T11 PCR DETECTION OF HERPES SIMPLEX VIRUS AND VARICELLA ZOSTER VIRUS IN CEREBROSPINAL FLUID

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ABSTRACT:

Introduction: Herpes simplex virus (HSV) type 1 is an important cause of encephalitis with a somber prognosis if not recognized and treated expeditiously. Due to the very small amounts of virus present in cerebrospinal fluid (CSF), detection of HSV-1 requires very sensitive nucleic acid amplification techniques. On the other hand, HSV-2 may also provoke a recurring, usually self-limiting, meningitis, included with Mollaret's meningitis. A third member of the Herpes family, Varicella Zoster Virus (VZV), may also cause encephalitis or vasculopathy associated with varicella or shingles. At the beginning of the infection, low amounts of viral DNA may be found in the CSF, but later only CSF antibodies and not virus can be detected. Treatment with acyclovir may benefit all of these patients. It would thus be useful to detect and distinguish these 3 herpes family viruses not only in CSF, but also in vesicular lesions. We evaluated 4 tests that simultaneously detect all 3 herpes viruses and are compatible with our Light Cycler 480 apparatus (Roche Diagnostics) or gel-based apparatus.

Methods: As we had only observed 14 HSV-1, 10 HSV-2 and 11 VZV positive samples in CSF over the last 5 years (1141 samples), we spiked CSF with clinical isolates of HSV-1, HSV-2 or VZV, and compared the efficiency of extraction with QIAamp DNA minikit columns (Qiagen) versus the NucliSENS Easy-Mag generic DNA/RNA extraction kit (BioMerieux,) and then amplified and detected with a homebrew, nested, gel based assay, the RealStar *alpha*-Herpesvirus kit (Altona Diagnositics, Hamburg, Germany) with the LC-480, the Seeplex Meningitis ACE detection kit (Seegene, Korea) using detection in agarose gels, and the LightMix HSV VZV kit (TIB MOLBIOL, Berlin, Germany) with the LC-480.

Results: Crossing point (Cp or Ct) values were obtained for 10 HSV-1 and 10 HSV-2 cultures spiked into CSF and extracted with the Easy-Mag or Qiagen columns. Values were also obtained for human mitochondrial genes using a homebrew assay. The values obtained with the Easy-Mag were generally 0.5 to 1 Cp lower than with the columns, indicating slightly better recovery. The Easy-Mag requires considerably less technical time than columns. Tenfold dilution series of 5 of the HSV-1, 5 of the HSV-2 and 1 VZV sample were analyzed with the 4 amplification tests. The RealStar and Seegene kits detected 9 of the 10 HSV samples at the highest dilution found positive for any kit. The Light Mix kit, and the homebrew were positive at this highest dilution for 4, and 2 samples respectively, while detecting the other samples at one dilution lower.

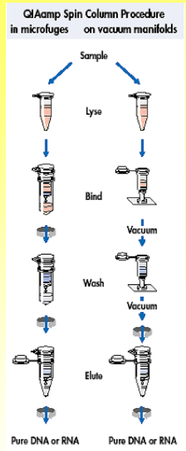
Conclusion: We adopted the Easy-Mag extractor and RealStar kit for our clinical laboratory, because of sensitivity, ease of use and compatibility with the Light Cycler 480 apparatus.

Protocols, Primers & Probes

P1	CGACTTTGCCAGCCTSTACC
P6	CCCAGCATCATCCAGGCCCA
P9	CATCATCATGGCCCACAACCT
894R	GTCCGTGTCCCCGTAGATGA

We have used a semi-nested protocol inspired by Rozenberg & Lebon, 1991 and by Johnson et al, 2000, targeting the polymerase gene for over 10 years.
We created a positive control based on the CMV polymerase gene that we lengthened by inserting about 200 bases of human DNA and added at 10 copies to our reaction mix.
We followed the manufacturer's recommendations for the RealStar *alpha*-Herpesvirus kit (Altona Diagnositics, Hamburg, Germany) with the LC-480, the Seeplex Meningitis ACE detection kit (Seegene, Korea) using detection in agarose gels, and the LightMix HSV VZV kit (TIB MOLBIOL, Berlin, Germany) with the LC-480.
Tenfold dilution series of 5 HSV-1, 5 HSV-2 and 1 VZV sample were analyzed with the 4 amplification tests.
Our non-nested homebrew is identified as Pr, our nested protocol as N, the RealStar protocol as RS, the LightMix protocol as TIB, and the Seegene protocol as See in the Table.

