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Evaluation of a New Alpha herpes virus Assay for Detection of HSV 1, 2 and VZV in CSF and Lesion Scrapings

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BACKGROUND & OBJECTIVES

Nucleic acid amplification testing has greatly facilitated the detection of herpes simplex viruses (HSV 1, 2) and varicella zoster virus (VZV) in cerebrospinal fluid (CSF). PCR methods vary by laboratory and there is little standardization. Real-time PCR assays are replacing conventional PCRs as they are less prone to contamination and do not require gel electrophoresis for detection. The goal of this study was to evaluate a new multiplex real time PCR assay that detects and identifies HSV 1 and 2 and VZV (altona Diagnostics, Hamburg, Germany) in csf and lesion scrapings.

METHODS

Spiking of CSF and shunt fluid: Pooled CSF and shunt fluids were prepared and spiked with ten-fold dilutions (10^{-3} to 10^{-7} for CSF and 10^{-5} to 10^{-9} for shunt fluid) of culture lysates of HSV 1, HSV 2, and VZV in order to assess the limit of detection of the conventional and real-time PCR assays.
Nucleic acid extraction: Nucleic acid was extracted from 450 µl of spiked viral suspension using the easyMag extractor (bioMérieux, Saint Laurent, QC, Canada) and eluted in 100 µl of elution buffer.

Each extracted nucleic acid sample was tested in triplicate with a conventional PCR assay and the commercial real-time RealStar® alpha herpes (altona Diagnostics (Hamburg, Germany).

Conventional PCRs: 10 µL of extracted nucleic acid was mixed with 40 µL of master mix containing the following primers:
HSV1/2 Master mix:
Forward primer (26 mer): 5'-GTG GTG GAC TTT GCC AGC CTG TAC CC-3'
Reverse primer (30 mer): 5'-TAA ACA TGG AGT CCG TGT CGC CGT AGA TGA-3'
The amplification parameters were as follows:
95°C 10 min
54°C 45 sec } 1 cycle
72°C 1 min
95°C 45 sec
54°C 45 sec } 40 cycles
72°C 1 min
VZV Master Mix:
Forward primer (29 mer): 5'-GTC GTC TTT GAT TTT CAA AGT TTA TAT CC-3'
Reverse primer (34 mer): 5'-ATA AAC ACA CAA TCC GTA TCA CCA TAA ATA ACC T-3'
The amplification parameters were as follows:
95°C 10 min
95°C 1 min
47°C 1 min } 40 cycles
72°C 1 min
72°C 3 min

PCR products were detected and visualized by ethidium bromide containing agarose gel.

***Real*Star®*alpha*Herpesvirus PCR kit:** a real-time based multiplex assay for the simultaneous detection of HSV-1, HSV-2 and VZV (Altona Diagnostics, Hamburg Germany) and an Internal Control provided with the assay. The purpose of IC was to rule out PCR inhibition. The probes were labeled with the following fluorophores:
- HSV-1 specific probe: ROX
- HSV-2 specific probe: Cy5
- VZV specific probe: FAM
- IC specific probe: JOE

10 µL of extracted nucleic acid was mixed with 20 µL of master mix (Master A + Master B) and the amplification was performed on a Rotor-Gene 6000 (Corbett Research, Australia) thermocycler according to the manufacturer's instructions. Except for positive and negative controls all reactions were performed in triplicate.

Clinical specimens:
CSF: Seventeen known positive CSF specimens from pediatric and adult patients were extracted with a manual guanidinium thiocyanate based extraction method (100 µL input and 25 µL output) and forty-two known negative CSF specimens were extracted with KingFisher Flex extractor (Thermo Scientific, Canada)(100 µL input and 40 µL output). The nucleic acid was tested with the conventional and the *Real*Star®*alpha*Herpesvirus PCR assays. Discordant results were repeated with both assays.
Skin lesions: Twenty-two DNA from known positive (by conventional PCR) skin scrapings from pediatric and adult patients were tested with *Real*Star®*alpha*Herpesvirus PCR for detection and characterization of the three viruses.

RESULTS

The RealStar®alpha herpesvirus assay was significantly more sensitive than the conventional PCR for all three targets; HSV 1 (2-3 logs difference), HSV 2 (1-2 logs difference), and VZV (2-3 logs difference) using the mock CSF and shunt fluid samples. The RealStar® real-time-PCR detected 19 (95.0%) and the conventional PCR detected 18 (90.0%) out of 20 positive CSF specimens. For the lesion scrapings, both assays detected and identified 6 VZV, 11 HSV 1 and 5 HSV 2 in 22 lesion scrapings.

TABLE 1. Number of positives when viral dilutions of spiked CSF (A) and shunt fluid (B) were tested in triplicate with the conventional and RealStar® PCRs.

A:

Virus	Dilution	RealStar®	Conventional
HSV1	10-3	3/3	3/3
	10-4	3/3	3/3
	10-5	3/3	3/3
	10-6	3/3	0/3
	10-7	2/3	0/3
HSV2	10-3	3/3	3/3
	10-4	3/3	3/3
	10-5	3/3	3/3
	10-6	3/3	3/3
	10-7	3/3	3/3
VZV	10-3	3/3	3/3
	10-4	3/3	3/3
	10-5	3/3	3/3
	10-6	3/3	3/3
	10-7	3/3	0/3

B:

Virus	Dilution	Realstar®	Conventional
HSV1	10-5	3/3	3/3
	10-6	3/3	0/3
	10-7	3/3	0/3
	10-8	1/3	0/3
	10-9	0/3	0/3
HSV2	10-5	3/3	3/3
	10-6	3/3	0/3
	10-7	1/3	0/3
	10-8	0/3	0/3
	10-9	0/3	0/3
VZV	10-5	3/3	3/3
	10-6	3/3	2/3
	10-7	3/3	0/3
	10-8	1/3	0/3
	10-9	1/3	0/3

TABLE 2. Performance of the conventional and RealStar® PCR assays using 59 CSF and 22 lesion scraping specimens

Specimen type	No. of samples	Conventional	RealStar®	Comment
CSF	40	Negative	Negative	
CSF	9	HSV1	HSV1	
CSF	8	HSV2	HSV2	
CSF	1	Negative	HSV1	On repeat testing, specimen was positive by both assays
CSF	1	Negative	HSV2	On repeat testing, specimen was positive by both assays
Lesion	11	HSV-1	HSV-1	
Lesion	6	VZV	VZV	
Lesion	5	HSV-2	HSV-2	

DISCUSSION & CONCLUSIONS

The new RealStar® alpha herpesvirus assay is a multiplex capable of detecting and characterizing HSV 1, HSV 2 and VZV in CSF and lesion scrapings. It also contains an internal control for monitoring inhibition. The assay was 1-3 logs more sensitive than the conventional PCRs for all targets in mock CSF and shunt specimens. The RealStar® assay also had a greater clinical sensitivity than the conventional PCRs for CSF specimens (100%; 19/19 vs. 89.5%; 17/19 positives) and was 100% specific for lesion scrapings. Use of a single assay for testing will save on the volume of CSF required for testing.

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