Candidozyma auris RUO PCR

REF #: OLM2010-RUO

qPCR kit for the detection of *Candidozyma auris* (*Candida auris*)

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

INTENDED PURPOSE

Candidozyma auris RUO PCR provides reagents for the detection of *Candidozyma auris* (formerly *Candida auris*) DNA from extracted research samples or cultured materials.

BIOLOGICAL PRINCIPLES

REAL-TIME PCR

Individual primer and probe designs for detection of *C. auris*, and a synthetic Internal Extraction Control template (introduced at the sample extraction stage) have been combined into a single assay and their DNA can be detected through the different fluorescent channels as described in the kit contents.

IMMY's *Candidozyma auris* RUO PCR test kit makes use of the most widely used qPCR chemistry, which is based on the detection of light emitted by hydrolysis probes (Figure 1).

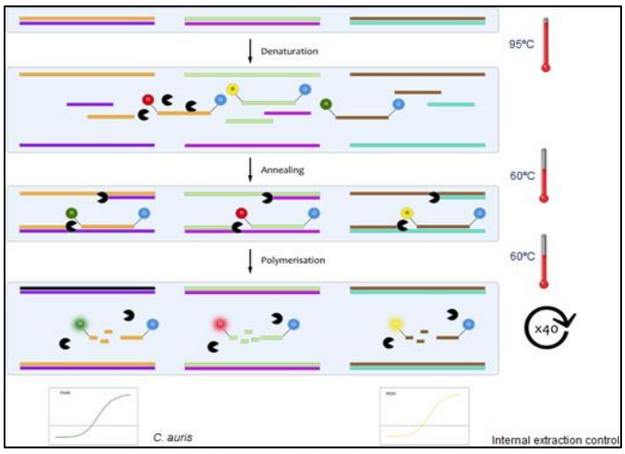

Figure 1. Principle of hydrolysis probe-based test. Double stranded genomic DNA, represented by the colored strands, is denatured as the sample is briefly heated to 95°C. During the annealing phase of the PCR the temperature is reduced and forward and reverse primers (short colored lines) hybridize to any complementary target DNA present. The same reaction mixture also contains fluorogenic probes, which consist of target-specific DNA oligonucleotides labelled with a 5`-fluorescent reporter dye (R) and a quencher (Q). In the case of the IMMY *Candidozyma auris* RUO PCR test kit, these probes are specific for *C. auris* (FAM) and the Internal Extraction Control (ROX). During the polymerization phase of the PCR, any bound probes are displaced and cleaved by the 5' nuclease activity of the Taq polymerase (black circles), resulting in the physical separation of the reporters and quenchers. This results in the emission of light at fluorophore- specific wavelengths, which can be detected in the appropriate channels of a qPCR instrument.

POSITIVE CONTROL (PC)

The kit contains a Positive Control tube with the template for the assay target (*C. auris*). The Positive Control is handled like a normal nucleic acid extract and indicates that the primers and probe for detecting *C. auris* are working properly in the run. Inclusion of the Positive Control depends on the preference of the end user and is not mandatory in every run. However, IMMY recommends its use in every run as it provides more confidence in the results if all samples are negative. The Positive Control does not need to be subjected to a nucleic acid extraction procedure. Care should be taken to avoid cross-contamination of other samples when adding the Positive Control to the run. Any risk can be minimized by sealing all other samples and Negative Controls before pipetting the Positive Control into the Positive Control well.

NO TEMPLATE CONTROL (NTC)

To confirm the absence of contamination, at least one No Template Control (NTC) reaction must be included in every PCR run. For this reaction, RNase/DNase free water should be used instead of template.

INTERNAL EXTRACTION CONTROL (IEC)

The Internal Extraction Control (IEC) is added to distinguish true negative samples from false negative samples, which can result from nucleic acid degradation, failure of nucleic acid extraction step, PCR inhibition or qPCR instrument malfunction. The primers and probe necessary to detect the IEC are included in the kit Primer/Probe mix. The IEC will give a quantification cycle (Cq) value of >25, with the variation depending on the efficiency of sample extraction and level of sample dilution.

