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Validation and implementation of a commercial real-time PCR assay for direct detection of *Candida auris* from surveillance samples

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Abstract

Background: Rapid and reliable laboratory methods are required for detecting the nosocomial yeast *Candida auris*. AurisID® (Olm Diagnostics) is a real-time PCR assay approved for detecting *C. auris* in fungal cultures and directly from blood samples, involving a nucleic acid extraction as a prior step.

Objectives: The purpose of this study is to validate the AurisID® kit for direct detection of *C. auris* from surveillance samples without prior DNA extraction and to analyse the results of implementing this methodology to our daily laboratory routine protocol for *C. auris* surveillance studies.

Methods: Our PCR method using the AurisID® kit was compared with our routine protocol, consisting of culture in CHROMagar® Candida and identification by mass spectrometry. A total of 113 swabs were used for validation and 136 pair of surveillance samples were tested. Limit of detection (LOD) was determined by using swabs in Amies transport medium, which were spiked in a series of dilutions of a *C. auris* standardised suspension (0.5 McFarland).

Results: The PCR method showed high sensitivity, specificity, predictive positive value and predictive negative value (96.6%, 100%, 100% and 98.2%, respectively) when compared with the routine protocol. LOD was 500 CFU/ml, which corresponds to approximately 1 CFU/PCR.

Conclusions: Our PCR method using the AurisID® kit allows a reduction in the turnaround time for surveillance of *C. auris* compared with other methods. These results are expected to contribute to control *C. auris* outbreaks, allowing isolation of patients and cleaning of environmental surfaces in advance.

KEYWORDS
Candida, colonisation, yeasts

1 | INTRODUCTION

Since its first report in 2009,[1] *Candida auris* has spread in many countries causing several outbreaks in healthcare settings.[2-4] Its resistance to the most commonly used antifungals and easy proliferation in a hospital environment coupled with the fact that it affects critically ill patients makes the establishment of infection control practices to prevent its dissemination highly recommended

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(eg, contact isolation of colonised/infected patients, patient cohorting, environmental cleaning and performance of environmental and surveillance cultures).[4,5] Surveillance cultures are crucial, since a minimum contact period of 4 h with an infected patient or a contaminated surface may be enough for the acquisition of *C. auris*.[4] Furthermore, colonisation with *Candida* species is a well-known risk factor for developing invasive candidiasis[6] and empirical antifungal therapy should be considered whether this is suspected.[7]

Reliable and rapid laboratory methods for the detection of *C. auris*, both in clinical and surveillance samples, are also essential. Although nowadays different methods can correctly determine the presence of *C. auris*, most of them are time-consuming since they require culture and 24–48 h of incubation prior to identification (eg MALDI-TOF or biochemical tests). Alternatively, different PCR assays have been developed in recent years,[8-10] allowing a reduction in the time-to-result. A commercial real-time PCR assay (AurisID®; Olm Diagnostics) has been approved for detecting *C. auris* from fungal cultures and directly from blood samples, with a previous step of nucleic acid extraction. The purpose of the present study is to validate the AurisID® kit for direct detection of *C. auris* from surveillance samples without previous DNA extraction and to analyse the results of implementing this methodology to our daily laboratory routine protocol for surveillance studies of *C. auris*.

2 | METHODS

Firstly, the method was validated by performing the real-time PCR assay (AurisID®; Olm Diagnostics) on 113 surveillance samples that had been previously cultured. The samples were pharyngeal or axillary-rectal swabs in Amies semisolid transport medium submitted to the clinical laboratory of Consorcio Hospital General Universitario de Valencia (Spain), from patients admitted to the ICU, where there was an established *C. auris* outbreak.[11,12] The surveillance cultures were performed by streaking the swabs in CHROMagar® Candida (Becton Dickinson), and the plates were incubated at 37°C and were read at 24 and 48 h. As this medium is not specific for *C. auris*, suspicious colonies (colour white to mauve) were identified by mass spectrometry (MALDI-TOF; Bruker). For the PCR assay, the same previously cultured swabs were spiked and shaken in Eppendorf tubes containing 0.5 ml of distilled water to suspend the cells. According to the manufacturer, a Master Mix solution was prepared by mixing 10 µl of AurisID qPCR Master Mix, 2 µl of Auris Prime Probe Mix and 1.8 µl of RNAse/DNAse-free water. As DNA was not extracted, the internal extraction control was added to the mix (0.2 µl/sample). Then, 6 µl of the resuspended sample were added to 14 µl of Master Mix and the PCR reactions were performed in the CFX96 Real-Time PCR System (Bio-Rad). A positive and a negative control supplied by the kit were also included. Limit of detection (LOD) was determined by reproducing the same sample handling process; thus, we used swabs in Amies transport medium, which were spiked in a series of dilutions of a *C. auris* standardised suspension (0.5 McFarland), then processed in triplicate as surveillance samples.

