Instructions for *Candida auris* Real-Time PCR Reagents

v 2.0

For Research Use Only.
Not for use in diagnostic procedures.

Follow the protocol included with the kit.

Key to symbols used

	Store at -25℃ to -15℃
	Consult instructions for use
	This way up
	Recyclable
	Contains sufficient for (n) test
	Catalog number
	Lot number
	Manufacturer
	Use by date
	Fragile
	Date of manufacture

Table of Contents

Product Name

Candida auris Real-Time PCR Reagents

Kit Contents

192 Tests for 15 µL PCR

Intended Use

The *Candida auris* Real-Time PCR Reagents is a real-time PCR test intended for the qualitative detection of *Candida auris* (*C. auris*) from extracted nucleic acid samples collected from human skin swabs, environmental surface swabs, or laboratory cultures.

The *Candida auris* Real-Time PCR Reagents is intended for research use only (RUO).

Principles of the Assay

The *Candida auris* Real-Time PCR Reagents is a real-time polymerase chain reaction test. The reagents utilize sequence-specific primer and TaqMan® probe to amplify the ribosomal RNA (rRNA) gene and the partial genetic region of the internal transcribed spacer (ITS) 1 & 2 of the fungus for *C. auris* identification[1]. A primer/probe set to detect Internal Control (IC), spiked-in to samples prior to extraction, is also included for monitoring extraction and PCR process. For human samples, an additional primer/probe set to detect endogenous human gene (RNase P) is included for human sample quality check.

The probes for the *C. auris*, Internal Control, and RNase P detection are labeled with FAM, HEX/VIC, and CY5 fluorescent dyes, respectively, to generate target-specific signal.

The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

Kit Components and Packaging Specifications

Candida auris Real-Time PCR Reagents (192 reactions for 15 µL PCR)
Part number: DXMDX-RGT-1001

Component Name	Specifications & Loading		Main Ingredients	Storage Conditions
Reagent A	950 µL	× 1 tube	Buffers, dNTPs, Mg^{2+}	-25 to -15°C

CAU Reagent B	230 µL	× 1 tube	TE buffer, primers, probes	-25 to -15°C
Reagent C	150 µL	× 1 tube	Taq DNA polymerase, UNG	-25 to -15°C
Negative Control	1.4 mL	× 1 tube	TE buffer	-25 to -15°C
Internal Control	1.4 mL	× 2 tubes	Synthetic plasmid of internal control.	-25 to -15°C
CAU Positive Control	200 µL	× 1 tube	Synthetic plasmid mixture that contains the fragments of *C. auris* ITS2 gene and the RNase P gene.	-25 to -15°C

Candida auris Real-Time PCR Reagents Positive Control (20 tests for 15 µL PCR)
Part number: DXMDX-CTL-1002

Component Name	Specifications & Loading		Main Ingredients	Storage Conditions
CAU Positive Control	200 µL	× 1 tube	Synthetic plasmid mixture that contains the fragments of *C. auris* ITS2 gene and the RNase P gene.	-25 to -15°C

Materials Required but Not Provided

1. DNA extraction reagents and instrument and related software that have been validated with the *Candida auris* Real-Time PCR Reagents: CMG-1033-G chemagic Pathogen NA H96 and chemagic™ 360 (2024-0020) with chemagic™ Rod Head Set 96 (CMG-370) (chemagic™ MSM I software version 6.1.0.5).
2. Real-time PCR Instruments with FAM™, HEX™/VIC™, and Cy5® channels (e.g., Applied Biosystems™ 7500 Fast or Fast Dx Real-Time PCR System, Applied Biosystems™ QuantStudio™ 3, 5, 6, 7 Flex, or Dx Real-Time Instrument, BioRad® CFX96™ or CFX384™ Touch Real-Time PCR Detection System, Revvity Eonis™ Q System, Analytik Jena qTower3 /3G or qTower3 84 / 3 84G Real-Time PCR System). The usage of the product will be defined by the available fluorescence detectors of the specific instrument.
3. Additional tools and consumables:
   a. Consumables for automated nucleic acid extraction using chemagic™ 360.
      - Microseal® 'F' PCR Plate Seal, foil, pierceable (Bio-Rad, MSF1001)
   b. Tools and consumables
      - Centrifuge (Eppendorf, Epp 5810/ 5810 R)
      - Vortex Mixer (VWR, 97043-562)
      - Heat-block or water bath incubator or equivalent
   c. Micropipettors (range between 1 to 20 µL, 20 to 200 µL and 100 to 1000 µL)
   d. Non-aerosol pipette tips
   e. Nuclease-free water or 1X TE buffer

