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Evaluation of ViveST™ Using the COBAS TaqMan HCV Test v2.0

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Introduction

With the advent of direct-acting antivirals for HCV treatment, HCV viral load testing will increase dramatically. Monitoring HCV usually requires frozen plasma to be shipped to central laboratories requiring dry ice and special packaging which is expensive and laborious. Herein we evaluated the performance of COBAS® TaqMan® HCV Test, v2.0 when using HCV infected samples stored and processed on ViveST™ a novel, second generation, dried sample collection and transportation device.

Method

- For all testing described below, HCV infectious plasma (0.8 mL) was loaded onto ViveST (ViveBio LLC, Alpharetta, GA), dried and stored at ambient temperature (RT). Samples were recovered with 0.8 mL recovery buffer and a 0.5 mL aliquot was analyzed using the COBAS® TaqMan® HCV Test, v2.0 for use with the High Pure System (Roche Diagnostics, Indianapolis, IN).
- To assess stability as well as inter- and intra-assay precision, HCV infectious samples with varying viral loads (3 levels) were analyzed in triplicate on 3 separate runs after storage (RT) on ViveST for 1, 3 and 7 days (n = 27).
- To assess linearity, a high titer HCV infectious plasma sample was diluted in HCV negative human plasma (7 levels). Each level was loaded onto ViveST in triplicate, stored for 7 days (RT), recovered and tested on a single run (n = 21).
- For determination of the Limit of Quantitation (LOQ), dilutions of an HCV infectious plasma were made in normal human plasma yielding dilutions of approximately 40 to 440 IU/mL. 20 replicates of each concentration were 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. HCV infectious samples processed through ViveST yielded reproducible results with a standard deviation of <0.15 LOG IU/mL (intra-assay) and <0.11 LOG IU/mL (inter-assay). The 95%CI were < ±0.17 (intra-assay) and < ±0.07 (inter-assay).

For HCV infectious plasma stored on ViveST at RT for a 7 day period, the maximum recorded reduction in viral load when compared to frozen plasma was 0.57 LOG IU/mL (See Table 2). The Standard Deviation across all levels/all test points ranged from 0.01 to 0.15 (data not shown). A linear fit (R^2 >0.97) was retained over the course of the 7 day study as indicated by linear regression analysis across all test points (Figure 1).

Testing diluted samples from 3.92 to 6.08 LOG IU/mL demonstrated a direct proportional relationship between the dilution factor and the number of HCV copies reported (R^2 = >0.99). (See Figure 2).

LOQ was determined by testing replicates of dilutions of HCV infectious plasma ranging from 40 to 440 IU/mL. Probit analysis (See Figure 3) revealed that the concentration of HCV RNA quantitated after 7 days with 95% probability was 161 IU/mL (2.21 LOG IU/mL) and with 70% probability was 96 IU/mL (1.98 LOG IU/mL).

Table 1. ViveST_Roche HCV Intra-assay and Inter-assay Precision

ViveST_Roche HCV Intra-assay and Inter-assay Precision												
	Intra-Assay Precision									Inter-Assay Precision		
Concentration	Low			Medium			High					
Run #	1	2	3	1	2	3	1	2	3	Low	Medium	High
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	3.65	3.59	3.48	4.17	4.22	4.06	4.48	4.49	4.38	3.57	4.15	4.45
Standard Deviation	0.15	0.04	0.07	0.13	0.04	0.07	0.01	0.03	0.09	0.11	0.11	0.07
95% Confidence Interval	0.17	0.04	0.08	0.15	0.04	0.08	0.01	0.03	0.10	0.07	0.07	0.04

Table 2. 7 Day Stability (ambient storage): ViveST_Roche HCV TaqMan v 2.0

Target HCV titre (LOG IU/mL)	Mean Results - Ambient Storage (LOG IU/mL)			
	Frozen	1 Day	3 Days	7 Days
4.70	4.94	4.48	4.49	4.38
4.30	4.63	4.17	4.22	4.06
3.78	3.96	3.65	3.59	3.48

Results (cont'd)

Figure 1. 7 Day Stability (ambient storage): ViveST_Roche HCV TaqMan v 2.0

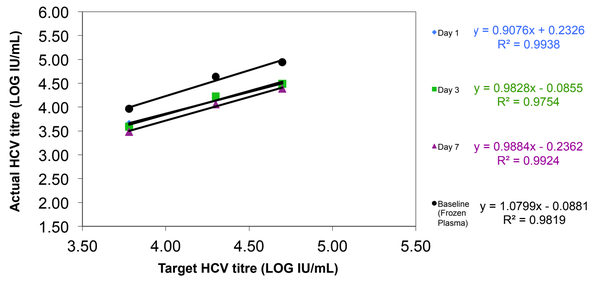


Figure 2. Analytical Measurement Range: ViveST_Roche HCV TaqMan v 2.0

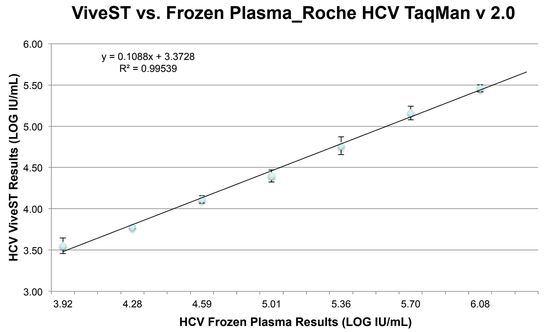
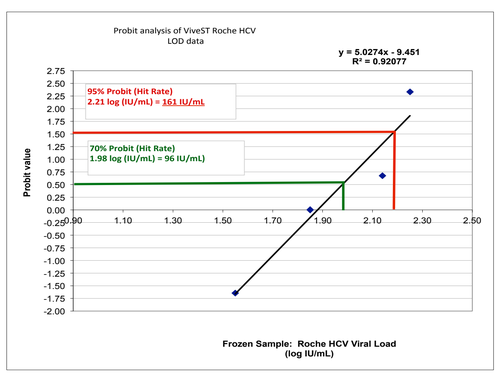


Figure 3. Probit Analysis: ViveST_Roche HCV TaqMan v 2.0



Conclusions

- ViveST sample transportation and storage device demonstrates utility for transporting plasma obtained from HCV infectious samples for viral load testing with the Roche® COBAS TaqMan® v2.0 assay.
- Plasma samples recovered from ViveST yielded reproducible results with a standard deviation of <0.15 LOG IU/mL (intra-assay) and <0.11 LOG IU/mL (inter-assay).
- For plasma samples stored at ambient temperature (7 days), the maximum reduction recorded when compared to frozen plasma was 0.57 LOG IU/mL.
- The concentration of HCV RNA quantitated with 95% probability after 7 days was 161 IU/mL (2.21 LOG IU/mL) and with 70% probability is 96 IU/mL (1.98 LOG IU/mL).
- The use of ViveST can offer a global solution and enhance access to healthcare and significantly reduce the burden associated with shipping frozen samples.

References

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