RUO

Application Note

When Time Matters - Fungiplex® Candida Auris for culture-free detection of *C. auris*

Introduction

Candida auris is an emerging global health threat. First isolated in 2009, clinical cases have been reported across five continents. The organism is often multi-drug resistant, can be difficult to identify, and its propensity to cause outbreaks has lead the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Control (ECDC) to issue alerts to healthcare institutions from 2016 onwards [1-4].

These alerts highlight that identification of *C. auris* by standard laboratory methods can be challenging. Phenotypic methods can misidentify the organism as closely related yeasts. Bruker's MALDI Biotyper® is the first MALDI-TOF method authorized by the FDA for use in the unambiguous identification of *Candida auris*.

The potential for *C. auris* to cause outbreaks means that active surveillance is recommended. Due to the transmission of *C. auris* within healthcare facilities and the associated healthcare implications, there is a need for methods that allow rapid detection of this species in high-risk areas, such as intensive care units. Those patient groups at risk of invasive candidiasis are at even greater risk if exposed to *C. auris*.

A rapid test, capable of sensitive and specific detection of *C. auris* directly from samples without waiting for culture, could prove beneficial for hospital hygiene and infection control management.

Keywords: Candida auris, multi-drug resistance, culture-free diagnostics, PCR, Candida PCR

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Bruker have developed **Fungiplex Candida Auris**, a real-time PCR Kit to assist laboratories with an interest in the rapid, culture-free detection of *C. auris*.

Methods

Sensitivity

The limit of detection (LOD) and PCR efficiency of the Fungiplex Candida Auris Kit was determined across a serial dilution of plasmid DNA in the range of 20 – 2 × 10^6 input copies (ipc) per PCR reaction. PCR was carried out in triplicate. Data shown in Figure 1 was acquired using the ABI 7500 thermocycler following the protocol provided in the Fungiplex Candida Auris Instructions for Use.

Specificity

Strains of fungal species listed in Table 1 were grown in Sabouraud Dextrose broth for 48 h at 24 °C. This resulted in a high concentration broth, 400 µL of which was extracted into 60 µL, using the EZ1 DSP Virus Kit on the Qiagen EZ1 automated extraction system. Each extract was analysed in triplicate using the Fungiplex Candida Auris Kit, according to the Instructions for Use.

Coverage

28 different strains of *C. auris* were grown in Sabouraud Dextrose broth for 48 h at 24 °C. 400 µL of broth was extracted into 60 µL, using the EZ1 DSP Virus Kit on the Qiagen EZ1 automated extraction system. Each extract was analysed in triplicate using the Fungiplex Candida Auris Kit, according to the Instructions for Use.

Detection from simulated swab samples

Candida auris isolates were grown in Sabouraud Dextrose broth for 48 h at 24 °C. These high concentration samples were diluted in Sabouraud Dextrose broth in a tenfold serial dilution. Swabs (Transwab®) were inoculated in 400 µL of the cultured broth in duplicate. The swabs were then added to 400 µL Tris-EDTA (TE) buffer and incubated at room temperature for approx. 30 min and vortexed at intervals to detach the cells. The samples were extracted by two methods (see below) and the PCR carried out in triplicate:

i) Qiagen EZ1 automated extraction system using the EZ1 DSP Virus Kit with 400 µL input volume, eluted in 60 µL. 5 µL was tested using the Fungiplex Candida Auris Kit.

ii) 400 µL was centrifuged to collect the cell pellets and resuspended in 60 µL TE buffer at 95 °C for 5 min before centrifuging. 5 µL of supernatant was tested using the Fungiplex Candida Auris Kit.

Results

Development and evaluation of Fungiplex Candida Auris

Due to the propensity of *C. auris* to cause outbreaks in high-risk settings, it is important that any detection method is designed to specifically detect this species, and to do so with high sensitivity. Real-time PCR primers and probes were designed and optimized for sensitivity and specificity to *C. auris*.

Analytical sensitivity

The analytical performance was first determined by testing plasmid DNA across a large dynamic range (20 – 2×10^6 ipc per reaction). The plasmid DNA samples were tested in triplicate across 6 commercially available thermocyclers*. Representative results obtained on the ABI(R) 7500 instrument is shown in Figure 1. A limit of detection of 20 ipc was determined with a PCR efficiency of 98%.

* i) ABI® 7500 Fast 2.3, (Applied Biosystems), ii) QuantStudio™ 5 1.3.1 (Applied Biosystems), iii) Mic qPCR Cycler 2.6.4 (Bio Molecular Systems), iv) CFX96™ 3.1 and IVD 6.1 (Bio-Rad), v) Rotor-Gene Q 5plex HRM Q-Series 2.3.1 and Q-Rex 1.0.0 (Qiagen), vi) LightCycler® 480 II 1.5.1 (Roche).