TABLE 1 Comparison of *C. auris* detection by rt-PCR and culture

C. auris detection by PCR	*C. auris* detection by culture			
	Validation study		Prospective study in surveillance samples	
	Positive	Negative	Positive	Negative
Positive	70	0	14	0
Negative	2	41	1	121

Once validated, the rt-PCR assay was tested prospectively by following the methodology of rt-PCR and culture previously described. In this case, two pairs of swabs (pharyngeal and axillary-rectal) were requested for each patient: one for the culture and one for the molecular assay. For the molecular assay, the two types of surveillance samples for every patient were pooled and tested together. Monitoring cultures were performed every 2 or 3 days to confirm PCR results.

Results were compared between the rt-PCR and the culture, and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with SPSS V27. The turnaround time involved the sum of the time required to complete sample preparation, device loading, DNA amplification, result reviewing and reporting. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

3 | RESULTS

For the validation of the method, a total of 113 samples from surveillance cultures were tested (Table 1). A total of 72 samples had a previous positive culture for *C. auris*, of which 70 were PCR positive. The remaining 41 samples were PCR negative for *C. auris*, consistent with the culture results. The culture of 22 swabs out of the 41 swabs with a negative PCR result for *C. auris* did not show any yeast growth, while 19 cultures were positive for a different *Candida* species (eight *Candida albicans*, five *Candida parapsilosis*, two *Candida lusitaniae*, one *Candida glabrata*, one *C. albicans* and *C. glabrata* and one *C. albicans* and *C. parapsilosis*). At 48 h of incubation, *C. auris*, *C. parapsilosis* and *C. lusitaniae* had a white to mauve colour in CHROMagar Candida, requiring further identification by MALDI-TOF. LOD of the PCR method was 500 CFU/ml, which corresponds to approximately 1 CFU/PCR, considering the volumes used in the process (Figure 1).

Once validated, the rt-PCR assay was performed for 136 pair of swabs (pharyngeal and axillary-rectal) from 113 patients and the result was positive for 14 samples from 12 patients (Table 1). The median C_t value was 32.3, ranging between 25.6 and 39.4. In nine of these patients, *C. auris* also grew in the culture. For the other three patients, although the culture of day one was negative, a monitoring culture obtained 2–7 days later was positive. For these samples, the C_t values were higher than 35 (35.4, 38.2 and 39.4), which is consistent with a low fungal load. In one patient, *C. auris* was not

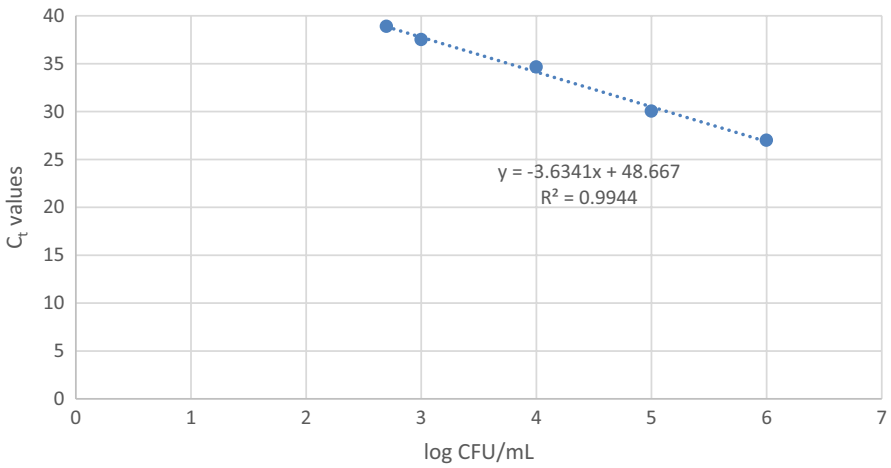

FIGURE 1 Limit of detection (LOD) of AurisID® rt-PCR kit using spiked samples in a series of dilutions of a *C. auris* standardised suspension (0.5 McFarland)