Reagent Storage & Handing Requirements

1. Store all reagents at -25 to -15°C.
2. Completely thaw reagents before use.
3. Reagents are stable within six cycles of freeze-thaw. **Reagent A may precipitate upon thawing. Mix reagent at room temperature until fully dissolved.** Once thawed, store at 2 - 8 °C for up to a week. Note that Reagent C (an enzyme mix) shall always be stored at -25 to -15°C.

Sample Collection and Storage

1. Before starting, perform hand hygiene and wear appropriate personal protective equipment.
2. Take out the clean swab from the package by grasping the plastic handle at the opposite end from the soft tip. Be careful not to touch the soft tip.
3. Firmly rub the soft end of the collection swab across the indicated site at least 5 times.

   Single swab axilla and groin composite collection:

   - Rub both sides of the swab tip over the left axilla skin surface targeting the crease in the skin where the arm meets the body. Swipe back and forth ~ 5 times per armpit. Repeat on the right side with the same swab.
   - With the same swab used on axilla, repeat the same procedure over the left groin skin surface targeting the inguinal crease in the skin where the leg meets the pelvic region. Repeat on the right side with the same swab.

4. Remove the swab collection tube cap and place the soft end of the collection swab into the tube. Be careful not to touch any material that may contaminate the sample and the collection device.
5. Bend the plastic handle against the edge of the collection tube to snap off the end of the swab at the marked line. Ensure the soft tip is submerged in the collection liquid.
6. Fasten the tube cap securely. Adjustment may be needed until the snapped end of the swab slides into place in the center of the cap.
7. Apply specimen information on the collection device.
8. Send or ship immediately to a testing laboratory.
   - Specimens should be stored at 4 - 25°C and shipped with a cold pack to the laboratory.
   - Specimens should be tested within 96 hours (4 days) of collection.

Detail procedures for the collection of swabs for *C. auris*[2] follow CDC recommendations at
https://www.cdc.gov/fungal/candida-auris/c-auris-patient-swab.html

Warnings and Precautions

1. This is a Research Use Only (RUO) kit.
2. Keep the kit upright during storage and transportation.
3. Before using the kit, check tubes for leakage or damage. Each kit component (except Reagent C), should be thawed at room temperature, thoroughly mixed, and centrifuged before use. **NOTE:** Reagent C (enzyme mix) is liquid at -25 to -15 °C and should be kept at that temperature or on ice until use.
4. Cross-contamination may occur when inappropriate handling of reference materials and specimens; which could cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
5. All specimens to be tested and the reference materials of the kits should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution and then cleaned with 70% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes.
6. ABI 7500 Fast and Fast Dx, QuantStudio™ 3, 5, 6, 7 Flex and Dx used for this assay should be calibrated regularly according to the instrument's instructions to eliminate crosstalk between channels.
7. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
8. Do not use reagents after the expiration date.
9. Do not use the kit if the outer box sealing label is broken upon arrival.
10. Do not use reagents if the tube caps are open or broken upon arrival.
11. Dispose of waste according to local, state, and federal regulations.

Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not

limited to) disposable powder-free gloves, hats, protective lab coats and goggles. Change gloves often when handling reagents or samples.
2. Wash hands thoroughly after handling specimens and reagents.
3. Handle all specimens and waste materials as if they could transmit infectious agents in accordance with Universal Precautions.
4. Follow national biological safety recommendations for handling biological samples.
5. Refer to the Clinical and Laboratory Standards Institute (CLSI) Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline (M29), for safety precautions.

Laboratory Precautions for Contamination Prevention

1. Do not handle human samples in a biosafety cabinet which is used for *C. auris* culturing purposes.
2. Prior to processing samples, thoroughly clean the work area with freshly prepared 10% bleach or 70% ethanol. Then wipe the work area with water.
3. Avoid excessive handling of the Positive Control to avoid contamination.
4. Change gloves after handling the Positive Control.
5. If spillage of specimen or Positive Control occurs, immediately disinfect the area with freshly prepared 0.5% sodium hypochlorite (bleach) or follow appropriate laboratory biosafety procedures.
6. After amplification is complete, immediately place the PCR plates in a sealable bag; ensure the bag is sealed, then discard the plates in a biohazard container.
7. Change gloves after handling a post PCR plate.
8. All materials used in one area should remain in that area and should not be moved or used in other areas. Never bring post PCR plates to other areas, such as PCR set up area and sample preparation area.

Assay Procedure

Sample Input Requirements

Extracted DNA is the test sample for the *Candida auris* Real-Time PCR Reagents. The kit Internal Control (IC) must be spiked-in to samples prior to extraction. Follow appendix for details on nucleic acid extraction recommendation and Positive Control (PC) preparation.

96- or 384-well platform PCR Setup and Amplification

Setup PCR Manually for 15 µL PCR Reactions with FAM™ and HEX™/VIC™ Channels for Non-human Samples, or FAM™, HEX™/VIC™ and Cy5® Channels for Human Samples

1) Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Note:
Reagent A may precipitate. Keep it at room temperature and mix well to ensure complete resuspension before use**.**

Component	Volume/ test	Volume for N Samples, Positive Control, and Negative Control *	110% of volume
Reagent A	3.75 µL	3.75 x (n + 2) µL	4.125 x (n + 2) µL
CAU Reagent B	0.75 µL	0.75 x (n + 2) µL	0.825 x (n + 2) µL
Reagent C	0.50 µL	0.50 x (n + 2) µL	0.55 x (n + 2) µL

*: the consumption of PCR mix for the no template control is not included in the "+2" reactions.

1) Vortex the prepared PCR mix to ensure it is fully mixed, then centrifuge briefly to collect in the bottom of the tube.

2) Pipette 5 µL PCR mix into each well of a 96- or 384-well PCR plate.

3) Add 10 µL of extracted nucleic acid of samples into assigned wells containing PCR mix.

4) Negative control (NC) (required): Add 10 µL of extracted Negative Control into the negative control well-containing PCR mix.

5) Positive Control (PC) (required): Add 10 µL of extracted CAU Positive Control into the positive control well-containing PCR mix.

6) No Template Control (NTC) (Optional): Add 10 µL of kit negative control stock into the no template control well-containing PCR mix.

7) Seal the PCR plate with an appropriate film.

8) Vortex the sealed plate for 10 seconds, and centrifuge for 3 minutes at 350x g.

<u>Amplification</u>

1) Set up and run the PCR instrument according to the instrument reference

guide.

Note: Some instruments may by default use ROX as passive reference. Make sure to select “NONE” on the passive reference setting.

2) Set the thermal cycling condition as below.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	94°C	10 minutes	1
3	94°C	10 seconds	40
	55°C	15 seconds	
	65°C*	45 seconds	

** Collect fluorescence signal during the final 65°C step.*

Note: Select “Fast” run mode if Fast mode is available on instrument with 384-well platform or select “Fast 96-well (0.1mL)” with 96-well platform for QuantStudio™ 3, 5, 6, 7 Flex or Dx instruments. Select “7500 Fast (96 wells)” for ABI 7500 Fast systems.

