AMD Ltd Zena Max Candida auris (C. auris) PCR Detection Kit

CE IVD

Product information:

Catalogue number KD919161-100

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

The assay is an in vitro qPCR detection kit for the qualitative determination of *C. auris* DNA in human samples. As the axilla and groin regions tend to have the highest colonisation rate, a skin swab covering these areas is optimal for testing in this assay. However, the pathogen has been isolated from other areas such as the oro- or nasopharynx, external ear canal, vagina and rectum and so these samples may be used subject to method validation.
This product is based on the hydrolysis probe detection method and is a highly sensitive one step qPCR kit.
For *in vitro* diagnostic use

Overview

Candida auris (*C. auris*) is an emerging fungal pathogen first reported in 2009 as a novel Candida species, following isolation of a strain retrieved from a patient's ear canal in a Japanese hospital. Although *C. auris* is less common than *C. albicans*, the yeast species responsible for thrush, there is an especially high risk for nosocomial infections associated with this species. As a result of this, *C. auris* has been declared a serious global health threat due to high mortality rates within patients and a high resistance to multiple drugs.
C. auris infection is usually asymptomatic, but can cause infections within the ear, blood and wounds. Importantly, the pathogen can spread through contaminated surfaces and fomites, highlighting the mechanism by which nosocomial infections occur. Typically, *C. auris* colonies are found in the groin and axilla regions of the body, predominantly on the skin. However, colonies have also been isolated from the urogenital tract and respiratory tract. Consequences of this colonisation include candidaemia, urinary tract infections, pneumonia and pericarditis.
C. auris is commonly mis-identified by microbiologists, and so the real prevalence of these infections is unknown. There is a clear lack of diagnostic services available in this area, and so Advanced Molecular Diagnostics Ltd have produced a PCR assay to fulfil that need.

Principles of the test

The Zena Max C. auris PCR Detection Kit is designed for the qualitative detection of C. auris by the real-time Polymerase Chain Reaction (PCR) method, which amplifies specific conserved DNA sequences, and fluorophore-labelled probes for the detection of amplified DNA. This assay contains two probes labelled with either the 5' FAM™ or 5' HEX™ reporter dye and a 3' quencher. The FAM™ labelled probe is specific for a conserved sequence within C. auris DNA, whereas the HEX™ labelled probe is specific for a control gene present in the IC transcript. Two sets of forward and reverse primers are provided, annealing either side of each target gene. As the new target cDNA strand is synthesized, the bound probes are cleaved by the 5' to 3' exonuclease activity of Taq polymerase, releasing the fluorescent reporter from the quencher and substantially increasing the fluorescent signal. Amplification of C. auris DNA is indicated in the FAM fluorescence channel, whereas amplification of the control gene is indicated in the HEX channel.

The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present. If, however, C. auris DNA is not present, a FAM signal or ROX signal will not be produced. These assays are both incorporated into a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity.

A Positive Control is provided alongside this assay, which consists of a transcript containing the conserved C. auris sequence used for detection. This control should be amplified in a separate reaction to allow verification of the C. auris specific PCR detection system. PCR grade water is also provided for use as a Negative Control for the PCR reaction to indicate contamination of PCR reagents. The ready-to-use master mix also contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products.

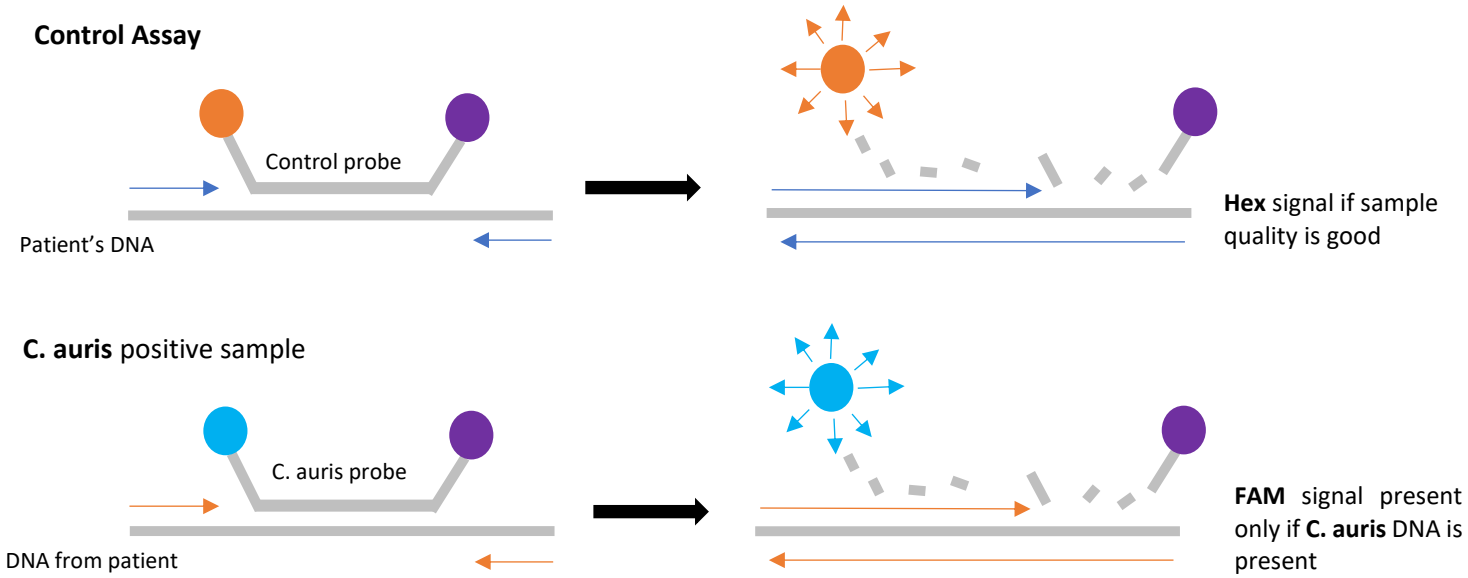

*Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of DNA. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. If a virus **positive** sample is tested, the C. auris Virus assay will produce a FAM fluorescent signal (Ct<38) to indicate the viral DNA is present in the patient sample. Due to assay competition, the HEX signal may be reduced or absent when other signals are strong. If a virus **negative** sample is tested, only the HEX signal will be detected.*

