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P0056 Clinical evaluation of a laboratory developed multiplex RT-PCR assay using the cobas omni Utility Channel: herpes simplex/varicella-zoster virus

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Background: Laboratory diagnosis of acute HSV-1/2 or VZV infection is based on molecular methods, but currently no CE labeled PCR test on a highly automated system is available. The open channel on the **cobas®** 6800 System, the **cobas omni** Utility Channel (UC), automates laboratory-developed tests using user-designed oligonucleotides. This study evaluated the clinical performance of multiplex assays using pre-designed primer/probe sets from IDT for the simultaneous detection of HSV-1/2 and VZV on the UC.

Materials/methods: The **cobas®** 6800 System with **cobas omni** Utility Channel Tool v3.0, was used for the study. Inter/Intra-run variability and LOD (2 fold dilution, n=63 repetitions per dilution) of the assay was determined using spiked M4RT contrived media (M4RT+0.15%Mucin+HCT15 1E4 cells/mL) and probit analysis (95%). Performance was further analysed using quality control (Instand, Germany) and clinical samples (M4RT swabs). Results were compared to routine PCR workflow (HSV/VZV-UKE) using lab developed primer/probes (extraction: MagnaPure, amplification/detection: LightCycler480). A CE-labeled PCR test (Altona Diagnostics) was used to analyze discrepant samples.

Results: LOD for HSV-1 was 0.026 TCID50/mL (95%CI,0.022– 0.033), HSV-2 0.582 TCID50/mL (95%CI,0.482– 0.755) and VZV 2344 cp/mL (95%CI,1962 – 2984 cp/mL). Inter/Intra-run variability determined at 3x LOD (n=5) was STD dev 0,5ct; 1ct (HSV-1) 0,3ct; 1ct (HSV-2) and 1ct; 1ct (VZV) respectively. All quality control panel specimens (n=10) were correctly identified. 334 swab specimens were tested for HSV/VZV with UC and HSV/VZV-UKE, yielding 333 valid results. After discrepancy analysis, there were 35 HSV-1 concordant positive, 292 concordant negative, 34 HSV-2 concordant positive, 298 concordant negative, 31 VZV concordant positive and 300 concordant negative samples. 11 samples remained discrepant, all with high CT values indicating low positive specimens. The sensitivity and specificity of the UC assay were 97.2%(95%CI:85.5–99.9%) and 98.3%(95%CI:96.1–99.5%) for HSV-1, 97.1%(95%CI:85.1–99.9%) and 99.7%(95%CI: 98.2–100%) for HSV-2 and 96.9%(95%CI:83.8–99.9%) and 99.3%(95%CI:97.6–99.8%) for VZV, respectively.

Conclusions: cobas omni Utility Channel is a convenient solution to automate LDT workflow. A multiplex assay using HSV/VZV primers/probes from IDT has favorable sensitivity and specificity.

Additional testing will be performed to assess cross-reactivity as well as analytical sensitivity of the multiplex assay using plasma and CSF samples.