3) Make sure the reaction volume setting is correct to 15 µL.

4) Fluorophore settings

- For culture or environmental samples (non-human samples), select the fluorophore setting as below.

Target Name or Detector	Channel
Candida auris	FAM
Internal Control	HEX/VIC

- For human samples without culture enrichment, select the fluorophore setting as below.

Target Name or Detector	Channel
Candida auris	FAM
Internal Control	HEX/VIC
RNase P	Cy5

i. For qTower$^{3/3}$G, qTower$^{384/384}$G, or Eonis™ Q systems with non-human samples, activate the following measurement detectors. The passive reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement

1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX_3	5	X
4	Orange	ROX	5	
5	Red	Cy5	5	
6	NIR1	Cy5.5	5	

- **Color compensation: Standard 1**

ii. For qTower^{3}/^{3}G, qTower^{384}/^{384}G, or Eonis^{TM} Q systems with human samples, activate the following measurement detectors. The passive reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX_3	5	X
4	Orange	ROX	5	
5	Red	Cy5	5	X
6	NIR1	Cy5.5	5	

- **Color compensation: Standard 1**

Note: All six Pos. and Channel options must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Pos. and Channel options may not show up in Scan setting.

5) Double check all settings and start the run.

Interpretation of Results

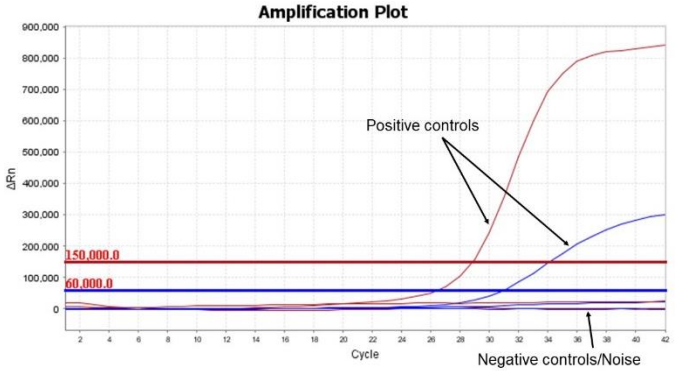
Baseline and threshold setting for ABI 7500 Fast or Fast Dx, QuantStudio^{TM} 3, QuantStudio^{TM} 5, QuantStudio^{TM} 6, QuantStudio^{TM} 7 Flex, QuantStudio^{TM} Dx, CFX^{TM} 96, and CFX^{TM} 384

After the run completion, save and analyze the data according to PCR instrument instructions.

1) Set baseline for each target:
The horizontal part of the baseline is used for the baseline range, which normally starts from 3-5 cycles and ends at 15-20 cycles. Baseline setting is normally automatically done by instrument. Manual baseline 3-15 is recommended as general.

2) Set threshold for each target:

Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal (refer to the background signal of true negative samples). The threshold value for different instruments varies due to different signal intensities. One example from QuantStudio™ Dx instrument is given below.

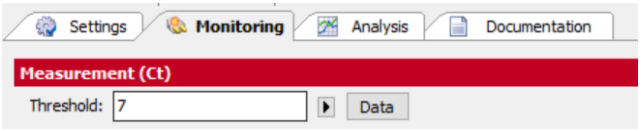


3) Interpret the results based on the tables listed in “Quality Control” and “Ct cutoff and result interpretation”.

Baseline and threshold setting for qTower$^{3/3G}$, qTower$^{3\ 84/84G}$, and Eonis™ Q

After the run completion, save and analyze the data according to PCR instrument instructions.
Under the **Settings** tab, for color compensation configuration, select “Standard1”.

