

DEVELOPMENT OF A NEW DIAGNOSTIC TOOL FOR THE QUANTITATIVE DETECTION OF HSV-1, HSV-2, AND VZV DNA.

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INTRODUCTION:

Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and Varicella-Zoster Virus (VZV) can cause life-threatening 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.

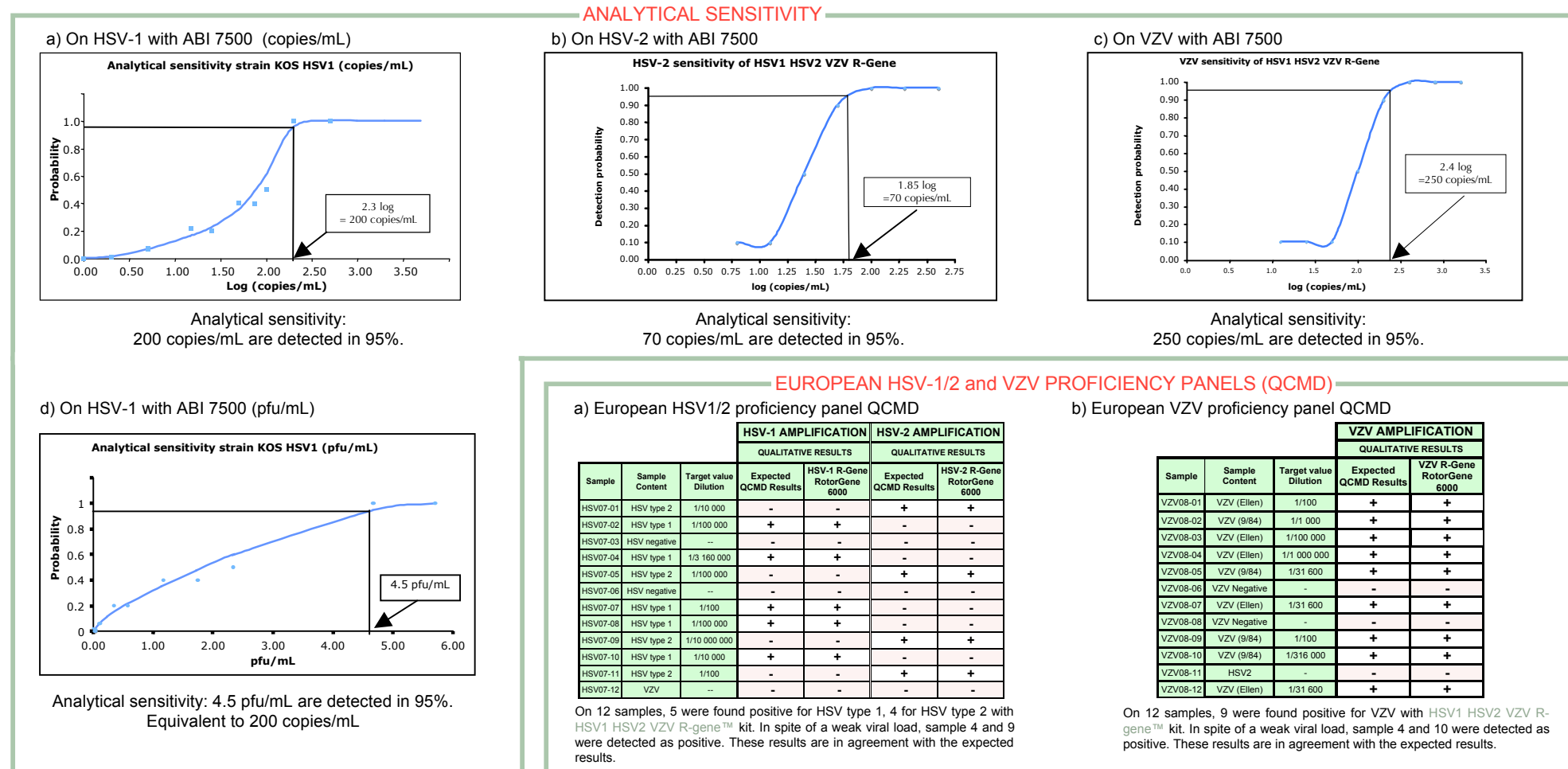
METHODS:

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



CONCLUSION:

Results presented in this study show the high sensitivity of the HSV-1 HSV-2 VZV R-gene™ 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.