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Automated Detection of *Candida auris* Direct from Whole Blood and Swab Specimens by T2MR

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Introduction

Background

Candida auris is now recognized worldwide as a virulent pathogen that can lead to serious disease and is associated with high mortality. The majority of *C. auris* isolates have exhibited resistance to one or more antifungal agents [1]. Nosocomial infections caused by *C. auris* are of particular concern, with both actively infected and colonized patients serving as sources of further environmental contamination. Compounding this problem is the difficulty in accurately and quickly identifying *C. auris* infections. To the best of our knowledge there are no direct from uncultured blood diagnostic tests available for *C. auris*. Microbiological cultures of *Candida* species are documented to take from 1-5 days and suffer from low sensitivity and specificity. Accurate diagnosis of a *C. auris* infection is also hampered by the misidentification *C. auris* as other species, commonly *C. haemulonii* and *Saccharomyces cerevisiae*, by current diagnostic assays [1].

Here we evaluate the use of the T2 Magnetic Resonance (T2MR®) platform for the highly sensitive, rapid species level identification of *C. auris*, *C. haemulonii*, *C. duobushaemulonii* or *C. lusitaniae* in whole blood samples or from common patient and environmental swab matrices.

Technology Description

The T2Candida® Panel is the first fully automated, FDA-cleared, direct from uncultured blood *Candida* assay run on the fully automated T2Dx® Instrument. T2Candida detects *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata* with a specificity ≥ 98.9%, an overall sensitivity of 91.1%, and a mean time to detection and species identification of 4.4 ± 1 hours. This assay has demonstrated cost savings and patient benefits in multiple hospital settings, in addition to identifying deep-seated candidiasis not detected by blood culture. T2MR biosensor technology is based upon superparamagnetic particle sensors and miniaturized magnetic resonance detection. Superparamagnetic particles offer unique opportunities as analyte-specific sensors because their clustering state can be detected by T2MR, a detection methodology impervious to the high backgrounds observed in biological specimens with optical detection methods, thus abrogating the need for analyte purification. Particles are derivatized with analyte-specific ligands (i.e. antibodies or oligonucleotides) and introduction of a biological specimen containing the analyte induces particle agglomeration and subsequent changes in T2 relaxation.

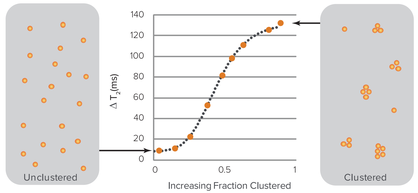

Figure 1: Particles bind to and cluster around the target.

The T2MR detector measures the signal from the nuclear spins of protons in water molecules. A short permanent magnet is used to align the nuclear spins of the hydrogen atoms in the sample. A small radiofrequency pulse is then used to rotate the spins 90 degrees into the transverse plane and thereafter a series of 180 degree radio frequency pulses create multiple spin echoes. The signal intensity of these echoes are fit with a simple algorithm to yield the T2 relaxation rate.

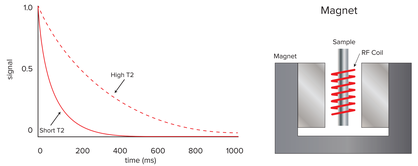

Figure 2: T2MR technology. a) High T2MR signal of clustered particles can be distinguished from a short T2MR signal. b) Schematic of T2MR reader.

T2MR superparamagnetic particles are magnetized by the detector and create microscopic non-uniformities in the magnetic field within the sample. The measured T2MR signal is influenced by the magnetic particles because they affect the average experience of a water molecule via the microscopic non-uniformities they create. Clustering depletes portions of the sample leading to an increase in average T2 relaxation of the sample.

Methods

Cell Preparation for Laboratory Testing

All isolates used were from the CDC-FDA AR Bank. *C. auris, C. lusitaniae, C. duobushaemulonii* and *C. haemulonii* cultured cells were spiked in K_2EDTA anticoagulated blood from healthy human donors or into Amies medium or a PBS Tween® buffer (PBST). *Candida* cells were grown overnight in Yeast-Peptone-Dextrose media at 30°C and cell concentration determined using an automated cell counter. From this stock, the culture was diluted to a target concentration to allow for a 1:100 addition to either healthy K_2EDTA-treated human whole blood, PBST, or Amies medium to achieve a final spike concentration. All spike concentrations were confirmed by plating of the cell solution used for spiking on YPD agar medium.

