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Detection of HCV RNA in Cerebrospinal Fluid (CSF) Using the Abbott™ RealTime HCV Assay

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Introduction

Understanding viral kinetics of latent virus residing in specific compartments within its host may be beneficial for monitoring immune compromised patients and development of new DAAs. HCV viral load testing is an invaluable diagnostic tool for accessing response to antiviral therapy. The Abbott RealTime HCV Assay is an in vitro RT-PCR assay intended for use in the management of HCV infected patient treatment and is currently FDA approved for quantitation of HCV in human plasma and serum only. For this study, the Abbott RealTime HCV Assay was assessed for its ability to quantitate HCV RNA in CSF.

Methods

To assess the ability of the Abbott RealTime HCV Assay to accurately quantitate HCV virus in CSF, HCV negative CSF (n=6) was spiked with an Abbott HCV low positive control (~2.65 LOG IU/ml), a high positive control (~6.05 LOG IU/ml) or Abbott HCV calibrator material (2.95 LOG IU/ml or 6.96 LOG IU/ml). For comparison, HCV negative human plasma (n=6) was also spiked with identical concentrations of the Abbott HCV controls and calibrators. The CSF and plasma samples were analyzed simultaneously.

For testing analytical measurement range, an Abbott HCV calibrator with viral concentration of ~ 10,000,000 IU/mL was serially diluted in HCV negative CSF (1:10, 1:100, 1:1,000, 1:10,000, 1:100,000 and 1:1M) and each dilution was tested in duplicate.

The Abbott RealTime HCV Assay was used on paired plasma and CSF from 20 treatment-naïve HCV patients to test the assay ability to detect HCV virus in CSF specimens, and to compare HCV viral loads between plasma and CSF samples of each patient.

HCV genotypes were determined for 16 of the 20 patient plasma samples using the Abbott RealTime HCV Genotyping II RUO Kit.

Results

The Abbott RealTime HCV Assay detected HCV RNA in all spiked CSF and plasma samples. CSF spiked with HCV yielded viral load values with an average difference of 0.21 LOG IU/mL when compared to plasma spiked with identical concentrations of HCV RNA (Table 1).

For analytical measurement range testing, all levels of virus diluted in CSF from ~7 to ~1 LOG IU/ml produced average results within +/- 0.22 log of the calculated concentrations based on dilution (Table 2). Linear regression analysis yielded a R^2 value of 0.9994 (Figure 1).

All paired plasma and CSF samples (n=20) resulted in a valid test with 85% (17/20) plasma samples yielding detectable HCV viremia while only 25% (5/20) of CSF samples had any detectable HCV RNA (Table 3).

Results (cont'd)

Of the 17 plasma samples with detectable virus levels, only 5 (29%) had paired CSF samples with detectable virus levels. There were 3 samples with undetectable virus (TND) in both plasma and CSF (Table 3).

Genotyping was performed on 16 of the 20 patient plasma samples (3 were not analyzed due to low viral loads and one sample had insufficient volume for analysis). Of the 16 samples,, 12 (75%) were genotype 1 [10 were 1a, 1 was 1b, 1 was undetermined], 1 (6%) was genotype 2, and 3 (19%) were genotype 3 (Table 3).

Table 1. HCV Spiked Human CSF and Plasma Samples Analyzed with Abbott's RealTime HCV Assay

HCV+ Material	Target LOG IU/mL	Plasma LOG IU/mL	CSF LOG IU/mL	Difference CSF vs. Plasma LOG IU/mL
+ Control	2.65	2.33	2.56	0.23
++ Control	6.05	5.65	6.49	0.84
Cal A, rep 1	2.95	2.56	2.65	0.09
Cal A, rep 2	2.95	2.62	2.70	0.08
Cal B, rep 1	6.96	6.73	6.71	-0.02
Cal B, rep 2	6.96	6.68	6.70	0.02
			AVERAGE	0.21

Table 2. Viral Load Results for Analytical Range Study of High Positive Control Diluted in CSF

Dilution Factor	Target LOG IU/mL	Rep 1 LOG IU/mL	Rep 2 LOG IU/mL	Average LOG IU/mL	Difference Avg vs. Target LOG IU/mL
Stock	7.03	7.05	7.01	7.03	0.00
1:10	6.03	6.13	6.15	6.14	0.11
1:100	5.03	5.11	5.08	5.10	0.07
1:1,000	4.03	4.06	4.08	4.07	0.04
1:1,0000	3.03	3.07	3.09	3.08	0.05
1:100,000	2.03	2.14	2.18	2.16	0.13
1:1M	1.03	1.23	1.27	1.25	0.22

Results (cont'd)

Figure 1. Abbott RealTime HCV Linearity (HCV RNA diluted in CSF)

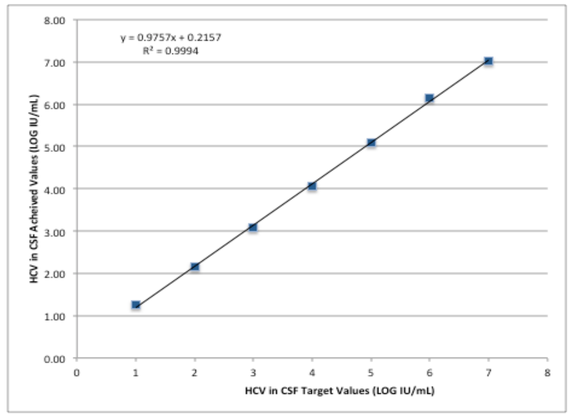

Table 3. Results of HCV Viral Load and Genotype Testing (Plasma Only) on Paired Patient Plasma and CSF

Sample	HCV Genotype (Plasma)	Plasma Viral Load Results Log (IU/mL)	CSF Viral Load Results Log (IU/mL)
1	Not tested	6.08	TND
2	2	4.98	TND
3	1,1a	6.26	<1.08
4	1,1a	5.48	<1.08
5	1,1a	4.78	<1.08
6	1,1a	5.48	TND
7	1,1a	5.31	TND
8	1,1a	6.19	TND
9	1,1a	6.03	TND
10	1,1a	5.73	TND
11	3	6.46	<1.08
12	3	6.76	1.18
13	1,1a	6.25	TND
14	Not tested	TND	TND
15	Not tested	TND	TND
16	Not tested	TND	TND
17	3	5.39	TND
18	1,1a	6.22	TND
19	1,1b	6.37	TND
20	1	6.09	TND

Conclusions

- The Abbott RealTime HCV Assay can be used to accurately detect HCV viral RNA in human cerebrospinal fluid (CSF).
- The Abbott RealTime HCV Assay is capable of detecting HCV virus in CSF over a range of viral loads from ~7 to ~ 1 LOG IU/mL.
- The concentration of HCV virus in plasma (average of 5.87 LOG IU/mL) is much greater than that found in CSF (average <1.08 LOG IU/mL) regardless of genotype.
- Monitoring CSF compartments for HCV RNA may be warranted to fully elucidate HCV viral kinetics and the Abbott HCV RealTime assay can be utilized for accurate CSF HCV RNA quantitation.

References

1. Package Insert for Abbott RealTime HCV (Ref # 1N30-90), Abbott Laboratories, Des Plaines, IL, USA.
2. Package Insert for Abbott RealTime HCV Genotype II RUO (Ref # 8K24-86), Abbott Laboratories, Des Plaines, IL, USA.

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