IDENTIFICATION OF COMMON FUNGAL PATHOGENS IN A SINGLE SAMPLE

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Introduction

The objective of this project was to develop a highly sensitive and specific single test for the direct detection of *Aspergillus* and *Candida* species within clinical specimens. This is the first assay of this kind using surface enhanced resonance Raman scattering (SERRS) as the detection technique.

The PCR amplification was designed to target the 18s ribosomal gene. Clinical plasma or serum samples are extracted from blood using standard methodologies. Simulated clinical specimens were used to assess the specificity within co-infection and analytical sensitivity of this assay and the results are reported.

Methods

Surface enhanced resonance Raman scattering (SERRS) allows more analytes to be detected at lower concentration than fluorescence. Multiplex assays were developed to detect a broad range of pathogenic fungal species, present in clinical samples, in low copy number.

Genus specific polymerase chain reaction (PCR) primers and species specific probes were designed to detect multiple targets from spiked serum/whole blood samples. Each probe has a unique spectrum allowing identification of multiple targets in a mixture.

One primer from each set was modified with biotin to allow capture of amplified DNA on micro beads. The specific probes were hybridised to the PCR products and captured. The probe is released from the micro beads and analysed by SERRS for rapid detection of multiple pathogenic fungal targets. A schematic of the assay is given in Fig 1.

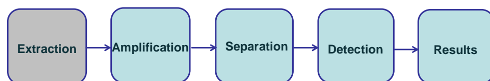

Fig 1. Flow diagram of the D3 Technologies' Assay.

Results

The assay was tested successfully against blind panels and is capable of detecting most clinically relevant species of *Candida* and *Aspergillus* as reported in Table 1. Further optimisation and testing is required for the fungal species in Table 2 and this work is ongoing at D3 Technologies.

For use in diagnostic and clinical testing applications, D3 Technologies are currently developing software to provide results as a patient report, requiring no interpretation of spectral data.

Table 1. *Candida* and *Aspergillus* Fungal Species Currently Detected by the D3 Assay.

Candida Species Detected by D3 Assay	*Aspergillus* Species Detected by D3 Assay
C. albicans	*A. fumigatus*
C. glabrata	*A. flavus*
C. krusei	*A. terreus*
C. tropicalis	*A. niger*
C. lusitaniae	*A. candidus*
C. parapsilosis	
C. dubliniensis	

Table 2. *Candida* and *Aspergillus* Fungal Species Undergoing Testing by D3 Technologies.

Candida Species Undergoing Testing by D3	*Aspergillus* Species Undergoing Testing by D3
C. fumata	*A. ustus*
C. kefyr	
C. guilliermondii	

An example of the spectra obtained for different targets is shown in Fig 2. The unique spectral fingerprint from each dye-labelled probe is observed.

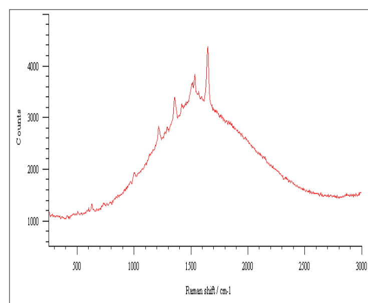

Fig 2. An example of the surface enhanced resonance Raman scattering (SERRS) spectrum obtained for *Aspergillus*.

The assay is designed to differentiate azole resistant species and can detect multiple aetiologies in one specimen. The detection limit is 2-3 copies input per reaction and can reproducibly detect 10^1 organisms within a simulated clinical specimen as reported in Table 3.

Table 3. The results of the duplexes dilution series tested through the D3 Assay.

Aspergillus and C. albicans Input Copy Concentration	*Aspergillus Detected*			*C. albicans Detected*		
	Rep 1	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
10^6	✓	✓	✓	✓	✓	✓
10^5	✓	✓	✓	✓	✓	✓
10^4	✓	✓	✓	✓	✓	✓
10^3	✓	✓	✓	✓	✓	✓
10^2	✓	✓	✓	✓	✓	✓
10^1	✓	✓	✓	✓	✓	✓
10^0	✓	✓	✓	✓	✓	✓

Further blind analysis was carried out on multiplex PCR samples from plasmid materials to assess the influence of competitive PCR amplification. The results are given in Table 4 and an example of one duplex spectrum is given in Fig 4.

Table 4. The results of the various duplexes tested through the D3 Assay.

Duplex Type Tested	*Replicate 1*	Replicate 2	Replicate 3
C. albicans / C. krusei	*Detected* ✓	*Detected* ✓	*Detected* ✓
C. krusei / C. glabrata	*Detected* ✓	*Detected* ✓	*Detected* ✓
A. fumigatus / C. krusei	*Detected* ✓	*Detected* ✓	*Detected* ✓
C. albicans / C. glabrata	*Detected* ✓	*Detected* ✓	*Detected* ✓
A. fumigatus / C. glabrata	*Detected* ✓	*Detected* ✓	*Detected* ✓

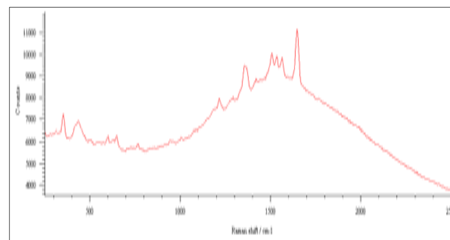

Fig 3. An example of the spectrum obtained from simulated clinical sample of *Aspergillus* and *C. glabrata* at 10^3 input PCR copies.

Conclusions

In this preliminary study, the assay successfully identifies the most common fungal pathogens with high efficiency and demonstrates sensitivity down to 2-3 PCR input copies. This assay is ready to go into pre-clinical testing with the addition of software designed to automate the analysis and the detection range will be increased to cover additional fungal agents. This is the first clinical application developed using D3 Technologies' powerful detection platform. The flexibility of this technology will enable ready adaptation to other diagnostic applications.