# REAL-TIME PCR BASED IDENTIFICATION OF *CANDIDA AURIS* USING APPLIED BIOSYSTEMS 7500 FAST REAL-TIME PCR PLATFORM

## 1.0 Purpose

The purpose of this procedure is to describe the process for identification of *Candida auris* using the Applied Biosystems 7500 Fast Real-time PCR platform.

## 2.0 Scope

This document is intended for laboratory personnel responsible for CLIA regulated processing and testing of clinical specimens for *C. auris,* using the ABI 7500 Fast Real-Time PCR System.

## 3.0 Responsibility

3.1 It is the responsibility of the Technical Supervisor to approve all deviations, reconcile conflicting results, determine supply substitutions, and review manufacturer's recommendations and procedures.

3.2 It is the responsibility of the Technical Supervisor and the General Supervisor to ensure the Testing Personnel performing this procedure are sufficiently trained and that training is documented prior to performance.

3.3 It is the responsibility of the Testing Personnel to follow all procedures listed in this document, document all deviations, complete all required documentation, and comply with all laboratory safety procedures.

3.4 It is the responsibility of the Testing Personnel to strictly follow all policies and safety practices governing laboratory operations, including the use of appropriate PPE; reporting to the supervisor any medical restrictions, reportable illnesses, and any event that may suggest a laboratory exposure or result in the creation of a potential hazard.

3.5 It is the responsibility of the Laboratory Safety Officer to review SOPs and Risk Assessments for safe work practices, provide support to lab personnel as needed, and routinely monitor laboratory spaces.

## 4.0 Definitions

4.1 ***BLB*** **–** Bacterial Lysis Buffer

4.2 ***BSC*** **–** Biological Safety Cabinet

4.3 ***NTC*** **–** No Template Control (blank)

4.4 ***PCR*** **–** Polymerase Chain Reaction

4.5 ***RCF –*** Relative Centrifugal Force, the amount of accelerative force applied to a sample in a centrifuge. RCF is measured in multiples of the standard acceleration due to gravity at the Earth's surface (x g). RCF and "x g" (units of gravity) are used interchangeably in centrifugation protocols.

## 5.0 References

5.1 Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition
5.2 Your laboratory's Biosafety Manual
5.3 Your laboratory's Chemical Hygiene Plan
5.4 *MagNa Pure System Application Note No. 5* (https://lifescience.roche.com/documents/High-throughput-detection-of-bacterial-fungal-and-viral-nucleic-acids-in-routine-microbiological-sample-types.pdf)
5.5 *MagNA Pure 96 Automated Nucleic Acid Extraction in the BSL-2*
5.6 Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument, Instructions for Use/User Guide (Publication# 4406991).
5.7 Leach L, Zhu Y, Chaturvedi S. Development and Validation of a Real-Time PCR Assay for Rapid Detection of *Candida auris* from Surveillance Samples. J Clin Microbiol., 2018 Jan 24; 56(2), e01223-17.

## 6.0 Equipment/Materials

6.1 Biological Safety Cabinet, Certified Class II A2
6.2 PCR enclosure
6.3 Applied Biosystems 7500 Fast Real-time PCR instrument with SDS Software version 1.4
6.4 Roche MagNA Pure 96 or equivalent extraction instrument
6.5 Shaking incubator (65°C)
6.6 Calibrated pipets (P2/10, P20, P200, P1000)
6.7 Aerosol barrier (filter) tips
6.8 96 well cooling rack
6.9 Bacterial Lysis Buffer (Roche; Cat# 04659180001)
6.10 2 mL Sarstedt tubes
6.11 RNase/DNase-free 1.5mL microcentrifuge tubes
6.12 ABI MicroAmp Fast Optical 96-Well Reaction Plate, 0.1mL
6.13 ABI MicroAmp Optical 8-cap strip
6.14 ABI MicroAmp Adhesive optical film
6.15 Molecular Grade RNase/DNase free water
6.16 MagNA Pure 96 DNA and Viral RNA Small Volume Kit (Roche; Cat# 06543588001) or equivalent kit to be determined
6.17 *Candida auris* primers and probes
6.18 *Bicoid* specific primers and probes

**6.18.1.1** If you do not have *Bicoid* DNA, it is acceptable to substitute another non-human non-fungal control DNA in this assay along with the appropriate primers and probes.

