Candida auris with Echinocandin Resistance: Design and Development of a Multiplex Real-Time PCR Assay for Detection and Differentiation.

B. Alabyev[1], Y. Belousov[1], and E. Lukhtanov[1].

[1]ELITechGroup MDx, Bothell, WA

AMP 2023, November 14-18, Salt Lake City, Utah

BACKGROUND

Fungal infections caused by *Candida auris* are emerging as a major problem in the healthcare field, leading to high mortality rates and expensive medical costs for governments and hospitalized patients. Left untreated, such infections can cause Candida systemic diseases which are frequently cited as the fourth leading cause of nosocomial bloodstream infections in the US. *Candida auris* has simultaneously emerged on five continents as a fungal pathogen causing nosocomial outbreaks. The challenges in the treatment of *C. auris* infections are the variable antifungal susceptibility profiles among clinical isolates and the development of resistance to single or multiple classes of available antifungal drugs. Echinocandins looked to be the answer for yeasts that were resistant to azoles, but recently there has been an emergence of Candida species that are resistant to echinocandins in both laboratory and clinical settings. A link has been found between reduced susceptibility of Candida isolates and mutations in the FKS1 gene. The mutation of S639F in the FKS1p subunit of b-(1,3) D-glucan synthase leads to the substitution of serine 639 for phenylalanine. This changes the target site, therefore inhibiting echinocandin susceptibility.

ASSAY PRINCIPLE

The MGB Alert Candida auris with echinocandin resistance RUO Detection Reagent is designed with dual **MGB Pleiades®** hybridization and **DSQ** hydrolysis PCR probe technologies. The assay includes a set of two primers, a duplex stabilizing quencher **("DSQ")** probe specific to a conserved region of the FKS1 gene, and an **MGB Pleiades** hybridization probe targeting the echinocandin resistance associated S639F mutation region of the same amplicon. The mutation is detected by post-PCR melting curve analysis. The probe is specifically designed to match an FKS1 S639F mutant with WT to be discriminated by the melt shift. The internal control primers and probe are included to monitor assay performance and the presence of inhibitors.

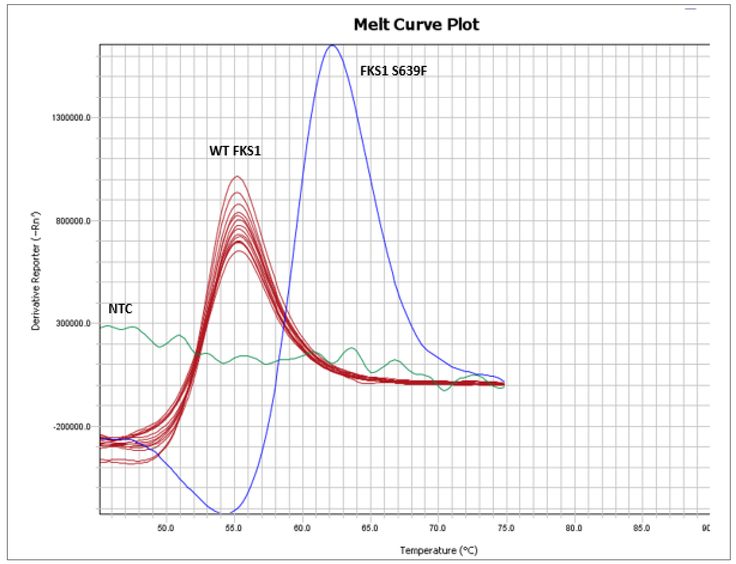
ASSAY COMPOSITION

Table 1 MGB Alert *Candida auris* with echinocandin resistance RUO Detection Reagent components description. The number in the AP fluorophore name indicates its peak excitation wavelength.

Target template	Probe type and chemistry	Probe fluorophore	Analogous fluorophore (for optical channel selection)
Candida auris FKS1 gene species-specific region	DSQ, hydrolysis	FAM	FAM
Candida auris FKS1 S639F point mutation	MGB Pleiades, hybridization	AP593	ROX, Texas Red
Internal control IC1	DSQ, hydrolysis	AP525	VIC, JOE, HEX

ANALYTICAL PERFORMANCE EVALUATION STUDY

Fig 1. Melt curve discrimination of the wild type and S639F FKS1 gene mutants. Fifteen isolates from the *C. auris* panel (ARISOLATE Bank, CDC) were tested. Clean separation of the wild type and mutant melt curve peaks indicate unambiguous differentiation. The results were obtained on the QuantStudio Dx (ThermoFisher).



Mutation	Melt Tm (C°)	ΔTm (C°)
FKS1 WT	55.4	7.2
FKS1 S639F	62.2	0

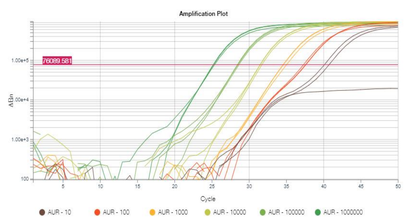
LIMIT OF DETECTION DETERMINATION

The limit of detection was determined by testing serial 3-fold dilutions of *Candida auris* genomic DNA) and calculated at 24 genome copies per PCR using Probit analysis approach. The calculated LoD was verified by testing 20 replicates of *C. auris* genomic DNA at LoD level.

LINEAR RANGE DETERMINATION STUDY

Fig 2. Linear range of *Candida auris* RUO reagents 1e6 – 10 genome copies per PCR

Target	Detection channel	Slope	R^2	Intercept
FKS1	FAM	3.045	0.998	43.648



CROSS-REACTIVITY EVALUATION STUDY

Specificity and inclusivity of the assay were evaluated by real-time PCR using the DNA extracted from organisms obtained from Zeptometrix and ARISOLATE Bank (CDC).

- *Candida albicans*
- *Candida dubliniensis*
- *Candida duobushaemulonii*
- *Candida glabrata*
- *Candida haemulonii*
- *Candida krusei*
- *Candida lusitaniae*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Saccharomyces cerevisiae*
- *Kodameae ohmeri*
- *Corynebacterium striatum*
- *Cryptococcus uniguttulatus*
- *Cryptococcus neoformans*
- *Malasezzia pachydermatis*
- *Escherichia coli*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*

The MGB Alert Candida auris with echinocandin resistance RUO Detection Reagent showed no cross-reactivity with the potentially interfering organisms tested.

MATERIALS AND METHODS

The RUO amplification reagents were tested on QuantStudio Dx and QuantStudio 7 Pro (ThermoFisher). The assay was also used in combination with the ELITe InGenius, a fully automated sample-to-result system with six optical channels for detection and melt curve capabilities (ELITechGroup).
A linear range and a limit of detection were established using serial dilutions of *Candida auris* gDNA extracted from the tittered live culture (Zeptometrix).

RESULTS

- Analytical sensitivity was determined and verified at 24 genome copies/reaction.
- The sample harboring the S639F mutation was clearly distinguished from the wild type by having a melting curve with the melting temperature (Tm) 7 degrees higher than the wild type.
- A linear range was established from 1e6 to 10 genome copies/reaction.

CONCLUSION

We have developed a robust real-time PCR assay for detection and differentiation of the wild-type *Candida auris* and its genotypic mutant conferring resistance to echinocandins. The assay was proven to be sensitive, specific, and easy-to-use. Analytical evaluation of the MGB Alert Candida auris with echinocandin resistance RUO Detection Reagent indicates potential as a valuable tool in the diagnosis of *C. auris* and resistance guided treatment.

*e-mail: b.alabyev@elitechgroup.com

ELITechGroup MDx

M501280 r0