detected by PCR even when it grew in the culture. For the remaining 121 samples, both the PCR and the culture for *C. auris* were negative. The turnaround time could be advanced from 24 to 48 h (time required for growth in CHROMagar® Candida) to 1–1.5 h on average. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), compared with culture, are shown in Table 2.

4 | DISCUSSION

Surveillance colonisation studies are essential to control a nosocomial pathogen such as *C. auris* and results should be given within the minimum possible time. Until 2017, *C. auris* was difficult to identify in the clinical laboratories because most commercial biochemical systems would misidentify it with other species.[13] Although nowadays commercialised systems such as MALDI-TOF or Vitek 2® (BioMérieux) include this novel yeast in their databases, these methods are time-consuming since they rely on growth. Therefore, there is still a need for reliable and rapid methods to identify *C. auris* in clinical or surveillance samples. Chromogenic methods are useful because they allow a relatively rapid presumptive identification. However, they require an incubation time of 24–48 h and complementary methods to confirm suspicious colonies, as CHROMagar® Candida does not specifically differentiate *C. auris*. However, recently a novel medium (CHROMagar® Candida Plus, CHROMagar, France) has proven to specifically differentiate *C. auris* in 36 h, thereby being able to reduce the use of complementary methods and the incubation time.[14]

Our methodology using the AurisID® kit has shown to directly detect *C. auris* in surveillance samples within 1–1.5 h as an average turnaround time, which is a significant reduction in time from the 24–48 h previously required for growth. The AurisID® kit had been validated in fungal colonies obtained from agar cultures and directly from blood samples according to the manufacturer, although no studies evaluating the clinical performance of this assay had been published so far.[15] Furthermore, we obtained promising results by using it directly from the swabs in Amies transport medium submitted for colonisation studies and without previous DNA extraction. This methodology has the advantage over the manufacturer's instructions of not requiring DNA extraction, which reduces the handling of the sample and the time-to-result. Only, some PCR assays with satisfactory results for direct detection of *C. auris* in surveillance samples have been published so far,[8,10,16] although they all involve a DNA extraction step. Leach et al developed an automated rt-PCR assay with the BD Max Open System,[17] which would also reduce the handling of the sample due to the automatisation of the DNA extraction. The time-to-result reported for the BD Max assay is that it allows the screening of 180 samples in a 12-h workday, while our methodology using AurisID® kit in the CFX96 Real-Time PCR System would allow the screening of 94 samples in approximately 2 h, considering the time required to complete sample preparation, device loading, DNA amplification, result reviewing and reporting.

LOD was 500 CFU/ml, which corresponds to approximately 1 CFU/PCR, which is similar to other reported methods.[8,10,16,17] Furthermore, the comparison with our reference method for surveillance studies, consisting of culture in CHROMagar™ Candida, showed excellent results. The overall sensitivity of the test was 96.6%. In the first part, two samples containing *C. auris* were not detected in the rt-PCR assay, which could be explained because the same swabs used for cultures were reused for the PCR, which could

TABLE 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the test

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Validation study	97.2	100	100	95.3
Prospective study in surveillance samples	99.3	100	100	99.2
Overall	96.6	100	100	98.2

have significantly reduced the fungal load. Because of this, two pairs of swabs were requested for each patient for the second part of the study: one for the culture and one for the rt-PCR assay. The specificity of the rt-PCR assay was 100%, which indicates that there is no cross-reaction with the most common *Candida* species or with other colonising microorganisms. The overall PPV and NPV were 100% and 98.2%, respectively. In the second stage, nine positive results could be informed 24–48 h before the culture, and we could advance the positive results to 2–7 days before the first positive culture in three patients.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

Juan V. Mulet Bayona: formal analysis (equal); methodology (equal); writing-original draft (equal). **Carme Salvador García**: conceptualization (equal); data curation (equal); methodology (equal); supervision (equal); writing-review and editing (equal). **Nuria Tormo Palop**: conceptualization (equal); methodology (equal); supervision (equal); writing-review and editing (equal). **Concepción Gimeno Cardona**: supervision (equal); visualization (equal); writing-review and editing (equal).