REAGENTS PROVIDED

Each kit contains sufficient reagents for 50 tests.

***Candidozyma auris* RUO PCR Primer/Probe Mix** FAM and ROX Labeled (see below) **Target** / **Fluorophore** / **Absorption (nm)** / **Emission (nm)** *C. auris* / FAM / 494 / 518 Internal Extraction Control / ROX / 575 / 602	> 100 µL
Positive Control *C. auris target template*	> 250 µL
Internal Extraction Control DNA (IEC)	> 250 µL
qPCR Master Mix	> 500 µL
RNase/DNase-Free Water	> 400 µL

Refer to the Safety Data Sheets for more information on hazards and warnings.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable gloves
- Protective glasses
- DNA extraction kit
- Pipette(s) and associated disposable tips
- 1.5 mL microcentrifuge tubes
- Vortex mixer
- Centrifuge
- qPCR plate and plate seals
- qPCR instrument
- Biohazard waste receptacle

REAGENT STABILITY AND STORAGE

The entire test kit should be stored at -20 °C until the expiration date printed on the product label (18 months from date of manufacture). Components should be kept at 0 °C, not be exposed to temperatures above -20 °C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. Please note that exposure to light leads to photo bleaching of the fluorescent reporters and so reduces the sensitivity of the assay.

The quality of the product cannot be guaranteed after the expiration date.

REAGENT PRECAUTIONS

1. IMMY cannot guarantee the performance of its products when used with materials purchased from other manufacturers. Do not interchange reagents from different kit lot numbers or other manufacturers.
2. The user assumes full responsibility for any modification to the procedures published herein.
3. Avoid reagent contamination by following contamination control practices for PCR and segregating workflow as appropriate.
4. Always ensure reagents are defrosted thoroughly, mixed, spun down, and kept on ice.
5. Do not use kit or any kit reagents after the stated expiration date.

WARNINGS AND PRECAUTIONS FOR USERS

1. Wear protective clothing, including lab coat, eye/face protection, and disposable gloves, and handle the kit reagents and research samples according to standard precautions. Wash hands thoroughly after performing the test.
2. Avoid splashing samples or solutions.
3. Biological spills should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach, 70% ethanol, or 0.5% Wescodyne Plus™. Materials used to wipe up spills may require biohazardous waste disposal.
4. Dispose of all samples and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous waste must be handled and discarded in accordance with all local, regional, and national regulations.
5. Standard precautions and local laboratory guidelines should be followed.
6. Low concentrations of DNA can be unstable if stored for long periods. IMMY advises that sample storage time should be minimized before testing.
7. Ensure all additional required consumables are RNase/DNase-free.
8. Use disposable filter tips for all pipetting.
9. Thaw DNA samples on ice and keep on ice.
10. Safety Data Sheets are available upon request.

SAMPLES

DNA should be extracted from culture or research derived samples. Extracted samples should be stored between -80 °C and -20 °C for long-term storage. The IMMY *Candidozyma auris* RUO PCR kit is designed for use with research samples prepared using nucleic acid extraction methods compatible with PCR amplification. Successful amplification depends on the quality of nucleic acids in the sample. Samples with degraded DNA, low concentration, or PCR inhibitors may yield invalid or negative results. Always run at least one negative (no template) control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase-free water.

PROCEDURE

1. For research protocols exploring direct detection of *C. auris* from swab samples without a DNA extraction step, refer to Appendix 1.
2. Remove the test kit from the freezer and allow the reagents to thaw. Thawed reagents must be kept on ice. Briefly vortex and centrifuge all reagent tubes prior to use.

