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P2185 The development of a real-time PCR assay for the diagnosis of *Pneumocystis* pneumonia: Fungiplex Pneumocystis

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Background: *Pneumocystis jirovecii is* the causative organism in *Pneumocystis* pneumonia (PCP); a life-threatening pneumonia in immunocompromised patients. Due to a lack of methods to propagate *P. jirovecii in vitro,* the most common current practice in diagnosis of PCP is the microscopic identification of *P. jirovecii* through staining of bronchoalveolar lavage (BAL) samples. This method lacks sensitivity to detect low fungal burdens, therefore PCR methods of detection would improve diagnosis of PCP and complement the current Bruker offering within the area of invasive fungal disease.

Materials/methods: Specific primer and probe sequences have been designed to target the mitochondrial large subunit (mtLSU) rRNA gene of *P. jirovecii*. During initial feasibility studies a range of plasmid concentrations were amplified in the presence of an amplification and extraction control using the standardised Fungiplex assay real-time PCR conditions. The Pneumocystis assay was also tested against QCMD's 2018 P. jirovecii pneumonia EQA panel.

Results: Figure 1 shows linear amplification plots for *P. jirovecii* plasmid concentrations of 20 to $2x10^6$ input copies (ipc) when tested on the ABI7500 real-time PCR instrument, demonstrating a limit of detection of 20 ipc of plasmid DNA in the presence of an internal amplification control (IAC). β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are also being investigated as potential endogenous controls.

When tested against the QCMD 2018 Pneumocystis panel, consisting of 9 saline samples with various concentrations of *P. jirovecii* and a single negative sample, the correct result was obtained in duplicate testing of each of the 10 samples, with IAC detected in all samples.

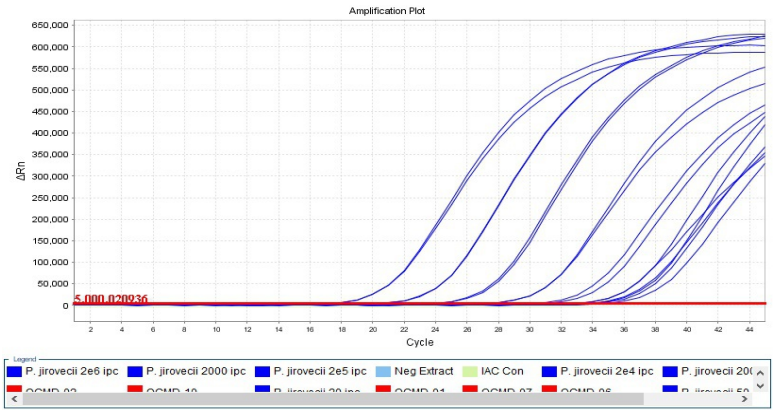

Figure 1: Linear amplification plots for *P. jirovecii* plasmid concentrations from 20 to $2x10^6$ ipc

Conclusions: The Bruker real-time PCR assay for the detection of *P. jirovecii* shows excellent analytical sensitivity at low sample concentration and is being further developed to produce a CE-IVD kit to aid the diagnosis of invasive fungal infection in a clinical setting.