The development of a real-time PCR assay for the diagnosis of a broad range of fungal infections

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Introduction

Invasive fungal diseases (IFDs) are a significant cause of morbidity and mortality in patients who are immunocompromised or undergoing intensive therapy. The diagnosis of IFDs is challenging as clinical manifestations are non-specific and standard methods of diagnosis are often slow or have low sensitivity. In recent years, the epidemiology of IFDs has changed; *Candida* species remain the most common causes of IFD, but the incidence of *Aspergillus* species and other filamentous fungi, such as *Zygomycetes*, *Fusarium* and *Scedosporium spp.* has increased.¹ Identifying a fungal infection as early as possible is critical for implementing appropriate antifungal therapy; early diagnosis can improve treatment outcomes and potentially reduce healthcare costs.²

Fig 1: Example of a Fungiplex Real-Time PCR Kit

The Fungiplex® Universal RUO PCR Kit provides a multiplex polymerase chain reaction (PCR) test for the rapid detection of fungal species in various sample matrices, such as whole blood, serum, plasma, bronchoalveolar lavage (BAL) and directly from isolates. This test is for research use only and covers a broad range of fungal pathogens providing rapid results with high sensitivity and specificity without isolation of the organism (Table 1).

The Fungiplex Universal RUO PCR Kit is validated to detect fungal species not detected by other diagnostic biomarkers, such as from the *Zygomycetes* or *Cryptococcus* genera.

Table 1: Genera detected by the Fungiplex Universal RUO PCR Kit

<i>Apophysomyces</i>	<i>Fusarium</i>	<i>Rhizomucor</i>
<i>Aspergillus</i>	<i>Histoplasma</i>	<i>Rhizopus</i>
<i>Candida</i>	<i>Lichtheimia</i>	<i>Saccharomyces</i>
<i>Cladosporium</i>	<i>Microsporum</i>	<i>Saksenaea</i>
<i>Coccidioides</i>	<i>Mucor</i>	<i>Scedosporium</i>
<i>Cryptococcus</i>	<i>Paecilomyces</i>	<i>Syncephalastrum</i>
<i>Cunninghamella</i>	<i>Penicillium</i>	<i>Talaromyces</i>
<i>Exophiala</i>	<i>Pneumocystis</i>	<i>Trichosporon</i>

Methods

Primers and probes were designed to detect the ribosomal RNA gene internal transcribed spacer region which was found to be highly specific.

The analytical performance of the Fungiplex Universal assay was determined by testing plasmid material to determine the Limit of Detection (LOD) and performance across a dynamic range (20 – 2 × 10⁶ input copies). Cross-reactivity studies were carried out as well as testing the assay robustness to inhibitors.

Simulated samples were prepared by spiking known concentrations of plasmid & gDNA for a variety of fungal species into serum, plasma and BAL. Extractions were performed on the Qiagen EZ1 platform.

Results

The LOD of plasmid DNA for 50 different fungal species targets was determined on five real-time PCR instruments with example data shown (Table 2). The overall sensitivity and specificity of the Fungiplex Universal RUO PCR assay has been reported (Table 3).

Table 2: Limit of detection data for example targets tested across 5 real-time PCR instruments

Fungiplex Universal Target	LOD (ipc / rxn) per instrument				
	CFX	LC480 II	QuantStudio	ABI7500	RotorGene
<i>Aspergillus fumigatus</i>	50	200	50	50	50
<i>Apophomyces elegans</i>	50	100	50	50	50
<i>Cryptococcus neoformans</i>	50	100	50	50	50
<i>Trichosporon asahii</i>	100	200	200	200	100
<i>Lichtheimia corybifera</i>	50	50	50	50	50
<i>Mucor circinelloides</i>	50	50	50	50	50
<i>Rhizomucor pusillus</i>	50	50	50	50	50
<i>Syncephalastrum racemosum</i>	50	50	50	50	50
<i>Pneumocystis jirovecii</i>	300	200	200	200	200

Table 3: Detection rates at the defined LOD for a selection of plasmid and gDNA samples tested

Fungiplex Universal Target	CFX	Quant Studio	LC 480 II	ABI7500	Rotor-Gene	All thermal cyclers
<i>Aspergillus fumigatus</i>	32/32	16/16	16/16	16/16	16/16	96/96
<i>Apophysomyces elegans</i>	30/32	16/16	16/16	16/16	16/16	94/96
<i>Cryptococcus neoformans</i>	32/32	16/16	16/16	16/16	16/16	96/96
<i>Trichosporon asahii</i>	16/16	16/16	32/32	16/16	16/16	96/96
<i>Lichtheimia corymbifera</i>	16/16	16/16	31/32	16/16	16/16	95/96
<i>Mucor circinelloides</i>	16/16	16/16	31/32	16/16	16/16	95/96
<i>Rhizomucor pusillus</i>	16/16	32/32	16/16	16/16	16/16	96/96
<i>Syncephalastrum racemosum</i>	16/16	30/32	15/16	16/16	16/16	94/96
<i>Pneumocystis jirovecii</i>	16/16	32/32	16/16	16/16	16/16	96/96
<i>Trichosporon asahii</i> gDNA	Not Tested	Not Tested	Not Tested	Not Tested	16/16	16/16
<i>Mucor circinelloides</i> gDNA	Not Tested	Not Tested	Not Tested	Not Tested	16/16	16/16
<i>Rhizopus oryzae</i> gDNA	Not Tested	Not Tested	Not Tested	16/16	Not Tested	16/16
<i>Candida albicans</i> gDNA	Not Tested	Not Tested	Not Tested	16/16	Not Tested	16/16
<i>Cryptococcus neoformans</i> gDNA	Not Tested	Not Tested	Not Tested	16/16	Not Tested	16/16
Total Number of Samples	192	192	192	192	176	944
Assay sensitivity (%)	98.3	100.0	97.5	100.0	100.0	99.1
Assay specificity (%)	98.2	99.5	99.7	99.2	97.4	98.8

Conclusions

- **Fungiplex Universal** reliably detects fungal DNA from genera not identified using (1-3)-beta-D-glucan assays
- Analytical sensitivity and specificity was > 97 % when tested with a broad range of fungal pathogens
- The **Fungiplex Universal RUO Real Time PCR** can be used when the presence of fungal species is suspected

References

(1) Ala-Houhala M et al., Clinical Microbiology and Infection 2017; 1-5.
(2) Drgona L et al., European Journal of Clinical Microbiology & Infectious Disease 2014; 33: 7-21.