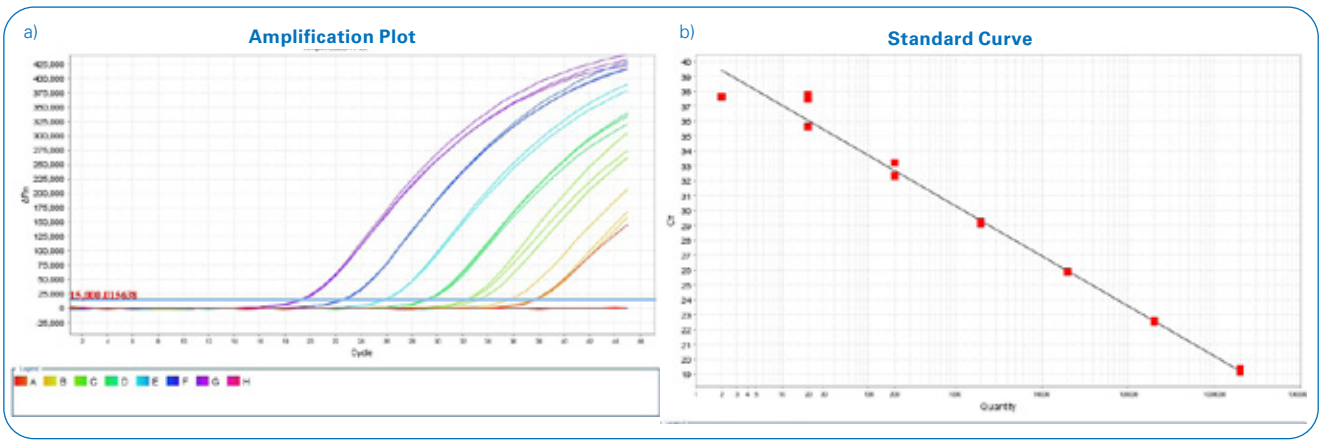

Figure 1: a) Real-time PCR amplification curves obtained from a concentration series of Candida auris plasmid DNA (20 - 2×10^6 ipc), b) Logarithmic concentration curve from which PCR efficiency was calculated.

Strain coverage & specificity

To ensure sufficient coverage by the Fungiplex Candida Auris Kit, the assay was challenged with a range of *C. auris* strains of diverse geographic origin. Genomic DNA was extracted from 28 different *C. auris* strains and tested in triplicate (Table 1). All replicates of the 28 strains were detected.

The Kit was also challenged to ensure sufficient specificity. A range of fungal species were tested for cross-reactivity (Table 1). Closely related yeast species that can be misidentified by other laboratory methods were tested at high concentration. No cross-reactivity was observed when 29 strains of non-*C. auris* fungi were tested with the Fungiplex Candida Auris RUO PCR Kit.

Reproducible detection and specificity were both 100% using the Fungiplex Candida Auris Kit. By comparison, a PCR designed to target the ITS gene (a common target for fungal PCRs) failed to detect all *C. auris* strains, and cross-reacted with some other yeast species (Table 1). This highlights the critical importance of both the sensitivity and specificity of PCR design.

Analysis of simulated swab samples

The ability of Fungiplex Candida Auris to detect *C. auris* DNA extracted from swabs was tested using simulated samples. A high concentration broth of *C. auris* was serially diluted and extracted, i) using a commercial system, and ii) manually by a boiled lysate protocol. Across a wide dynamic range, *C. auris* was detected in all replicates. In the lowest load sample, with a 1:100,000 dilution, detection was achieved in triplicate with a correlated detected concentration of 10 copies from the commercial extraction system, and 28 copies using the boiled lysate protocol.

Table 1: Coverage and specificity of Fungiplex Candida Auris tested against 57 strains from different fungal species (28 C. auris strains, 29 non-C. auris strains)

Species tested	ITS real-time PCR	Fungiplex Candida Auris RUO PCR Kit
***C. auris* (28 strains)**	63/84 reps	84/84 reps
***C. haemulonii/haemulonis* (10 strains)**	4 reps	-
***C. duobushaemulonii* (6 strains)**	-	-
C. dubliniensis	1 reps	-
C. lipolytica	1 reps	-
C. lusitaniae	-	-
C. albicans	2 reps	-
C. glabrata	-	-
C. krusei	-	-
C. sake	-	-
C. parapsilosis	1 reps	-
C. tropicalis	1 reps	-
Kodameae ohmeri	-	-
Rhodotorula glutinis	-	-
Saccharomyces cerevisiae	1 reps	-
Aspergillus fumigatus	-	-

Table 2: The detection rate of a dilution series inoculated on swabs (Transwab®) and extracted either by i) commercial extraction, or ii) boiled lysate. Number of copies detected was calculated via standard curve.

Commercial extraction			Boiled lysate		
Dilution	**Number of Copies**	**Detection**	**Dilution**	**Number of Copies**	**Detection**
1:10	1,179,515	3/3	1:10	86,391	3/3
1:100	399,962	3/3	1:100	72,177	3/3
1:1,000	5,885	3/3	1:1,000	42,810	3/3
1:10,000	542	3/3	1:10,000	1,992	3/3
1:100,000	**10**	**3/3**	**1:100,000**	**28**	**3/3**

Summary and Conclusion

Concerns about *Candida auris* have arisen due to the fact that the organism is often multi-drug resistant, causes outbreaks and can be misidentified by standard laboratory methods. Even where identification is possible, the time required for organism growth means that there is a need for tools capable of rapidly detecting *C. auris* in healthcare settings. Fungiplex Candida Auris is a sensitive and specific real-time PCR Kit for the culture-free detection of *C. auris*. The kit does not cross-react with closely related yeasts that are commonly misidentified by other laboratory systems. The potential for detection from swabs has been demonstrated using simulated swabs. The Fungiplex Candida Auris PCR Kit is for research use only. Not for use in clinical diagnostic procedures.

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Check out the link or scan the QR code for more details.

https://www.bruker.com/products/molecular-diagnostics/real-time-pcr

References

[1] June 2016, *C. auris* Clinical Update, Centres for Disease Control and Prevention, Atlanta
[2] European Centre for Disease Prevention and Control. *Candida auris* in healthcare settings – Europe – 19 December 2016. Stockholm: ECDC; 2016.
[3] September 2017, *C. auris* Clinical Update, Centres for Disease Control and Prevention, Atlanta
[4] European Centre for Disease Prevention and Control. Candida auris in healthcare settings – Europe – first update, 23 April 2018. Stockholm: ECDC; 2018

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