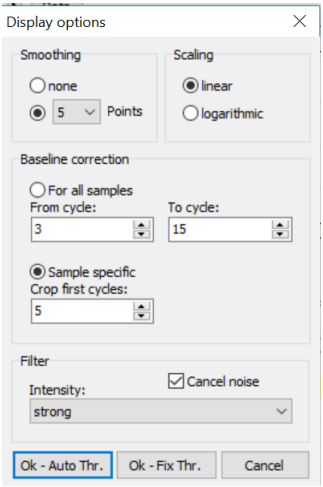
Under the **Monitoring** tab, click “Calculate Ct”, then the following view shows up.



1) Set baseline for each target:
In most cases, the default baseline can be used. In order to adjust baseline, click icon 

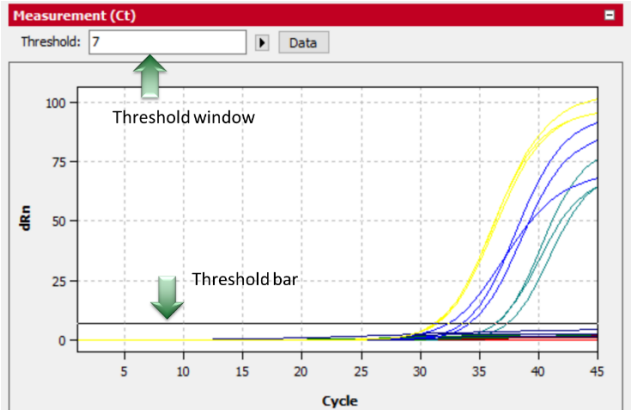
The default setting is “Sample specific crop first cycles”, which is good for most of the cases. The default is 5, which can be adjusted, for example 10 or 15 to minimize background noises in some cases.

To set up a different baseline, click “For all samples”, from cycle X (default 3) to Y (default 15) as the following window.



To switch back to the default setting, click “Sample specific Crop first cycles”.

2) Set threshold for each target:
   Under the **Monitoring** tab, view the threshold values under “linear” scaling (shown in above figure) for each target. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signals. The threshold value for different instruments varies due to different signal intensities. It is recommended to set up threshold manually instead of default settings. For manual threshold setup, either move threshold bar up and down, or manually input threshold number to the “Threshold” window, shown in the following figure. It is recommended to set up the threshold in the range of 5-15 as general.



3) Interpret the results based on the tables listed in “Quality Control” and “Ct cutoff and result interpretation”.

Quality Control

The provided Negative Control (TE buffer), Internal control, and Positive Control monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification. All test controls must be examined prior to interpretation of test sample results. Positive Control and Negative Control must meet the requirements listed in the table below to ensure valid results. If the controls are not valid, the results cannot be interpreted.

1) Negative Control (NC): A negative control is required to monitor reagent and/or environment contamination. One PCR plate should include one extracted NC. The final negative control (spiked-in with IC) should be included from sample extraction to PCR. The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the negative control is invalid. See Appendix A for NC preparation prior to extraction.

2) Positive Control (PC): A positive control is required to monitor reagent performance. It can be used to assist the threshold setup to differentiate the amplification signal vs. instrument background noises or signal drift. One PCR plate should include one extracted PC. The final PC (spike-in with IC) should be included from sample extraction to PCR. The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the positive control is invalid. See Appendix A for PC preparation prior to extraction.

3) Internal Control (IC): IC serves as a process control for monitoring entire process from sample extraction to PCR amplification and detection. It is required to spike into Negative Control, Positive Control, and test specimens. It is not used for monitoring the lysis efficiency. See Appendix A for IC preparation prior to extraction.

4) Endogenous Control (human reference gene RNase P): The endogenous human gene RNase P detection is required for samples collected from humans only. The RNase P serves as an endogenous internal control that is used together with the data from other targets for interpretation of an individual specimen. RNase P must be positive (≤ 35 Ct) for all human specimens in order to report negative results for the target analytes.

   If RNase P is negative in the presence of a positive result for *C. auris* target, the target result should be considered valid.