T2MR *Candida auris* Panel

Spiked blood or swab eluent buffer samples were processed on a T2Dx® Instrument, which automates the following steps: chemical lysis of blood cells (if required), concentration of target cells through centrifugation, release of target cell DNA through mechanical lysis, and amplification of target DNA (Fig. 4). The T2Dx detects and identifies the presence of each individual species by hybridizing the amplicon with DNA probe conjugated superparamagnetic particles. The particles cluster only in the presence of the species they are directed against, and the resulting clustering is identified by the T2 relaxation signal. Target-specific DNA induced clustering results in 30x higher T2MR signal versus dispersed particles allowing for sensitive detection and identification of *Candida* species direct from whole blood.

Figure 3: T2Dx Instrument.

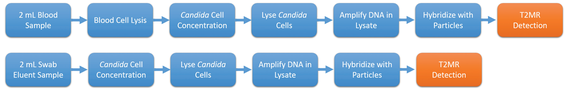

Figure 4: Process for detection of *Candida auris* using T2 Magnetic Resonance.

Results

Highly Sensitive DNA Deteciton by T2MR

T2MR detection is highly sensitive to small amounts of target DNA that have been amplified in the presence of background human DNA. Titrations of oligomers representing the *Candida* target sequences indicate that concentrations as low as 1E+10 copies per reaction can be reliably detected with T2MR (Fig. 5).

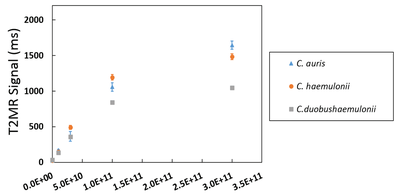

Figure 5: Detection of *Candida* target oligomers.

C. auris cells were spiked into K_2EDTA anticoagulated whole blood at decreasing concentrations. 100% detection on the T2Dx Instrument was observed for samples spiked at 5 CFU/mL direct from whole blood without blood culture (Table 1). T2MR signals of samples spiked with target were approximately 30 times higher than samples with no target present, no cross reactivity was observed between *C. auris, C. haemulonii* and *C. lusitaniae*.

Table 1: Detection of *Candida auris* in K_2EDTA anticoagulated whole blood

Target	Concentration (CFU/mL)	T2MR Detection Channel (# Positive/# Run)		
		C. auris	*C. haemulonii*	*C. lusitaniae*
C. auris	21	**10/10**	0/10	0/10
C. auris	11	**7/7**	0/7	0/7
C. auris	5	**4/4**	0/4	0/4

Differentiation of Individual Species

The panel is designed in a manner such that the detection of the targets are species specific. Particles can be functionalized with probes to detect a single target or dual targets in a detection channel. No cross-reactivity was observed when particles directed toward one or two species are tested against other *Candida* species, even in high concentration spikes (Table 2). Clades I, II and III of *Candida auris* were tested and shown not to cross react. The panel is designed in a manner such that amplification and detection of the targets are species specific. No cross-reactivity is observed when particles directed toward one species are tested against the other two species (Table 2).

Table 2: Species level differentiation of targets

Target in Spike (> 700 CFU/mL)	T2MR Detection Channel (# Positive/# Run)						
	C. auris	*C. haemulonii*	*C. duobushae-mulonii*	*C. lusitaniae*	*C. albicans/ C. tropicalis*	*C. krusei/ C. glabrate*	*C. parapsilosis*
C. auris (I/II/III)	**12/12**	0/12	0/12	0/12	0/12	0/12	0/12
C. haemulonii	0/4	**4/4**	0/4	0/4	0/4	0/4	0/4
C. krusei	0/4	0/4	0/4	0/4	0/4	**4/4**	0/4
C. lusitaniae	0/4	0/4	0/4	**4/4**	0/4	0/4	0/4
C. duobushaemulonii	0/7	0/7	**7/7**	0/4	0/4	0/4	0/4

High Sensitivity Detection in Common Swab Eluents

The flexible T2Dx platform allows for high sensitivity detection of *Candida* species in common swab eluents such as Amies medium and PBS Tween buffer. Rapid environmental and patient sampling will enable a timelier implementation of prevention and infection control measures and potentially help prevent the spread of infection within affected healthcare facilities. Sensitivities of below <10 CFU/mL will allow for the pooling of multiple samples and screening of patients and surfaces (Table 3).