6.19 5× PerfeCTa Multiplex qPCR ToughMix (Quanta Biosciences; Cat# 95147-250)

6.20 Template DNA (DNA from Specimens)

6.21 *Bicoid* inhibition control plasmid DNA

**6.21.1.1** If you do not have *Bicoid* DNA, it is acceptable to substitute another non-human non-fungal control DNA in this assay along with the appropriate primers and probes.

6.22 BleachRite, 10% bleach solution, or other *C. auris*-approved cleaning agent

6.23 ELIMINase or similar reagent for removal of DNase and RNase

6.24 70% Ethanol

## 7.0 Safety Precautions

7.1 Clinical *Candida auris* specimens (Swabs/Sponge) or isolates have the potential to transmit infectious diseases. Wear protective gloves, lab coat, and eyewear when handling samples.

7.2 All BSL-2 practices, safety equipment, and facility design must follow the requirements described in the BMBL and the Biosafety Manual.

7.3 All biohazardous liquid and solid waste and sharps are handled per agency-specific waste handling policies.

7.4 Laboratory personnel should complete appropriate safety training and *Candida*-specific laboratory training.

7.5 Review safety section of equipment manuals prior to performing procedure.

7.6 Follow the instrument specific guidance from Roche for decontamination of the MagnaPure 96, or equivalent extraction system.

7.7 Review all SDS for chemicals used in this procedure.

7.8 Follow institutional guidelines for Spills and Incident Reporting.

7.9 Work with unidentified and pathogenic yeast must be performed in an operational Class II A2 BSC.

7.10 BSCs should be decontaminated before and after use with 10% Bleach for a contact time of 10 minutes.

7.11 10% Bleach must be prepared daily for effective decontamination of C. auris or a bleach stabilized solution like Oxivir TB should be used according to the manufactures recommendations.

## 8.0 Procedure

### Sample Information

8.1 Specimen tubes received for testing must be intact, labeled with two unique identifiers (e.g. patient ID and specimen ID) and must be accompanied by a submission form.

8.2 All patient swabs are collected using BD ESwab collection and transport system (Catalog# 220245; Becton Dickinson and Company, Sparks, MD).

8.3 Following collection, swabs are placed in 1mL Amies buffer for storage and transport.

8.4 Specimens should be stored at 4°-25°C, and shipped with a cold pack to the laboratory for processing within 96 hours of collection.

8.5 Specimen rejection criteria:

- 8.5.1 Specimens received >4 days after collection
- 8.5.2 Damaged or visible leakage of transport tubes
- 8.5.3 Specimen without submission forms
- 8.5.4 Specimen without identifiers

8.6 Individual institutions submitting swabs are responsible for the compliance with local human subjects/Institutional Review Board (IRB) regulations or applying for the IRB exemption for public health outbreak surveillance and emergency response.

**Reagent Handling and Storage**

8.7 Store all primers and probes at 2-8°C until re-hydrated for use; store all control materials at -20°C.

8.8 Always check the expiration date prior to use. Do not use expired reagents.

8.9 Protect fluorogenic probes from light.

8.10 Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on ice or cold block at all times during preparation and use.

8.11 Control DNA must be thawed and kept on ice at all times during preparation and use.

8.12 Clean and decontaminate all work surfaces, BSC, pipettes, and other equipment with a proper *C. auris*-approved decontamination solution such as 10% bleach solution before and after use.

8.13 Use separate and dedicated equipment (e.g., BSC, pipettes) and supplies (e.g., microcentrifuge tubes, pipette tips) for master mix preparations, setup of Optical 96-Well reaction plate, and addition of DNA template in the wells and decontaminate with UV light and/or ELIMINase to prevent DNA contamination after work is completed.

8.14 Reagents, master mix, and DNA should be maintained on cold block during preparation and/or use to ensure stability.

**Quality Control**

8.15 Extraction Controls

8.15.1 A Positive and Negative Extraction control ESwab must be processed each day a new set of specimen swabs is processed.

