*Auris*ID – A qPCR kit for the detection of *Candida auris*

Gemma Johnson [1], Sara Kirvell [2], Stephen Bustin [2]

1 OLM Diagnostics, Newcastle, UK

2 Department of Medical Science and Public Health, Faculty of Medical Science, Anglia Ruskin University, Chelmsford, UK

Introduction

• *Candida auris* is a globally emerging multidrug resistant yeast species causing a wide spectrum of infections, especially in intensive care settings.
• Since it was first isolated in 2009, *C. auris* has been associated with bloodstream and wound infections globally and has caused hospital outbreaks in several countries.
• *C. auris* is commonly resistant to the first-line antifungal drug Fluconazole, and multidrug resistant strains have been reported.
• Our objective was to develop a qPCR kit designed to detect genomic DNA of *C. auris*.

Materials/Methods

Assay designs, optimisations and validations were performed in strict compliance with the MIQE guidelines (1). Suitable target sequences were identified by aligning sequences of the target organism as well as those to be avoided in CLC Sequence Viewer (Qiagen). Primer and probe sequences were designed by Beacon Designer (Premier Biosoft). *In silico* analysis was performed using nucleotide BLAST and the target secondary structure/template accessibility was assessed using MFOLD and DINAMelt. SYBR Green chemistry, PCR gradients and melt curve analyses were used to determine optimal annealing temperatures and optimal primer/probe concentrations. A *Candida auris* (FAM) hydrolysis probe assay was combined with our own internal extraction control assay (ROX) and optimal conditions were established to create *Auris*ID. The assay was extensively validated using DNA extracts from fungal cultures.

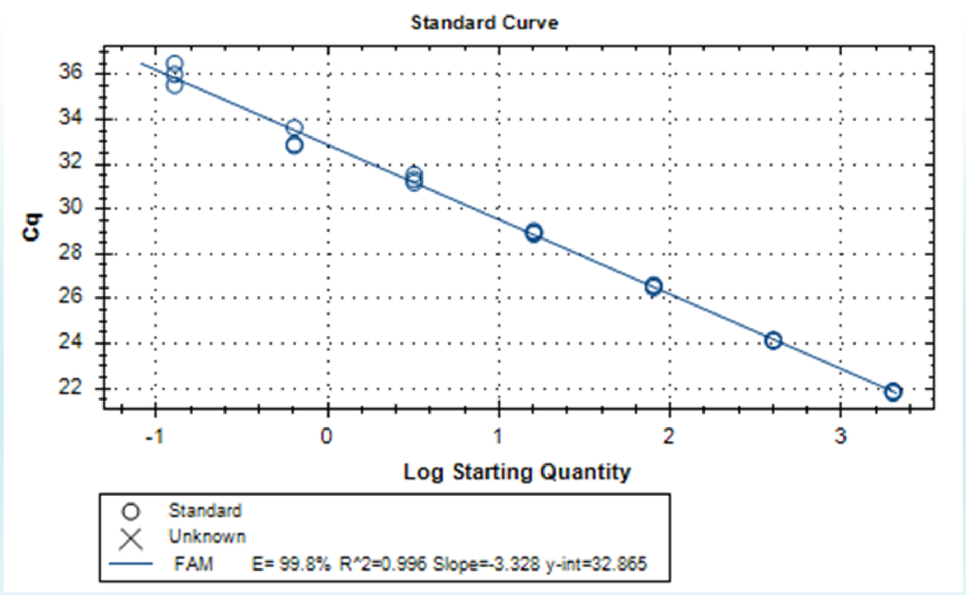

Figure 1. *C. auris* standard curve. 7-point standard curves were produced using *C. auris* DNA extracts with 5-fold serial dilutions from 2 ng to 128 fg, amplified in the FAM, *C. auris,* channel only, giving an amplification efficiency of 99.8% and $r^2 = 0.996$.

Results

• Under optimal PCR conditions the primers in *Auris*ID result in **priming efficiencies of >95%** (Figure 1).
• Optimum primer annealing temperature was established by temperature gradient analysis (Figure 2).
• *Auris*ID has a **broad dynamic range** of at least **six orders of magnitude** and is **sensitive to <10 *Candida auris* genome copies.**
• Amplification specificity for the *C. auris* primer set was validated with SYBR Green I chemistry and melt curve analysis using 20pg of genomic DNA (Figure 3).
• *Auris*ID **does not cross-react** with any other *Candida* species or the non-*Candida* fungal species *Saccharomyces cerevisiae, Aspergillus fumigatus, Absidia spp, Pencillium spp, Fusarium oxysporum* or *Scedosporium prolificans.*

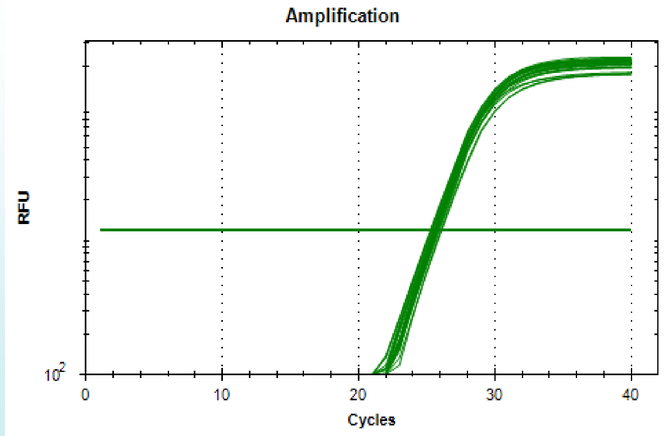

Figure 2. Amplification plots across a temperature gradient. A temperature gradient ranging from 58ºC to 64ºC was used to determine optimal annealing temperature for *C. auris* primers.

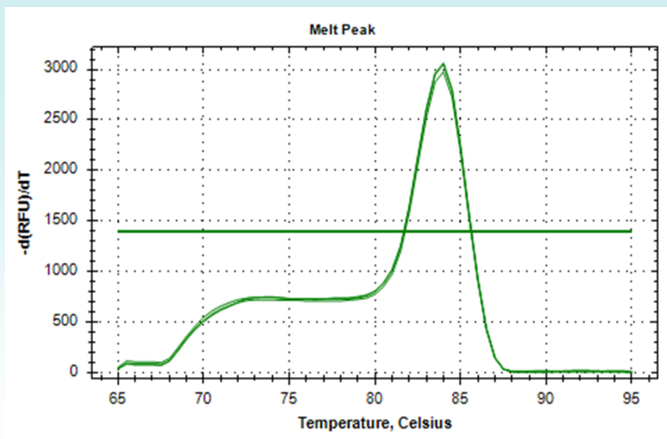

Figure 3. Melt curve analysis of amplification products using 20pg *C. auris* genomic DNA. Melting temperature 84ºC.

Conclusions

• The *Auris*ID qPCR test kit sensitively and specifically detects genomic DNA of *Candida auris*.

• *Auris*ID has been commercialised by OLM Diagnostics (Newcastle, UK) and enables **direct detection in clinical nucleic acid extracts within 45 minutes of nucleic acid extraction**.

(1) Bustin et al. Clin Chem 2009;55(4):611-22. Epub 2009/02/28

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