   However, if *C. auris* target generate negative results and RNase P is also negative (> 35 Ct or not determined), the test is considered as invalid. Failure to detect RNase P in the specimens could indicate:

   - Insufficient nucleic acid extraction from tested samples
   - Poor specimen quality or loss of specimen integrity
   - Improper assay execution
   - Reagent or equipment malfunction

   If the result for any test specimen collected from human is invalid, repeat testing of specimen nucleic acid and/or re-extract and repeat PCR. If repeat test is invalid, collection of a new specimen and subsequent testing should be considered.

5) No Template Control (NTC): A no template control is an optional control to be included to monitor PCR reagent and/or environment contamination. The NC without IC spike-in is used as NTC, without the process of sample extraction. The Ct requirements are listed in the following table. If one of the targets fails the Ct requirements, the no template control is invalid.

The Ct requirements for each control and specific sample types are listed in the following tables.

Ct cutoff and result interpretation

Regardless of sample type, all required controls must be valid prior to sample result interpretation.

i. For cultured sample or environmental sample (non-human sample)

Control Type	Ct	
	***Candida auris* (FAM)**	**Internal Control (HEX/VIC)**
No Template Control*	Undet/blank or >40	Undet/blank or >35
Negative Control	Undet/blank or >40	≤ 35
Positive Control (with IC spiked-in)	≤ 35	≤ 35

Undet: Undetermined.

*: Only if included for PCR.

Ct		Result interpretation
***Candida auris* (FAM)**	**Internal Control (HEX/VIC)**	
≤ 40	/	*Candida auris* detected.
Undet/blank or >40	≤ 35	*Candida auris* not detected.
Undet/blank or >40	Undet/blank or > 35	Invalid. Sample needs to be re-tested by repeating PCR if there is sufficient extracted DNA. Otherwise, sample needs to be re-tested by re-extraction or re-collection from the original source.

Undet: Undetermined

/: No requirements on the Ct value.

ii. For human sample without culture enrichment

Control Type	Ct		
	***Candida auris* (FAM)**	**Internal Control (HEX/VIC)**	**RNase P (Cy5)**
No Template Control*	Undet/blank or >40	Undet/blank or >35	Undet/blank or >35
Negative Control	Undet/blank or >40	≤ 35	Undet/blank or >35
Positive Control (with IC spiked-in)	≤ 35	≤ 35	≤ 35

Undet: Undetermined.

*: Only if included for PCR.

Ct			Result interpretation
***Candida auris* (FAM)**	**Internal Control (HEX/VIC)**	**RNase P (Cy5)**	
≤ 40	/	/	*Candida auris* detected.
Undet/blank or >40	/	≤ 35	*Candida auris* not detected.
Undet/blank or >40	≤ 35	Undet/blank or > 35	Extraction valid but sample quality invalid. Sample needs to be re-tested by re-extraction or re-collection from specimen.
Undet/blank or >40	Undet/blank or > 35	Undet/blank or > 35	Extraction invalid. sample needs to be re-tested by re-extraction or re-collection from specimen.

Undet: Undetermined

/: No requirements on the Ct value.

Kit Limitations

1. This kit is used for qualitative detection of *C. auris* DNA.
2. The specimens to be tested shall be collected, processed, stored, and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
3. The DNA extraction method listed in the Appendix is validated with *C. auris* culture in liquid amies solution. It has not been validated with *C. auris* positive human skin sample.
4. *C. auris* primer/probe for this kit targets a highly conserved ITS region within the *C. auris* genome. Mutations occurring in this conserved region may result in DNA being undetectable. Other standard laboratory methods (i.e., phenotypic-base, whole genome sequencing, MALDI-TOF) for *C. auris* identity confirmation are highly recommended.
5. This kit uses a UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories.

6. This kit was not tested for all known/unknown cross-reactants i.e., some fungi, bacteria, and viruses.
7. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics, or immunosuppressant drugs have not been evaluated.