8.15.2 Prepare the positive extraction control:

8.15.2.1 Using a *C. auris* B11220 stock plate that is < 1 month old, touch a single 1 mm colony with the swab and place the soft end of the collection swab into the tube containing transport medium.

8.15.2.1.1.1 Any *C. auris* isolate from your laboratory may be substituted here. This isolate is pan susceptible.

8.15.2.2 Snap off the end of the swab at the marked line by bending the plastic handle against the edge of the transport tube and secure the tube cap.

8.15.2.3 There should be a *C. auris* positive PCR result for the positive extraction control.

8.15.2.4 If the result is *C. auris* negative, invalidate patient specimen results and retest starting from the primary swab.

8.15.3 Prepare the negative extraction control:

8.15.3.1 Place a clean swab into the ESwab transport medium. Take care not to contaminate the negative control by touching the swab or cap.

8.15.3.2 Snap off the end of the swab at the marked line by bending the plastic handle against the edge of the transport tube and secure the tube cap.

8.15.3.3 The negative extraction control results should be *C. auris* negative and *Bicoid* positive.

8.15.3.4 If there is a *C. auris* positive or *Bicoid* negative result, invalidate patient specimen results and retest starting from the primary swab.

8.15.3.5 Process the positive and negative control the same way as clinical specimens following *Specimen Processing* procedure below.

8.16 PCR controls

8.16.1 A Positive and Negative PCR control should be included with each PCR reaction

8.16.2 Prepare a *C. auris* positive PCR control

8.16.2.1 Genomic DNA should be prepared from a pure culture of *C. auris* B11220 every 6 months and stored at – 20 °C for use as a positive control for each PCR reaction performed. Previous positive extraction controls which were found to be positive for C. auris can be used for the positive PCR control.

8.16.2.2 There should be a *C. auris* positive result for the PCR positive control.

8.16.2.3 If there is a *C. auris* negative result, invalidate patient specimen results and rerun the PCR, starting post extraction.

8.16.3 Prepare a *C. auris* negative PCR control

8.16.3.1 Prepare a PCR negative control by adding Molecular grade water into the PCR master mix.

8.16.3.2 The negative PCR control results should be *C. auris* negative and *Bicoid* positive.

8.16.3.3 If there is a *C. auris* positive or *Bicoid* negative result, invalidate patient specimen results and retest starting from the primary swab.

8.16.3.4

8.16.4 Prepare a No Template Control (NTC)

8.16.4.1 NTCs consist of 5µL PCR grade water spiked into the reaction mix in place of 5µL extracted DNA.

8.16.4.2 NTC should be included in each run NTCs

8.16.4.3 NTCs should return C. auris negative and *Bicoid* negative results.

8.16.4.4 If result is C. auris positive or *Bicoid* positive, invalidate patient specimen results and rerun the PCR, starting post extraction.

8.17 Replication

8.17.1 All samples should be run in triplicate. If there are discordant values between the triplicates then the majority two values take precedence.

**Specimen Processing**

8.18 Assign an Experiment Number for testing.

8.19 Specimens may be stored at 2-8$^{o}$C or ≤ -20 $^{o}$C prior to analysis. Freeze thaw cycles have been shown to not affect the functionality of the assay, as described by Leach L, Zhu Y, Chaturvedi S. Development and Validation of a Real-Time PCR Assay for Rapid Detection of *Candida auris* from Surveillance Samples. J Clin Microbiol., 2018 Jan 24; 56(2), e01223-17.

8.20 Perform all specimen handling steps below in a BSC.

8.21 Verify all specimen information on the tube matches the specimen form.

8.22 Record the ID for each specimen on a Nucleic Acid Extraction Log

**DNA Extraction from Dermal Swabs**

* If you do not have a MagnaPure96, you may validate another extraction method or platform.