DNA EXTRACTION

1. The IEC DNA can be added either to the DNA lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.
   NOTE: DO NOT add the Internal Extraction Control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.
2. Add 5 µL of the Internal Extraction Control (IEC) DNA to each sample in DNA lysis/extraction buffer for an elution volume of 50 µL. For eluting into different volumes adjust volume of the IEC DNA accordingly.
3. Complete the DNA extraction according to the manufacturer's recommended protocols.

PCR DETECTION PROTOCOL

1. Prepare a PCR premix according to the table below. Volumes are given per reaction well and should be multiplied by the number of reaction wells required. Include sufficient reactions for positive and negative controls. The prepared PCR premix should be thoroughly mixed and briefly spun down.

Component	Volume/Reaction
qPCR Master Mix	10 µL
Primer/Probe Mix	2 µL
RNase/DNase-Free Water	2 µL
Final Volume	14 µL

Due to small variations in pipetting accuracy, we recommend that you allow an additional 10% for the final volume, i.e. if you are assaying ten samples, make up sufficient reaction mix for 11 tests.

2. Pipette 14 µL of prepared PCR premix into each reaction well according to your qPCR experimental plate set up.
3. Prepare sample DNA templates for each of your samples (see DNA Extraction, above).
4. Pipette 6 µL of DNA template into each well according to your experimental plate set up.
5. For negative control wells use 6 µL of RNase/DNase-free water. For Positive Control use 6 µL of Positive Control. The final volume in each well is 20 µL.
6. Ensure that your PCR reaction plate is sealed and briefly centrifuged before transferring to a validated thermocycler for amplification.

AMPLIFICATION PROTOCOL

1. If using an instrument that uses ROX as a passive reference then the passive reference must be turned off or set to "none" for no passive reference, as the IEC uses the ROX channel.
2. Please refer to your instrument manual for instructions on setting up an amplification run. Amplification should be carried out according to the conditions detailed the table below.

	Step	Time	Temp
Cycling x40	Enzyme Activation	2 mins	95 °C
	Denaturation	5 secs	95 °C
	Data Collection*	20 secs	60 °C

** Fluorogenic data should be collected during this step through the FAM and ROX channels*

RESULTS

Refer to the instruction manual for your thermocycler for information on how to operate the qPCR instrument and perform data analysis.

It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

NOTE: Cq estimations stated below are calculated with threshold positioned at the center of the log-linear range of the PCR amplification curve.

POSITIVE CONTROL (PC)

The supplied Positive Control should record a characteristic amplification plot through the FAM (*C. auris*) channel, with Cq of 27±2. There is no Internal Extraction Control template within the Positive Control so the ROX channel should record no signal (flat amplification plots). The Positive Control signals indicate that the kit is working correctly for *C. auris* detection.

NO TEMPLATE CONTROL (NTC)

The NTC should give a flat line (flat amplification plots) through both FAM and ROX channels. Signals in the NTC may indicate cross contamination during plate set up.

SAMPLE DATA

1. Successful sample extraction is indicated by a positive signal in the ROX channel (Cq >25), with lower Cqs indicative of more efficient extraction.
2. Absence of a signal in the FAM channel suggests that the sample does not contain *C. auris*.
3. A signal in the FAM channel indicates the presence of *C. auris*.
4. It is possible to obtain a signal in the FAM channel without also recording a signal in the ROX channel. Typically, this occurs when a high fungal load results in early amplification (i.e. low Cqs) and the accumulation of target DNA inhibits the amplification of the IEC DNA. This result is still valid.
5. If there is no signal in either channel, the assay has failed and no conclusions are possible.

