DEVELOPMENT OF A NEW DIAGNOSTIC TOOL FOR THE QUANTITATIVE DETECTION OF HSV-1, HSV-2, AND VZV DNA. VIGNOLES M., BES J., MAGRO S., RAOUX N., BARRANGER C. and JOANNES M. ARGENE, Parc Technologique Delta Sud, 09340 VERNIOLLE, France

INTRODUCTION:

Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and Varicella-Zoster Virus (VZV) can cause lifethreatening infections of the central nervous system. Mortality of encephalitis is still very high, despite the development of efficient antiviral treatment. HSV-1, HSV-2 and VZV have been successfully identified by PCR for few years in cerebrospinal fluid (CSF), particularly with Herpes Consensus[™] kit.

We present here a new and complementary diagnostic tool, HSV-1 HSV-2 VZV R-gene™ kit, for the quantitative detection of these viruses by real-time PCR.

METHODS:

*Analytical sensitivity:

- HSV1 KOS strain titrated in pfu/mL was quantified and diluted in negative CSF sample. Dilutions were extracted with QIAamp® DNA Blood mini kit (Qiagen) and tested in 20 replicates on ABI® 7500.

- HSV-2 culture cells (ATCC, VR-734) were extracted and quantified. Then several serial dilutions of this quantified sample were tested in 20 replicates on ABI® 7500.

-Quantified QCMD samples VZV was extracted with QIAamp® DNA Blood mini kit (Qiagen). Serial dilutions were performed and tested in 20 replicates on ABI® 7500.

-KOS strain was kindly provided by F Rozenberg and P Lebon, St Vincent de Paul hospital - Paris.

*European HSV-1/2 (2007) and VZV (2008) Proficiency Panels:

200 μ L of each panel sample were extracted using QIAamp® DNA Blood mini kit (Qiagen) and eluted in 50 μ L. An additional control to check extraction and amplification inhibitors was added before extraction to each samples. Then, 10 μ L of extracted DNA was added to 15 μ L of the ready-to-use amplification premix. The study was performed on RotorGene® 6000 (Corbett Research).









Analytical sensitivity: 4.5 pfu/mL are detected in 95%. Equivalent to 200 copies/mL



70 copies/mL are detected in 95%.

EUROPEAN HSV-1/2 and VZV PROFICIENCY PANELS (QCMD)

Sample Sample Content Target value Dilution 4SV07-01 HSV type 2 1/10 000 4SV07-02 HSV type 1 1/100 000 4SV07-03 HSV negative	QUALITATIN Expected QCMD Results - +	/E RESULTS HSV-1 R-Gene RotorGene 6000	QUALITATIN Expected QCMD Results	/E RESULTS HSV-2 R-Gene RotorGene
Sample Sample Content Target value Dilution 4SV07-01 HSV type 2 1/10 000 4SV07-02 HSV type 1 1/100 000 4SV07-03 HSV negative	Expected QCMD Results - +	HSV-1 R-Gene RotorGene 6000 -	Expected QCMD Results	HSV-2 R-Gene RotorGene
HSV07-01 HSV type 2 1/10 000 HSV07-02 HSV type 1 1/100 000 HSV07-03 HSV negative	-+	-		6000
HSV07-02 HSV type 1 1/100 000 HSV07-03 HSV negative	+		+	+
HSV07-03 HSV negative	-	+	-	-
	-	-	-	-
HSV07-04 HSV type 1 1/3 160 000	+	+	-	•
HSV07-05 HSV type 2 1/100 000	-	-	+	+
HSV07-06 HSV negative	-	-	-	-
HSV07-07 HSV type 1 1/100	+	+	-	-
HSV07-08 HSV type 1 1/100 000	+	+	-	-
HSV07-09 HSV type 2 1/10 000 000	-	-	+	+
HSV07-10 HSV type 1 1/10 000	+	+	-	-
HSV07-11 HSV type 2 1/100	-	-	+	+
HSV07-12 VZV	-	-	-	-

CONCLUSION:

Results presented in this study show the high sensitivity of the HSV-1 HSV-2 VZV R-geneTM kit: For HSV-1, sensitivity is 200 copies/mL, for HSV-2 70 copies/mL and for VZV 250 copies/mL. The limit of detection (LOD) for these viruses is 50 copies/mL. For HSV1 KOS1 strain, we determined correlation between the number of infectious virus and the genome copy number: 4.5 pfu/mL equivalent to 200 copies/mL and the LOD was 1 pfu/mL corresponding to 50 copies/mL. The results obtained for the panels underline the high sensitivity, robustness and practicability of the technique, all positive and negative samples were correctly identified.

HSV-1 HSV-2 VZV R-gene™ kit, compatible with the major real-time PCR platforms, is a useful tool for routine diagnostic laboratories.