8.23 Label a 2mL tube with the specimen ID.
8.24 Working in a BSC, vortex the ESwab to release the specimen from the swab into the Amies buffer.
8.25 Transfer 100µL of the Amies buffer into the labeled 2mL tube.
8.26 Add 100µL Bacterial Lysis Buffer (BLB) to each tube.
8.27 Add 20µL Proteinase K to each tube and cap each tube.
8.28 Vortex for few seconds.
8.29 Place the capped tube in 65°C incubator for 10 min with shaking.
8.30 Return the tube to the BSC and transfer 200µL liquid sample to a MagNA Pure 96 processing cartridge.
8.31 Follow the procedure for *MagNA Pure 96 Automated Nucleic Acid Extraction* SOP using the following specifications:
8.31.1 Reagent Kit: DNA and Viral RNA Small Volume Kit
8.31.2 Protocol: Pathogen Universal 200 3.1
8.31.3 Elution Volume: 50µL

**Master Mix Preparation and Setup of 96-Well reaction plate**

8.32 Prior to use, decontaminate PCR enclosure and pipets with ELIMINase and 70% ethanol to minimize the risk of nucleic acid contamination.
8.33 Working in a PCR enclosure, place 5× PerfeCTa Multiplex qPCR ToughMix, Primers and Probes on cold block at 4-8°C.
8.34 Briefly centrifuge 5× PerfeCTa Multiplex qPCR ToughMix, Primers and Probes, then place on cold block.
8.35 Complete a Reaction Plate Setup Table
8.36 Assign a detector and a control or sample to a designated well.
8.36.1 All analyses should be run in triplicate. Triplicates for a sample should be within 1 Ct.
8.37 Determine the number of reactions being performed per assay. Be sure to account for all positive and negative controls, and triplicates.
***Note: If the number of samples and controls is <14 make enough master mix for one extra reaction, if the total number is >14 make enough for two extra reactions. This will account for pipette error.***
8.38 Calculate the amount of each reagent needed to make up each master mix using the Master Mix Preparation Worksheet, *Attachment # 1*.
8.39 Combine all reagents listed on *Attachment # 1* in the corresponding labeled tube using an appropriate size pipette.
8.40 Pulse vortex master mix 3 times for 5 seconds to homogenize.
8.41 After all master mixes have been prepared, prepare the NTC reactions.
8.41.1 Refer to the Reaction Plate Setup Table and dispense appropriate volume of NTC master mix into the wells designated for NTC reactions.
8.41.2 Securely cap all NTC reactions.

**DNA Template Addition**

8.42 Working in a BSC, gently vortex extracted nucleic acid samples and positive control tubes and place on ice or in a cold rack.

8.43 Set up reaction plate on ice or in a cooler rack.

8.44 Refer to Master Mix Preparation Worksheet, *Attachment #1* and add appropriate volume of *Bicoid* DNA (1µL/Reaction) in appropriate master mix tubes.

8.45 Pulse vortex master mix 3 times for 5 seconds to homogenize.

8.45.1 Dispense 15µL of prepared master mix containing *Bicoid* DNA into each corresponding well.

8.45.2 Carefully pipette 5µL of samples (*Candida auris* DNA) and positive controls into the corresponding wells. Change tips between each sample.

8.45.3 After template addition, securely cap the wells to prevent cross contamination.

8.45.4 Spin down the capped/sealed plate in a centrifuge for 30 seconds at 1000 rcf.

**ABI 7500 Fast Run Setup**

8.46 Create a run template**.**

***Note: Use Reporter dye FAM and Cy3 for Candida auris and Bicoid, respectively.***

8.47 Define the Instrument Settings

8.47.1 Select the **Instrument tab** to define thermal cycling conditions.

8.47.2 Modify the thermal cycling conditions as follows:

8.47.2.1 *In Stage 1, Set to 20 Sec at 95°C; 1 Rep*

8.47.2.2 *In Stage 2, Step 1 set to 03 Sec at 95°C*

8.47.2.3 *In Stage 2, Step 2 set to 30 Sec at 60°C*

8.47.2.4 *In Stage 2, Reps should be changed to 45*

8.47.2.5 *Under Setting, bottom left box, set volume to 20 µL*

8.47.2.6 *Stage 2 Step 2 should be highlighted in yellow to indicate data collection*

8.47.3 Save the file by selecting Save As and give file name as CAP000X_Date (X = Experiment number). Save as type should be SDS Templates (*.sdt).