Kit Contents

Item	**KD919167- 100**
C. auris qPCR Master Mix	2x 1 ml
C. auris Positive control	1 x 0.05 ml
Nuclease-free water	1 x 1 ml

Reagent storage and handling

- The kits should be transported and stored at temperatures between -18°C and -25°C.
- The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept accordingly.
- Do not use the reagents beyond their expiration dates.
- Repeated freeze-thawing of the kit components may result in lower detection quality.
- Avoid exposure to light.

- Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and equipment required (not provided)

DNA extraction: For optimal results use a suitable DNA Extraction Kit to elute the DNA from the sample. Other leading kits, or in-house methods are acceptable for use with this diagnostic kit, providing that it has been validated prior to use on patient samples.

PCR Instrument: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes. It is also compatible with both low and high ROX instruments.

Consumables: Use nuclease free PCR consumables appropriate to the qPCR instrument.

Other Laboratory Equipment: Vortex (for mixing samples), centrifuge (to collect components from the bottom of the tube), micro pipettes and sterile tips, tube rack, PCR tube/plate rack and spectrophotometer, disposable gloves, refrigerator (to store samples)

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to precisely follow the instructions in this manual, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD C. auris PCR Detection Kit.

Instrument compatibility

The AMD C. auris qPCR detection kit is compatible with the most common Real Time PCR equipment with the capability of detecting FAM and HEX fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Assay Procedure

Sample Collection

The sample for AMD Candida auris (C. auris) qPCR detection Kit should be collected via human samples, such as the axilla and groin regions tend to have the highest colonisation rate, a skin swab covering these areas is optimal for testing in this assay. However, the pathogen has been isolated from other areas such as the oro- or nasopharynx, external ear canal, vagina and rectum and so these samples may be used subject to method validation. Please ensure that samples are stored correctly and kept away from any contamination.

Sample Storage

As a general rule, multiple freeze-thaws should be avoided. The most practical way to address this concern is by aliquoting samples after collection.

Sample transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of the sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material. The samples should be shipped within 6 hours. It is not recommended to store the samples where they have been collected. It is possible to ship the samples by mail, following the legal

instructions for the transport of pathogen material. We recommend the sample transport with a courier.

PCR Set up

Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
Set up the reactions using the table below.

Product	Volume
C. Auris Master Mix	20µl
DNA Sample/control	5µl

Add the DNA samples and at least one replicate of the C. auris control to the PCR tubes/plate. As a No Template Control (NTC), add 5µl nuclease-free water in place of DNA. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile:

Stage/step	Temperature	Time
Stage 1: Step 1	30°c	2 min
Stage 1: Step 2	95°c	2 mins
40 cycles		
Stage 2: Step 1	95°c	10 secs
Stage 2: Step 2	56°c	35 secs

When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

This is a qualitative assay which indicates the presence or absence of Candida auris.
The results should be interpreted as follows:

- If there is a signal in the **HEX** channel and no signal in **FAM**, the sample is negative for C. auris.
- If there is a signal in the **HEX** and **FAM** channels, the sample is positive for C. auris.
- The internal control assay signal in the **HEX** channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay (**FAM**) signal is strong. This is an inconclusive result. If there is no signal in either channel, the result is also inconclusive.
- Inconclusive or ambiguous sample results should be repeated.

HEX (Internal control)	FAM (C. auris)	Interpretation
+	-	Sample Negative for C. auris
+	+ (Ct<38)	Sample Positive for C. auris
-	+ (Ct<38)	Sample Positive for C. auris
-	-	Result inconclusive

Performance Characteristics

Quality: All AMD kits are manufactured under high-quality standard methods and unique precision, comparable with other leading commercial C. auris PCR Detection Kits.
Sensitivity: The AMD C. auris PCR detection kit is highly sensitive, able to detect a minimum of 10 copies/rxn "rxn volume 25µl" under our validation methods and devices.
Specificity: The Advanced Molecular Diagnostics Candida auris C. auris kit is very specific up to 100% for Candida auris DNA under our validation methods and devices.

References

Chowdhary, A., Sharma, C., & Meis, J. F. (2017). Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS pathogens, 13(5), e1006290. https://doi.org/10.1371/journal.ppat.1006290

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Sikora, A. and Zahra, F., 2022. Candida Auris. [online] Ncbi.nlm.nih.gov. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK563297/> [Accessed 17 June 2022].

Product Limitations

This kit is for in vitro diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally, mutations may arise in the region of the genome targeted by the primers and probes of this assay, leading to under-quantification or failure to detect the presence of the virus in these cases. Assay design and efficacy are reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA and population genotyping studies to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity.

Contact

Any queries, comments or complaints please refer to our website at:
www.am-diagnostics.co.uk
info@am-diagnostics.co.uk

Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	Name and address of EU Representative	Advena Ltd Tower Business Centre, 2nd Floor, Tower Street, Swatar BKR 4013 Malta
	UDI-DI number for the product given	Basic: 506105998CAURA9 UDI-DI: (01)05061059980458 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD919161-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC