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Differential quantitative diagnosis of human alpha herpes viruses by qPCR

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Background: The alphaherpesvirinae subfamily comprises 2 genera, simplex virus with herpes simplex virus 1 and 2 (HSV-1 and HSV-2), and varicellovirus with varicella-zoster virus (VZV), all three being double stranded DNA enveloped viruses. Primary infection may occur at any age, followed by latent infection which is reactivated under a variety of stimuli, linked or not to the host immune status. HSV affects the respiratory tract, genital epithelium, eye, skin and the central nervous system (encephalitis).

This study aims to characterize the performances of Eurobioplex HSV1/HSV2/VZV kit for sensitivity and specificity.

Material/methods: Eurobioplex HSV1/HSV2/VZV is a real-time PCR multiplex for the qualitative and quantitative detection of these 3 viruses using fluorescence (FAM, HEX, Texas Red respectively), with in addition an internal control of DNA extraction and PCR inhibition (Cy5). The study aims at: 1/ defining the detection limits for qualitative and quantitative analysis using plasmids and 2/ assessing the performances on biological samples pretested in reference laboratories. A comparative study was performed with a CE marked kit. A total of 101 samples were used to determine the performances on respectively, 36, 29 and 23 positive samples for HSV1, HSV2, or VZV. DNA was extracted from various tissue origins (e.g. skin, genital tissues, eye, amniotic fluid, cerebrospinal fluid, bronchial aspiration, blood).

Results: The PCR run lasts about 1h10 minutes and uses only 5 microliters of DNA in a single 25 microliter PCR reaction. Detection limits are 300 copies/reaction for HSV1, HSV2 and VZV.

Linear quantification range is from 300 to $3 \cdot 10^8$ copies/PCR for HSV1 and HSV2, and from 1000 to $3 \cdot 10^8$ copies/PCR for VZV. Depending on the channel of fluorescence, the coefficient of variation is 1,05 to 1,25% for intra-experiment reproducibility, and 1,82 to 2,97% for inter-experiments reproducibility. No cross reactivity between the 3 alpha herpes viruses was observed. Sensitivity and specificity on biological samples are >98 % for all 3 viruses.

Conclusions: Eurobioplex alpha herpes virus is a rapid and sensitive qPCR multiplex to simultaneously detect HSV1, HSV2 and VZV, usable on any biological sample.