Assay Performance

Limit of Detection (LoD)

The analytical sensitivity of the *Candida auris* Real-Time PCR Reagents was evaluated with synthetic material quantified by ddPCR. The assay can detect as low as 30 copies per PCR reaction when synthetic control was spiked-into PCR directly without sample extraction process.

Analytical Reactivity (Inclusivity)

Inclusivity of the *Candida auris* Real-Time PCR Reagents was evaluated using *in silico* analysis by BLASTn against the assay ITS primer/probe sequences to the publicly available *C. auris* full genome sequences (42), including all five clades host by human, in GenBank as of May 8, 2023. All five clades (I: South Asian, II: East Asian, III: African, IV: South American, and V: Iran) are predicted to be detectable by the reagents, with the exception to the 10 strains in which were identified in Lebanon in 2021 (see table below).

#	1	2	3	4	5	6	7	8	9	10
Strain	CA14LBN	CA19LBN	CA21LBN	CA24LBN	CA25LBN	CA29LBN	CA2LBN	CA4LBN	CA6LBN	CA9LBN

Analytical Specificity (Cross-reactivity)

Cross-reactivity of the *Candida auris* Real-Time PCR Reagents was evaluated using *in silico* analysis by BLASTn against the assay ITS primer/probe sequences to the full genome of 103 non-*C. auris* microorganisms at which belongs to the candida family or are often found on skin infections. Cross-reactivity is not predicted.

References

1. A TaqMan Probe-Based Real-Time PCR Assay for the Rapid Identification of the Emerging Multidrug-Resistant Pathogen *Candida auris* on the BD Max System. *Journal of clinical microbiology,* May 8, 2019.
2. CDC: Procedure for Collection of Patient Swabs for *Candida auris*. Reviewed version: December 14, 2022.

Appendix

A. Nucleic Acid Extraction with chemagic™ 360 system

CMG-1033-G chemagic Pathogen NA H96

Please follow chemagic™ 360 User Manual for extraction setup. The protocol file must be installed prior to starting the chemagic™ 360 software.

A quick-start instruction to support skin swabs in liquid Amies solution or resuspended culture is described below.

1) Prepare Positive Control (PC) for extraction in a biological safety cabinet. If the kit control stock is frozen, completely thaw it at room temperature. Vortex the kit control stock 3 seconds x 3 times at maximum speed, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes. Follow the table below to prepare one PC (total 300 µL) per extraction in a DNase/RNase-free microcentrifuge tube.

   Please note:
   i. Nuclease-free water is not provided in the kit. Liquid Amies or 1X TE buffer can be used to substitute for nuclease-free water.
   ii. A master mix of PC can be prepared followed by aliquoting 300 µL per tube, only if multiple extraction runs are planned at the same time.

One Positive Control (PC) per Extraction		110% Volume for N >1 Extraction runs
Nuclease-free water*	290 µL	319 x (n) µL
CAU Positive Control	10 µL	11 x (n) µL
Total Volume	**300 µL**	-

*: Nuclease-free water can be replaced by 1X TE buffer

2) Prepare Negative Control (NC) for extraction in a biological safety cabinet. If the kit control stock is frozen, completely thaw it at room temperature. Vortex the kit control stock 3 seconds x 3 times at maximum speed, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes. Follow the table below to prepare one NC (total 300 µL) per extraction in a DNase/RNase free microcentrifuge tube.

One Negative Control (NC) per Extraction	
Negative Control	300 µL
Total volume	**300 µL**

   Please note:
   i. Nuclease-free water can be used to substitute the kit negative control.
   ii. Multiple NCs can be prepared with 300 µL each, only if multiple extraction runs are planned at the same time.

3) Prepare specimens and place them in a biological safety cabinet. Vortex the collection tube for 3 seconds x 3 times at maximum speed to release samples into the liquid solution, then centrifuge the tube briefly at 1000 rpm to collect the liquid to the bottom of the tubes before use.