Table 3: High sensitivity detection in swab eluents

Target	Concentration (CFU/mL)	Matrix	T2MR Detection Channel (# Positive/# Run)		
			C. auris	*C.haemulonii*	*C. dubushaemulonii*
C. auris	5-9	Amies	**16/16**	0/16	0/16
C. auris	6-7	PBS Tween	**17/17**	0/17	0/17
C. duobushaemulonii	4	Amies	0/7	0/7	**7/7**
C. duobushaemulonii	3	PBS Tween	0/7	0/7	**7/7**
C. haemulonii	7	Amies	0/6	**6/6**	0/6
C. haemulonii	7	PBS Tween	0/6	**6/6**	0/6

Broad Species Level Inclusivity

The panel is designed to provide broad coverage of all known *Candida auris* clades (Table 4).

Table 4: Comparison of fresh and frozen spiked whole blood samples

Candida auris Clade	Concentration (CFU/mL)	Matrix	T2MR Detection Channel (# Positive/# Run)		
			C. auris	*C. duobushaemu-lonii*	*C. haemulonii*
Clade I South Asia	24	PBS Tween	**7/7**	0/7	0/7
Clade II Asia	8	PBS Tween	**7/7**	0/7	0/7
Clade III Africa	6	PBS Tween	**7/7**	0/7	0/7
Clade IV South America	4	PBS Tween	**6/6**	0/6	0/6

Testing of Clinical Samples

Frozen K_2EDTA anticoagulated whole blood samples from patients with a suspicion of candidemia were collected at La Fe University and Polytechnic Hospital (Valencia, Spain). The clinical samples were tested with the T2MR *Candida auris* panel and results were compared to blood culture (Table 5). Results were found between blood culture and T2MR for 3 positive and 7 negative samples. The time to positive blood culture ranged from 19 to 83 hours prior to subsequent identification, which required additional time. The average time to identification by positive T2MR was < 5 hours. A single sample determined to be *C. auris* positive by blood culture, but not detected by T2MR was from a patient that was admitted with candidemia caused by *C. parapsilosis* and had been previously identified as being colonized with both *C. parapsilosis* and *C. auris*. Blood cultures for this patient taken both before and after the positive *C. auris* blood culture were negative. Further clinical review is underway to understand the clinical presentation and confirmation of the causative agent of candidemia for this patient.

Table 5: Results of testing clinical remainder samples from patients with a suspicion of candidemia

Patient code *C. auris* Study	Location of T2 Blood Draw	Previous candidemia	Previous colonization	Time Blood Culture Result	Blood Culture Results	T2 *C. auris* Results
ID001	Arterial catheter	No	No	5 days	**Negative**	**Negative**
ID002	Venous catheter	No	No	5 days	**Negative**	**Negative**
ID003	Venous catheter	No	No	83 hours	***C. albicans***	**Negative**
ID004	Arterial catheter	No	*C. glabrata*	5 days	**Negative**	**Negative**
ID008	Arterial catheter	*C. auris*	*C. auris*	56 hours	***C. auris***	***C. auris***
ID009	Arterial catheter	*C. auris*	*C. auris*	5 days	**Negative**	**Negative**
ID010	Venous catheter	*C. auris*	*C. auris*	5 days	**Negative**	**Negative**
ID011	Arterial catheter	*C. glabrata*	*C. auris, C. parapsilo-*	33 hours	***C. auris***	***C. auris***
ID012	Arterial catheter	*C. parapsilosis*	*C. auris, C. parapsilosis*	39 hours	*C. auris*	**Negative**
ID013	Venous catheter	No	*C. glabrata, C. auris*	5 days	**Negative**	**Negative**
ID014	Venous catheter	No	*C. glabrata, C. auris*	17 hours	***C. auris***	***C. auris***

Conclusions

A prototype assay for the rapid detection of *C. auris* and based on the T2MR technology, has been used to detect *Candida auris* direct from whole blood and common swab matrices at concentrations <10 CFU/mL. Initial testing with clinical samples indicates that this test may be used to identify *Candida auris* from patient blood samples without requiring blood culture. This rapid and sensitive test may enable detection of *Candida auris* in candidemic patients and assist in screening, isolating and monitoring the spread of this emerging multidrug resistant pathogen.

Disclosures

B.M., J.L.S., B.C., T.H., R.P.S., C.W., D.G., and T.J.L. are employees of T2 Biosystems.

Sources

1. Centers for Disease Control and Prevention. Clinical Alert to U.S. Healthcare Facilities - Global Emergence of Invasive Infections Caused by the Multidrug-Resistant Yeast *Candida auris*. https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris-alert.html (Accessed on July 08, 2016).
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The Candida auris panel in this study is not FDA cleared.

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