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REFERENCES

1. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol Immunol*. 2009;53(1):41-44.
2. Ruiz-Gaitán A, Moret AM, Tasias-Pitarch M, et al. An outbreak due to *Candida auris* with prolonged colonisation and candidaemia in a tertiary care European hospital. *Mycoses*. 2018;61(7):498-505.
3. Calvo B, Melo ASA, Perozo-Mena A, et al. First report of *Candida auris* in America: clinical and microbiological aspects of 18 episodes of candidemia. *J Infect*. 2016;73(4):369-374.
4. Schelenz S, Hagen F, Rhodes JL, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrob Resist Infect Control*. 2016;5(1):35.
5. European Centre for Disease Prevention and Control. *Candida auris in healthcare settings – Europe – first update, 23 April 2018*. Stockholm: ECDC; 2018.
6. Yapar N. Epidemiology and risk factors for invasive candidiasis. *Ther Clin Risk Manag*. 2014;10:95-105.
7. Bretonnière C, Lakhal K, Lepoivre T, Boutoille D, Morio F. What is the role of empirical treatment for suspected invasive candidiasis in non-neutropenic non transplanted patients in the intensive care unit? – Empiricus strikes back!. *J Thorac Dis*. 2016;8(12):1719-1722.
8. Malczynski M, Dowllow N, Rezaeian S, et al. Optimizing a real-time PCR assay for rapid detection of *Candida auris* in nasal and axillary/groin samples. *J Med Microbiol*. 2020;69(6):824-829.
9. Mahmoudi S, Agha Kuchak Afshari S, Aghaei Gharehbolagh S, Mirhendi H, Makimura K. Methods for identification of *Candida auris*, the yeast of global public health concern: a review. *J Mycol Med*. 2019;29(2):174-179.
10. 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;56(2):e01223-17.
11. Salvador García C, Tormo Palop N, Mulet Bayona JV, et al. *Candida auris*: descripción de un brote. *Enferm Infecc Microbiol Clin*. 2020;38(suppl 1):39-44.
12. Mulet Bayona JV, Tormo Palop N, Salvador García C, et al. Characteristics and management of candidaemia episodes in an established *Candida auris* outbreak. *Antibiotics*. 2020;9(9):558.
13. Lockhart SR, Berkow EL, Chow N, Welsh RM *Candida auris* for the clinical microbiology laboratory: not your grandfather's Candida species. *Clin Microbiol Newsl*. 2017;39(13):99-103.
14. Mulet Bayona JV, Salvador García C, Tormo Palop N, Gimeno CC. Evaluation of a novel chromogenic medium for Candida spp. identification and comparison with CHROMagar™ Candida for the detection of *Candida auris* in surveillance samples. *Diagn Microbiol Infect Dis*. 2020;98(4):1-16.
15. Camp I, Spettel K, Willinger B. Molecular methods for the diagnosis of invasive candidiasis. *J Fungi*. 2020;6(3):101.
16. Sexton DJ, Kordalewska M, Bentz ML, Welsh RM, Perlin DS, Litvintseva AP. Direct detection of emergent fungal pathogen *Candida auris* in clinical skin swabs by SYBR green-based quantitative PCR assay. *J Clin Microbiol*. 2018;56(12):1-6.
17. Leach L, Russell A, Zhu Y, Chaturvedi S, Chaturvedi V. A rapid and automated sample-to-result *Candida auris* real-time PCR assay for high-throughput testing of surveillance samples with BD MAX™ open system. *J Clin Microbiol*. 2019;57(10):e00630-19.

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