COMPARISON OF THREE LABORATORY PROTOCOLS FOR THE DETECTION OF HERPES SIMPLEX VIRUS IN LOW VOLUME CEREBROSPINAL FLUID SPECIMENS

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Abstract

Introduction: Detection of Herpes Simplex virus (HSV) 1/2 in cerebrospinal fluid (CSF) by real-time PCR is the gold standard for the diagnosis of HSV encephalitis. While extremely sensitive and specific, most real-time PCR assays require preanalytic extraction of nucleic acid, with the volume of specimen needed for extraction varying between assays. The volume of CSF required for analysis can be problematic, especially in neonates from whom specimen recovery is often limited. Recently, the Food and Drug Administration (FDA) cleared the first real-time PCR assay (Simplexa™ HSV-1/2 Direct, Focus Diagnostics [Cypress, CA]) for the detection and differentiation of HSV-1/2 directly from CSF. The Focus assay does not require preanalytic nucleic acid extraction, and is approved for testing 50 µL of CSF. In this study, we compared the performance of the Focus HSV-1/2 Direct assay to two alternative procedures (heat-treatment and dilution) used for low-volume (<200 µL) CSF specimens.

Methods: CSF specimens (n=150) were submitted for routine HSV-1/2 real-time PCR, which consisted of extraction of 200 µL of sample on the MagNA Pure (Roche Diagnostics) followed by analysis of 5 µL of extract using the Roche HSV-1/2 analyte specific reagents (ASR) on the LightCycler 2.0 (Roche). Following routine testing, samples of sufficient volume were divided into three aliquots; two 50 µL aliquots and one 100 µL aliquot. The first 50 µL aliquot (Process 1) was tested by the Focus HSV-1/2 Direct assay on the 3M Integrated Cycler (Focus) according to manufacturer's instructions. The second 50 µL aliquot (Process 2) was heated to 95^{0}C for five minutes, cooled to room temperature, and 5 µL of the processed specimen tested directly using the Roche ASR reagents on the LightCycler 2.0. The 100 µL aliquot (Process 3) was diluted to 200 µL with PCR-grade water, extracted using the MagNA Pure, and 5 µL subsequently tested using the Roche ASR reagents on the LightCycler 2.0. Results were analyzed by comparing each process to the results obtained by routine testing, which was considered the reference standard for this study.

Results: Following testing, Process 1, 2, and 3 demonstrated a sensitivity of 100% (20/20), 75% (15/20), and 85% (17/20) for HSV-1, respectively, and 100% (59/59), 56% (33/59), and 69% (41/59) for HSV-2, respectively. The percent specificity of Process 1 ranged from 94% to 98% for HSV-1 and HSV-2, respectively, while processes 2 and 3 showed 100% specificity compared to our routine method.

Conclusions: Testing 50 µL of CSF by the Focus HSV-1/2 Direct assay (Process 1) showed superior performance to both heat-treatment (Process 2) and dilution (Process 3) of low-volume (<200 µL) CSF. The Focus HSV-1/2 Direct assay offers a sensitive and specific means of detecting HSV-1/2 directly from 50 µL CSF and provides a rapid (~65 min) turnaround time of results.

Objectives

1. Compare the performance of the Focus HSV-1/2 Direct assay to two alternative procedures (heat-treatment and dilution) used for low-volume (<200 µL) CSF specimens.
2. Determine if heat-treatment or CSF dilution are acceptable for processing and testing of low-volume CSF.

Materials and Methods

Specimens: Cerebrospinal fluid specimens (n=150) submitted for routine real-time PCR for HSV-1/2 were divided into three aliquots; two 50 µL aliquots and one 100 µL aliquot (Figure 1).

Routine testing: 200 µL of CSF was extracted by the MagNA Pure (Roche) and 5 µL subsequently tested using the Roche HSV-1/2 ASR reagents on the LightCycler 2.0 (Figure 1 and 2A).

Process 1: The first 50 µL aliquot was tested by the Focus HSV-1/2 Direct assay on the 3M Integrated Cycler (Focus) according to manufacturer's instructions (Figures 1 and 2B).

Process 2: The second 50 µL aliquot was heated to 95^{0}C for five minutes, cooled to room temperature, and 5 µL of the processed specimen tested directly using the Roche HSV-1/2 ASR reagents on the LightCycler 2.0 (Figures 1 and 2A).

Process 3: The 100 µL aliquot was diluted to 200 µL with PCR-grade water, extracted using the MagNA Pure, and 5 µL subsequently tested using the Roche HSV-1/2 ASR reagents on the LightCycler 2.0 (Figures 1 and 2A).

Figure 1. Study Design

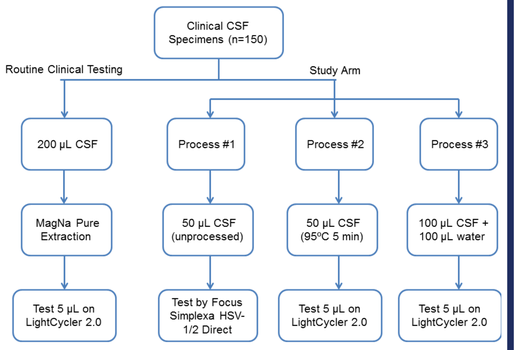

Figure 2. PCR platforms

A. Roche LightCycler 2.0

A. Focus Integrated Cycler

Table 1: Comparison of 3 Processes to the Gold Standard for the Detection of HSV-1

	Routine Testing (Gold Standard)			
HSV 1	Positive	Negative	% Sensitivity (95% CI)	% Specificity (95% CI)
Process 1				
Positive	20	4	100(81-100)	94(85.4-98.1)
Negative	0	64		
Process 2				
Positive	15	0	75(52.8-89.2)	100(93.6-100)
Negative	5	68		
Process 3				
Positive	17	0	85(63.1-95.6)	100(93.6-100)
Negative	3	68		

Table 2: Comparison of 3 Processes to the Gold Standard for the Detection of HSV-2

	Routine Testing (Gold Standard)			
HSV 2	Positive	Negative	% Sensitivity (95% CI)	% Specificity (95% CI)
Process 1				
Positive	59	1	100(92.7-100)	98(91.4-100)
Negative	0	67		
Process 2				
Positive	33	0	56(43.3-67.9)	100(93.6-100)
Negative	26	68		
Process 3				
Positive	41	0	69(56.8-79.8)	100(93.6-100
Negative	18	68		

Conclusions

- Testing 50 µL of CSF by the Focus HSV-1/2 Direct assay (Process 1) showed superior performance to both heat-treatment (Process 2) and dilution (Process 3) of low-volume (<200 µL) CSF.
- The Focus HSV-1/2 Direct assay offers a sensitive and specific means of detecting HSV-1/2 directly from 50 µL CSF and provides a rapid (~65 min) turnaround time of results.

References

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