DETECTION OF HERPESVIRUSES IN CLINICAL SAMPLES : EVALUATION OF REAL-TIME PCR METHODS

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INTRODUCTION

Herpes Simpex Virus Types 1 and 2 (HSV1 and HSV2) and Varicella Zoster Virus (VZV) belong to the family of Herpesviridae, the genuses Simplexvirus and Varicellovirus, respectively and their genetic material is DNA. Herpes Simplex Virus Types 1 and 2 are transmitted by direct contact through lesions or body fluids of an infected individual. The usual site of infection by HSV1 is the face (lips, mouth and eyes), whereas the site of infection by HSV2 are the genitals, as this type of herpes is transmitted from one individual to the other through sexual intercourse. The most characteristic symptom of the infection is the formation of multiple round sores on the site of infection, accompanied by malaise and fever. Varicella Zoster (in adults, usually after the 50th year of age) and chickenpox (in children) are caused by the same virus and the symptoms are the same round sores with the ones caused by the HSV infection. It is possible that these herpesviruses may cause neurological disorders, such as myelitis, aseptic meningitis and encephalitis.

OBJECTIVE

To evaluate commercial Real-Time PCR methods for the detection of herpes simplex and herpes zoster viruses in clinical samples.

CLINICAL SAMPLES

A total of 24 clinical samples – 18 samples from lesions of the mouth, the trunk and the genital area and 6 cerebrospinal fluid samples – were processed.

METHODS

The DNA extraction was performed using the QIAamp DNA Blood Mini kit (Qiagen) for the in-house methods and the Affigene® DNA extraction kit for the real-time PCR methods by Affigene (Cepheid A.B., Sweden).

In this study, the following methods were performed :
- In-house TaqMan Real Time PCR for the DNA detection of Herpes Simplex Virus Types 1 and 2
- In-house conventional PCR for the DNA detection of Varicella Zoster Virus, and
- A Direct Immunofluorescence kit (BioRad, BIOS) for the detection of Herpes Simplex Virus Types 1 and 2 and Varicella Zoster Virus.

The combined results of the methods mentioned above were the "gold standard", that was compared to the Affigene commercial kits :
- Real Time PCR HSV1/2 tracer for the DNA detection of Herpes Simplex Virus Types 1 and 2 and
- Real Time PCR VZV tracer for the DNA detection of Varicella Zoster Virus

Finally, the same commercial kits were used for the DNA detection of HSV1, HSV2 and VZV, without prior extraction ("Direct Affigene").

RESULTS

The combination of results from Direct Immunofluorescence and the in-house Real-time PCR was the "Gold Standard" which was compared to the two different types of the commercial Real-time PCR. As shown in the tables below, the commercial Real-time PCR without prior DNA extraction ("Direct Affigene") had the exact same results with the "Gold Standard".

AFFIGENE	IFA & IN-HOUSE REAL TIME (GOLD STANDARD)					
	HSV1		HSV2		VZV	
	+	-	+	-	+	-
+	8	0	5	0	3	0
-	1	15	1	18	0	21

Table 1: Compared results of the Affigene Real-time PCR with the "Gold Standard" method.

"DIRECT" AFFIGENE	IFA & IN-HOUSE REAL TIME (GOLD STANDARD)					
	HSV1		HSV2		VZV	
	+	-	+	-	+	-
+	9	0	6	0	3	0
-	0	15	0	18	0	21

Table 2: Compared results of the "Direct" Affigene Real-time PCR with the "Gold Standard" method.

	AFFIGENE (with prior extraction)			"DIRECT" AFFIGENE (without prior extraction)		
	HSV1	HSV2	VZV	HSV1	HSV2	VZV
SENSITIVITY (95%CI)	88.8 (84.6%-93%)	83 (80%-86%)	100	100	100	100
SPECIFICITY	100	100	100	100	100	100
POSITIVE PREDICTIVE VALUE	100	100	100	100	100	100
NEGATIVE PREDICTIVE VALUE (95%CI)	94.4 (90.8%-98%)	95 (92.5%-97.5%)	100	100	100	100

Table 3: Results of the two types of the Affigene Real-time PCR for Herpes Simplex Virus types 1 and 2 and Varicella Zoster virus.

Characteristic results of Real time PCR and Direct Immunofluorescence, are shown in the figures that follow.

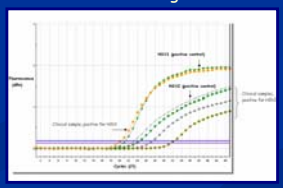

Figure 1: Amplification plots of clinical samples, positive for HSV1 and HSV2, using the in-house Real-time PCR.

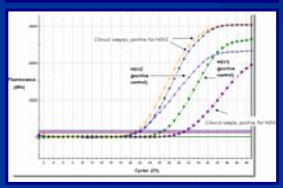

Figure 2: Amplification plots of clinical samples, positive for HSV1 and HSV2, using the Affigene Real-time PCR, with prior DNA extraction.

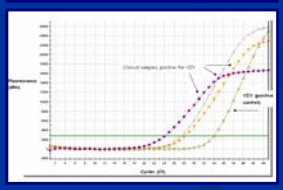

Figure 3: Amplification plots of clinical samples, positive for VZV, using the Affigene Real-time PCR, without prior DNA extraction.

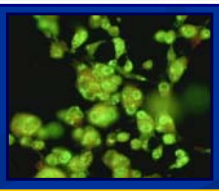

Figure 4: Detection of Herpes Simplex Virus type 1 using direct immunofluorescence, from a mouth lesion.

CONCLUSIONS

- The commercial Real-time PCR methods by Affigene, HSV 1/2 tracer and VZV tracer show high sensitivity and specificity, as well as high negative and positive predictive values.
- The Real-time PCR method by Affigene, without prior DNA extraction has 100% sensitivity, specificity and negative and positive predictive values.
- The commercial kits by Affigene (Cepheid A.B., Sweden) can be used with or without prior DNA extraction.