Detection Channels		
FAM	**ROX**	**Result**
+	+	*C. auris POSITIVE sample. IEC PASS.* ***Valid result***.
-	+	*C. auris NEGATIVE sample. IEC PASS.* ***Valid result***.
+	-	*C. auris POSITIVE sample.* IEC outcompeted by high *C. auris* target DNA load. ***Valid result***.
-	-	***Invalid result.***

LIMITATIONS OF THE PROCEDURE

1. For research use only. Not for use in diagnostic procedures.

INTERNATIONAL SYMBOL USAGE

	Manufactured by		Lot Number
	Reference Number		Expiration Date
	Sufficient for "#" Tests		Research Use Only
	Consult Instructions for Use		Irritant

Rev. Date 2025-11-21
Rev. 1
For a list of IFU changes, email info@immy.com

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Appendix 1 – Direct detection from research swab samples

Supplementary Protocol for direct detection of *C. auris* from research swab samples, without the DNA extraction step.
For research use only. Not for use in diagnostic procedures.

PROCEDURE

1. Remove the *C. auris* test kit from the freezer and allow the reagents to thaw. Thawed reagents must be kept on ice. Briefly vortex and centrifuge all reagent tubes prior to use.

SAMPLES

1. Swabs collected for research should be stored in Amies semi-solid transport medium at 4-25°C and processed within 96 hours of collection.

SAMPLE PROCESSING

1. Transfer swab into an Eppendorf tube containing 0.5mL sterile distilled water.
2. Vortex vigorously for 30 seconds to suspend the cells and then discard the swab.
3. The resuspended sample forms your template for direct load into the PCR reaction mix.

PCR DETECTION PROTOCOL

1. Prepare a PCR premix according to the table below. Volumes are given per reaction well and should be multiplied by the number of reaction wells required. Include sufficient reactions for positive and negative controls. The prepared PCR premix should be thoroughly mixed and briefly spun down.

Component	Volume/Reaction
qPCR Master Mix	10 µL
Primer/Probe Mix	2 µL
RNase/DNase-Free Water	1.8 µL
Internal Extraction Control (IEC) DNA	0.2 µL
Final Volume	14 µL

Due to small variations in pipetting accuracy, we recommend that you allow an additional 10% for the final volume, i.e. if you are assaying ten samples, make up sufficient reaction mix for 11 tests.

2. Pipette 14 µL of prepared PCR premix into each reaction well according to your qPCR experimental plate set up.
3. Pipette 6 µL of sample template (from Sample processing step) into each well according to your experimental plate set up.
4. For negative control wells use 6 µL of RNase/DNase -free water. For Positive Control use 6 µL of *C. auris* Positive Control. The final volume in each well is 20 µL.
5. Ensure that your PCR reaction plate is sealed and briefly centrifuged before transferring to a validated thermocycler for amplification.

AMPLIFICATION PROTOCOL

1. If using an instrument that uses ROX as a passive reference then the passive reference must be turned off or set to "none" for no passive reference, as the IEC uses the ROX channel.
2. Please refer to your instrument manual for instructions on setting up an amplification run. Amplification should be carried out according to the conditions detailed the table below.

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It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds.

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NO TEMPLATE CONTROL (NTC)

The NTC should give a flat line (flat amplification plots) through both FAM and ROX channels. Signals in the NTC may indicate cross contamination during plate set up.

SAMPLE DATA

1. The IEC will amplify in the ROX channel, with a quantification cycle (Cq) value >25. Variation will be dependent on the level of inhibition within the reaction.
2. Absence of a signal in the FAM channel suggests that the sample does not contain *C. auris*.
3. A signal in the FAM channel indicates the presence of *C. auris*.
4. It is possible to obtain a signal in the FAM channel without also recording a signal in the ROX channel. Typically, this occurs when a high fungal load results in early amplification (i.e. low Cqs) and the accumulation of target DNA inhibits the amplification of the IEC DNA. This result is still valid.
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-	+	*C. auris NEGATIVE sample. IEC PASS.* ***Valid result***.
+	-	*C. auris POSITIVE sample.* IEC outcompeted by high *C. auris* target DNA load. ***Valid result***.
-	-	***Invalid result.***

LIMITATIONS OF THE PROCEDURE

1. For research use only. Not for use in diagnostic procedures.

INTERNATIONAL SYMBOL USAGE

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