Development and evaluation of a TaqMan based qPCR assay for specific quantification of *Candida auris*

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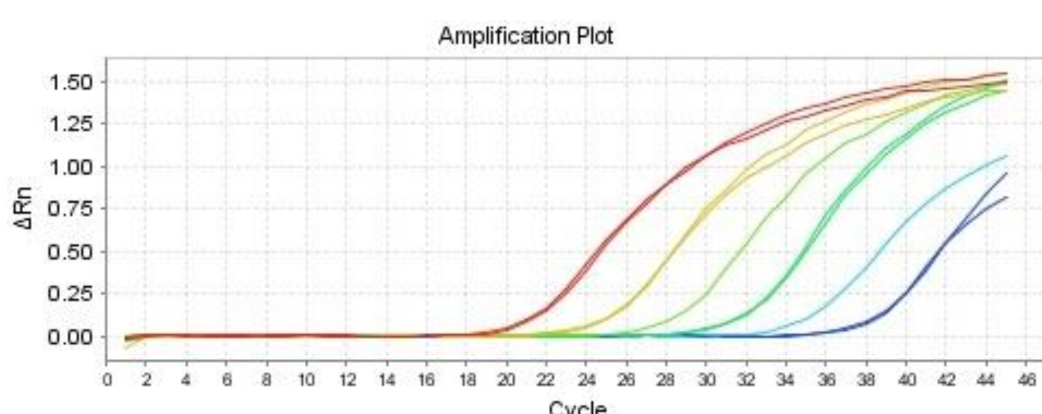
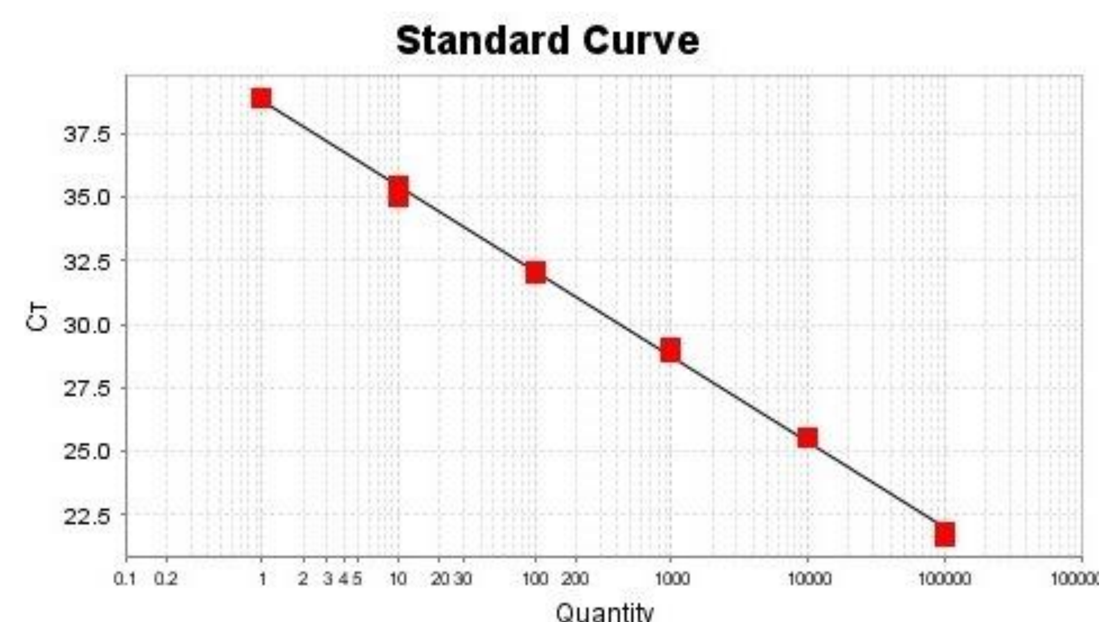
Objectives: *Candida auris* is multidrug-resistant yeast causing invasive nosocomial infections. This emerging opportunistic pathogen has been rapidly spread across the world. Although standard microbiologic methods commonly misidentify *C.auris* as other yeast, Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) method has made precise identification of the yeast possible. In the lack of access to the MALDI-TOF in routine clinical laboratories, rapid and accurate methods are demanded for detecting and identifying of *C. auris*. Thus, we developed and validated a quantitative real-time PCR (qPCR) assay targeting the internal transcribed spacer 2 (ITS2) region of the ribosomal gene of *C. auris*.

Methods: The internal transcribed spacer2 (ITS2) region of the nuclear ribosomal DNA of *C. auris* and other related yeasts were analyzed for finding an amplifiable specific target in *C.auris*. A123 base pair target was selected and primers and probe designed according to TaqMan chemistry. Serial dilutions of counted targets containing from 10^5 to 10^0 CFU of the yeast were used to establish a standard curve for quantifying the yeast. The qPCR reaction was based on the simultaneous detection of a specific ITS target and also contained an internal control to compensate for variations in DNA extraction and the various compounds from human that inhibit PCR.



Results: The qPCR assay was able to identify and quantify *C.auris* with the detection limit of one *C. auris* CFU per reaction. The specificity was confirmed by the lack of amplification of other *candida* species, other yeast and molds, bacteria and human DNA. A qPCR using DNA extracted from a suspension contains one CFU of *C. auris* resulted in steady Ct-value (Ct) of 34. The assays resulted in a standard curve showed a highly significant linearity between the Ct-values and the dilution rates ($R^2 = 0.99$; slope = −3.32).



Target: C.auris rRNA **Slope**: -3.32 **Y-Inter**: 40.141
R^2: 0.996 **Eff**%:94.32

Conclusions: The TaqMan qPCR assay could rapidly and accurately identify and quantify emerging opportunistic *C. auris* from a wider variety of specimen. The Assay time considering sample processing and DNA extraction would take less than 4 h with greater sensitivity and specificity in comparison with microbiological based identification and conventional PCR. This method shows great promise as a tool for rapid diagnosing exposures to *C. auris* in clinical laboratories.