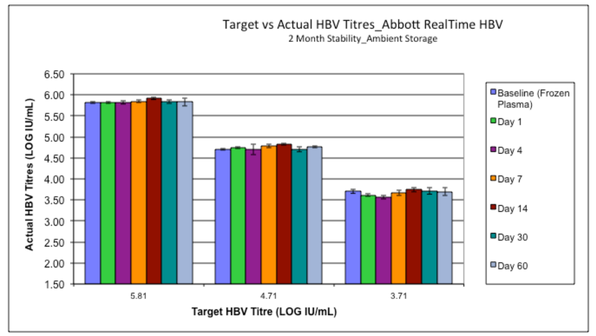
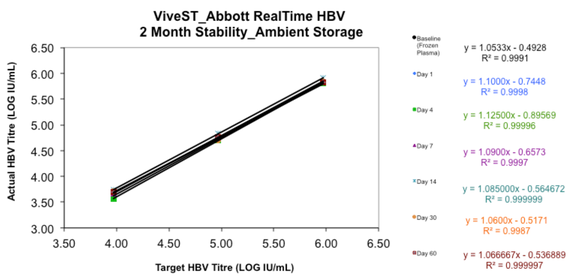


Figure 4. Linear Regression Analysis of ViveST HBV 60 Day Stability Study



For the LOD/LOQ study, 14 of 15 samples (93%) with an estimated viral load of 6 IU/mL were detected and yielded a manually calculated mean viral load of 4 IU/mL. All samples (15/15) with an estimated viral load of 7 IU/mL were detected and yielded a manually calculated mean viral load of 6 IU/mL (see Table 3).

Probit analysis (see Figure 5) revealed that the concentration of HBV DNA quantitated after 7 days with 95% probability was 13 IU/mL (1.10 LOG IU/mL).

Results (cont'd)

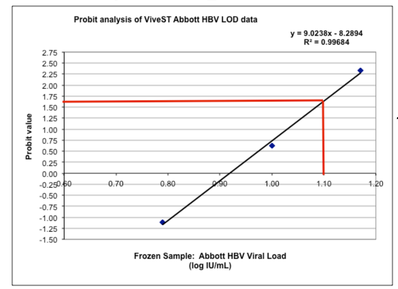
Table 3. Summary of ViveST Abbott RealTime HBV Assay LOD/LOQ Data

Level	Frozen Sample Viral Load (LOG IU/mL)	Number tested	Number Detected	Percent Detected (%)	Mean Viral Load (LOG IU/mL)
6	0.67	15	14	93%	0.62*
5	0.79	15	15	100%	0.71*
4	0.99	15	15	100%	1.06*
3	1.17	15	15	100%	1.42
2	1.68	15	15	100%	1.72
1	1.85	15	15	100%	1.97

Level	Frozen Sample Viral Load (IU/mL)	Number tested	Number Detected	Percent Detected (%)	Mean Viral Load (IU/mL)
6	6	15	14	93%	4*
5	7	15	15	100%	6*
4	11	15	15	100%	12*
3	19	15	15	100%	27
2	49	15	15	100%	54
1	70	15	15	100%	95

* = Results reported as <1.00 LOG IU/mL (<10 IU/mL) by the Abbott RealTime HBV data analysis software were manually calculated using a stored HBV calibration curve.

Figure 5. Probit Analysis: ViveST_Abbott HBV RealTime



95% Probit (Hit Rate)
1.10 LOG IU/mL (13 IU/mL)

Conclusions

- This study demonstrates the utility of ViveST for transportation and storage of HBV infectious plasma.
- These data demonstrate the excellent sample stability and recovery achieved with ViveST, enabling levels of precision to be obtained with commercially available HBV viral load assays comparable to those obtained with frozen plasma after 60 days storage at ambient temperatures.
- The use of ViveST offers a global solution to sample transportation for diagnostics enhancing access to healthcare while significantly reducing the burden associated with shipping frozen samples.

Acknowledgements

The authors would like to thank ViveBio, LLC for their support during this evaluation study.

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