DNA EXTRACTION: EASYMAG versus MAGNAPURE COMPACT



QIAamp DNA minikits

Sample Prep: 0.4 ml of CSF is mixed with 40 µl proteinase K then added to 400 µl of AL buffer, heated at 56°C for 10 min. 465 µl ethanol is added and 2 600 µl aliquots are passed through a spin column by centrifuging at 8,000 rpm for 1 min, spin washed with AW1 then AW2 buffers and finally eluted in 80 µl of AE buffer. This extraction could also be performed on a QIAcube apparatus.

Preparation takes approximately 50 minutes for 1 or 2 samples.

EasyMag

Tha apparatus delivers lysis buffer into 8 chamber reaction vessels. Patient sample (0.4 ml) is added to each vessel, mixed, and incubated 10 min. Silica beads are then added to each vessel. Different sample types can be extracted simultaneously, even RNA extracts for respiratory viruses!

Preparation 15-20 minutes for 24 samples then 45 minutes in the robot

10 HSV-1 and 10 HSV-2 cultures were spiked into CSF and extracted with the Easy-Mag or Qiagen columns and amplified with homebrew RT-PCR. Values were also obtained for human mitochondrial genes using a homebrew assay. In the Table the Cp value of the sample extracted with the EasyMag was subtracted from the Cp value of the QIAamp extract, so a negative value would indicate that the more DNA was recovered by the EasyMag extraction. The values obtained with the Easy-Mag were generally 0.1 to 2 Cp lower than with the columns indicating slightly better average recovery.,

Viral DNA detection

Sample	HSV1	HSV2
1	-1,82	0,08
2	1,5	-2,97
3	-1	-2,03
4	-4,82	-2,08
5	-0,78	1,49
6	-2,95	3,28
7	-2	4
8	1,81	4,61
9	-2,97	-6,4
10	3,15	-1,6
Average	-1,00	-0,16

Mitochondrial DNA detection

Sample	HSV1	HSV2
1	-2,98	0,65
2	-1,59	0,43
3	-1,19	-2,21
4	-4,09	-0,85
5	-0,48	2,8
6	-4,04	-0,34
7	-4,8	-5,7
8	0,11	0,5
9	-1,6	0
10	-1,02	1,32
Average	-2,17	-0,34

CONCLUSIONS and PERSPECTIVES

: We adopted the Easy-Mag extractor and RealStar kit for our clinical laboratory, because of sensitivity, ease of use and compatibility with the Light Cycler 480 apparatus.
•In light of its equivalent sensitivity and ability to detect other herpes viruses, it would be interesting to test the Seegene kit with their detection apparatus that is more friendly to clinical laboratory technicians than the agarose gels we employed and may even have enhanced its sensitivity,
•Automated DNA extraction with the QIAcube would probably also yield equivalent DNA samples at a reduced time compared to the spin columns, and at a reduced cost compared with the EasyMag!.

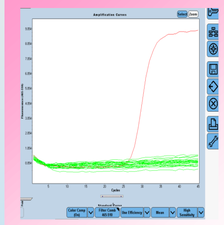
HSV1

	#1					#2					#3					#4					#7				
	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See
ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-5	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
-6	-	-	+	+	+	+	+	+	-	+	-	-	+	-	+	-	-	+	+	+	-	-	+	+	+
-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

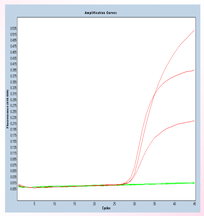
HSV2

	#1					#2					#3					#4					#5				
	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See	Pr	N	RS	TIB	See
ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-5	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+
-6	-	-	+	+	+	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+
-7	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-
-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

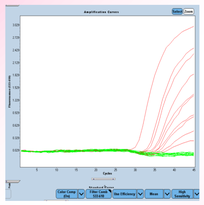
Typical Experiment with the RealStar Kit



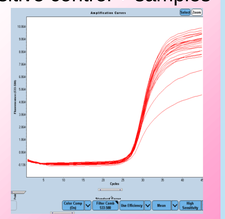
VZV positive control



HSV2 positive control + samples

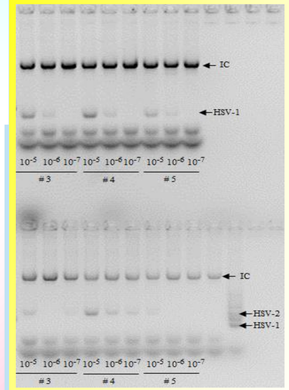


HSV1 positive control and samples



Internal control

Typical experiment with Seegene kit



Shown here are dilutions 10^{-5}, 10^{-6}, and10^{-7}, of HSV1 samples #3-5 (top panel) and HSV2 #3-5 (bottom panel). The marker with herpes virus 1 & 2 identified is at the right of the lower panel.