*Pneum*ID – A qPCR kit for the detection of *Pneumocystis jirovecii*

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Introduction

• *Pneumocystis jirovecii* is a common cause of life-threatening pneumonia in individuals with impaired immune systems.

• This organism cannot be cultured *in vitro* and consequently laboratory detection relies heavily on microscopic identification directly from patient specimens using fluorescent stains or antibodies.

• The lack of sensitivity of this method has led to *Pneumocystis*-specific real-time quantitative PCR (qPCR) being proposed as an additional tool to aid conventional diagnostic procedures for *Pneumocystis pneumonia,* by providing sensitive and specific detection of *P. jirovecii* DNA in clinical specimens.

• Our objective was to develop a qPCR kit designed to detect genomic DNA of *P. jirovecii*.

Materials/Methods

Assay design, optimisation and validation were performed in strict compliance with the MIQE guidelines (1). A *P. jirovecii* (FAM) hydrolysis probe assay was combined with a human β-globin gene assay (HEX), as an endogenous internal control, and our own internal extraction control assay (ROX) and optimal conditions were established to create *Pneum*ID. The assay was extensively validated using DNA extracts from fungal cultures and the QCMD PCP 2015 panel (Qnostics Ltd, UK). For QCMD panel testing the samples were subjected to DNA extraction on the Qiagen EZ1, using the DNA bacteria card and tissue kit, prior to loading into the *Pneum*ID assay. In a small-scale demonstration of clinical sample testing, five confirmed *Pneumocystis*-positive clinical respiratory samples were put through DNA extraction on the Qiacube (Qiagen) and tested in the *Pneum*ID assay.

Results

• Under optimal PCR conditions the *P. jirovecii* primers in *Pneum*ID result in **priming efficiencies of >95%** (Figure 1).

• The test has a **broad dynamic range** of at least **six orders of magnitude** and can detect *P. jirovecii* specifically, down to **a single *P. jirovecii* genome** (Figure 2).

• *Pneum*ID **does not cross-react** with *Candida albicans*, *C. glabrata, C. parapsilosis, C. tropicalis, C. dubliniensis, C. krusei, C. auris, Saccharomyces spp, Aspergillus fumigatus, Absidia spp, Pencillium spp, Fusarium oxysporum* or *Scedosporium spp.*

• *Pneum*ID **detects all targets** in retrospective testing of the EQA programme **QCMD PCP 2015 panel**; a **100% score.**

• *Pneum*ID **successfully detected *Pneumocystis* DNA** in 4/5 clinically confirmed *Pneumocystis* respiratory extracts, with **simultaneous detection of the human control gene** marker achieved in all 5 extracts.

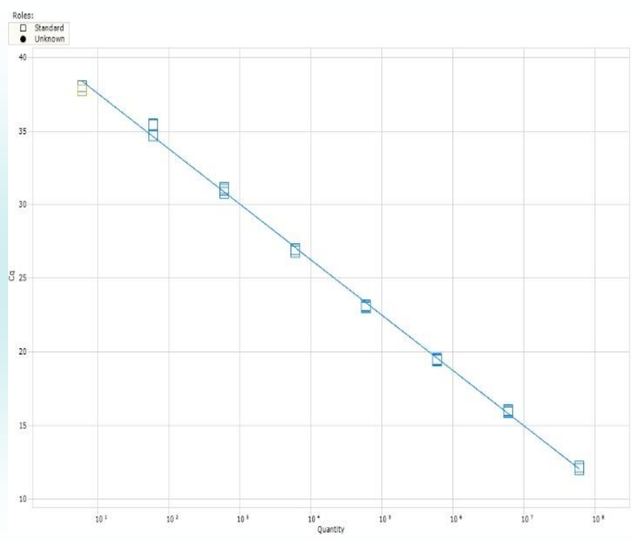

Figure 2. *Pneum*ID standard curve. 8-point standard curves were produced using quantified *P. jirovecii* amplicon, with 10-fold serial dilutions from $6x10^7$ – 6 copies/µL. All standards were successfully amplified and detected.

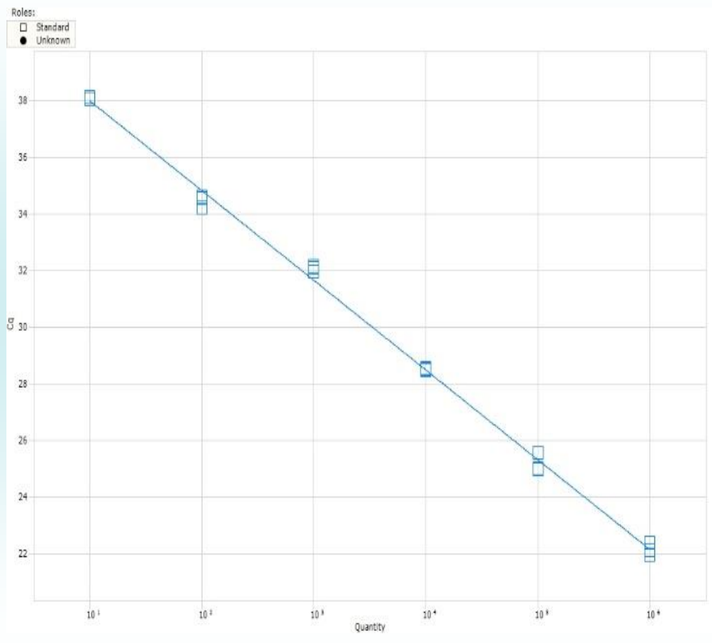

Figure 1. *P. jirovecii* standard curve. 6-point standard curves were produced using *P. jirovecii* amplicon with 10-fold serial dilutions down to single copy target, amplified in the FAM channel only, giving an amplification efficiency of 106% and r^2 = 0.997.

Conclusions

• The *Pneum*ID qPCR test kit sensitively and specifically detects genomic DNA of *Pneumocystis jirovecii*.

• *Pneum*ID has been commercialised by OLM Diagnostics (Newcastle, UK) and enables **direct detection in clinical nucleic acid extracts within 45 minutes of nucleic acid extraction**.

•*Pneum*ID includes both an **endogenous internal control** (human gene target) and an **exogenous internal extraction control** (synthetic sequence), together with a ***Pneumocystis* quantification standard.**

(1) Bustin et al. Clin Chem 2009;55(4):611-22. Epub 2009/02/28

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