4) Prepare extraction premix in a DNase/RNase-free container in a biological safety cabinet according to the following table. Gently invert the premix tube up and down 5 times to mix well followed by a quick spin down.

Please note:

i. Dissolve lyophilized Proteinase K in H_2O before use (volume is given on the label).
ii. Keep extraction premix at room temperature. It may form precipitation if placed on ice.

Extraction premix (for N sample, one PC, and one NC)		
Component	**Volume/ well**	**110% Volume for N+2 reactions**
Tissue Lysis Buffer	300 µL	330 x (n+2) µL
Proteinase K	10 µL	11 x (n+2) µL
Internal Control (IC)	10 µL	11 x (n+2) µL
Final input volume per extraction well	**320 µL**	**-**

5) Transfer 320 µL of the extraction premix to new 1.5 mL (or 2 mL) DNase/RNase-free microcentrifuge tubes. One for each sample.

6) Add 300 µL of sample to each microcentrifuge tube containing the 320 µL extraction premix.

7) Transfer 320 µL of the extraction premix to the tube containing the 300 µL Negative Control (NC) prepared for extraction.

8) Transfer 320 µL of the extraction premix to the tube containing the 300 µL Positive Control (PC) prepared for extraction.

9) Vortex the tubes containing the sample or controls in the extraction mix at maximum speed for 3 seconds x 3 times, followed by a quick spin down.

10) Incubate samples and control tubes at 56°C for 30 minutes.

11) After incubation, spin down the tubes at maximum speed for 15 seconds to collect liquid from the cap.

12) Transfer 600 µL of individual heat lysed samples, PC and NC to corresponding wells in a new 2 mL deep-well-plate (riplate SW)

13) In a low-well plate, add 150 µL magnetic beads into each well.

14) In a new 2 mL deep-well plate (riplate SW), add 60 µL Elution Buffer 6 into each well.

15) Turn on the chemagic 360, double click the software icon “chemagic_360”, select username and enter password to start. Follow the chemagic 360 User Manual to select the appropriate protocol.

16) Load the magnetic rod disposable tips box onto the tracking system (table) according to the instructions given by the chemagic software, the tip rack should be in the position indicated in the table below.

17) Load the plates manually onto the tracking system (table) according to the instructions given by the chemagic software. The plates should be in the positions indicated in the table below.

Please note:

i. Specimens and Magnetic Beads should be thoroughly vortex mixed before use.
ii. Never move the tracking system (table) manually. All movements must be performed with the [Turn Table] function in the instrument software.

chemagic™ 360 layout:

Position 1	Magnetic rods disposable tips
Position 2	Low-well-plate (MICROTITER SYSTEM) prefilled with 150 µL Magnetic Beads
Position 3	Deep-well-plate (riplate SW) containing: 600 µL of each heat-lysed sample and controls only [Binding Buffer 2 will be added automatically]
Position 4	Deep-well-plate (riplate SW) containing: Empty deep-well-plate (riplate SW) [Wash Buffer 3 will be added automatically]
Position 5	Deep-well-plate (riplate SW) containing: Empty deep-well-plate (riplate SW) [Wash Buffer 4 will be added automatically]

Position 6	Deep-well-plate (riplate SW) containing: Empty deep-well-plate (riplate SW) [Wash Buffer 5 will be added automatically]
Position 7	Deep-well-plate (riplate SW) prefilled with 60 µL Elution Buffer 6

18) Double-check the positions and directions of all consumables according to the tracking system.

19) Click “Start” to start the extraction process.

20) Proceed to the downstream assay with the extracted nucleic acids or seal the plate and store the nucleic acids at 2 - 8 °C within 48 hours or at –25 to -15 °C for long-term storage.

Revision history:

Revision	Date	Description
1.0	June 2023	New document for *Candida auris* Real-Time PCR Reagents
2.0	January 2024	Updated contact email address.

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