**Data Analysis**

8.48 After the run has completed perform data analysis.

8.49 Cutoff values must be determined by each laboratory during the validation phase. Cutoff values can vary between different extraction methods and by sample type. Determine the specific cutoff value for each extraction method and sample type. The cutoff value established for this assay is XX:

8.49.1 A sample with a Ct value ≤ XX is positive for *C. auris*

8.49.2 A sample with a Ct value > XX is negative for *C. auris*

8.50 Sample triplicates that do not have corresponding outcomes must be rerun for confirmation using the extracted DNA. If still no result, report as Inconclusive.

8.51 In the event that the sample and the Bicoid are negative, the sample should be processed by culture. This result could indicate the presence of inhibitors.

## 9.0 Primer/Probe Sequences

*C. auris* Primers/Probe:

V2424F (CAURF), 5'-CAG ACG TGA ATC ATC GAA TCT-3'
V2426 (CAURR), 5'-TTT CGT GCA AGC TGT AAT TT-3'
V2425P (CAURP), 5'-/56-carboxyfluorescein (FAM)/AAT CTT CGC /ZEN /GGT GGC GTT GCA TTC A /3IABkFQ/-3'

Bicoid Primers/Probe:

V2375 (BICF), 5'-CAG CTT GCA GAC TCT TAG-3'
V2376 (BICR), 5'-GAA TGA CTC GCT GTA GTG-3'
V2384 (BICP), 5'/Cy3/AAC GCT TTG ACT CCG TCA CCC A /3IAbRQSp/-3'

3IABkFQ: Iowa Black® FQ
3IAbRQSp: Iowa Black RQ

ZEN/Iowa Black FQ is a Double-Quenched Probe, which provides superior performance compared to traditional single-quenched probes

## 10.0 Attachments

Attachment 1: Master Mix Preparation Worksheet (1 Page)

## 11.0 Revision History

| Revision Level | Document Section | Changes Made to Document Section |
|---|---|---|
| 01 | Quality Control | Addition of specifics regarding PCR controls and replication |
| 00 | New | New Document |

## 12.0 Approval Signatures

Approved By: _____ Date: _____
Author

_____
Print Name and Title

Approved By: _____ Date: _____

Technical Reviewer

_____

Print Name and Title

Approved By: _____ Date: _____

Quality Manager / Designee

_____

Print Name and Title

# Master Mix Preparation Worksheet

| Technician: | Exp. ID: | Date: |
|---|---|---|

| Equipment Information | | |
|---|---|---|
| **Equipment ID** | **Calibration Date** | **Calibration Due** |
| **PCR Enclosure:** | | |
| **Pipette:** | | |
| **Pipette:** | | |
| **Pipette:** | | |
| **Pipette:** | | |

| Reagent Information | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Reagent (Stock Conc.)** | **Catalog Number** | **Lot Number** | **Exp. Date** | **Vol./Rxn. (µL)** | | **Total Rxn.** | | **Total Vol. (µL)** |
| Nuclease Free Water | | | | 6.0 | × | | = | |
| 5× PerfeCTa Multiplex qPCR ToughMix | | | | 4.0 | × | | = | |
| *C. auris* Forward primer CAURF (10µM) | | | | 1.0 | × | | = | |
| *C. auris* Reverse primer CAURR (10µM) | | | | 1.0 | × | | = | |
| *C. auris* Probe CAURP (2.5µM) | | | | 0.8 | × | | = | |
| *Bicoid* Forward primer BICF (10µM) | | | | 0.2 | × | | = | |
| *Bicoid* Reverse primer BICR (10µM) | | | | 0.2 | × | | = | |
| *Bicoid* Probe BICP (2.5µM) | | | | 0.8 | × | | = | |
| *Bicoid* DNA | | | | 1.0 | × | | = | |
| **Total Volume** | N/A | N/A | N/A | **15.0** | × | | = | |

Team Lead or Designee Review (Sign/Date):_____

Quality Review (Sign/Date):_____