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HardyCHROM™ Candida + auris: a Novel Culture Medium for Detecting *Candida auris*

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Revised Abstract

Introduction: *Candida auris* is a serious global threat and is often difficult to correctly identify in clinical laboratories; it is routinely misidentified on culture media or rapid identification systems and this can lead to increased morbidity and mortality.(1,2) In order to meet the need for improved detection of this pathogen, HardyCHROM™ (HC) Candida + auris was developed to screen specimens for *C. auris*, which develops a white colony with a teal center, or "bullseye" pattern, that fluoresces under UV light after 48-72 hours of incubation. The performance of HC Candida + auris was evaluated in a series of analytical studies: analytical reactivity, cross reactivity, and a contrived specimen study.

Methods: For the analytical reactivity study, *C. auris* strains (*n* = 16) were streaked for isolation with a 1µL loop at the limit of detection (LoD) (1.5x10^3 CFU/mL) to HC Candida + auris. To evaluate any potential cross reactivity, non-target organisms (*n* = 65) were tested at high concentrations (1.5x10^6-10^8 CFU/mL) and streaked for isolation with a 10µL loop to HC Candida + auris. For the contrived specimen study, *C. auris* strains (*n* = 16) were spiked into ear, nares, and axilla-groin specimens at 1.5x10^4 CFU/mL and spread-plated (100µL) onto HC Candida + auris to evaluate the recovery of each strain from each specimen type. The same 16 *C. auris* strains were also spiked into tryptic soy broth (15mL) with human blood (5mL) at 1.5x10^2 CFU/mL, incubated for 24 hours at 35°C, and then streaked to HC Candida + auris with a 10µL loop. HC Candida + auris plates were examined for growth and results were recorded at 48 and 72 hours of incubation at 35°C for all analytical studies. For all studies, SabDex agar was used as a control plate.

Results: For the analytical reactivity study, all *C. auris* strains (*n* = 16) were recovered at 1.5x10^3 CFU/mL. All non-target bacterial species evaluated (*n* = 33) and one yeast species were inhibited in the cross reactivity study. The remaining yeasts and filamentous fungi species (*n* = 31) grew on HC Candida + auris but 65/65 (100%) and 62/65 (95.4%) of all species tested did not replicate the same reaction expected of the *C. auris* strains at 48 and 72 hours of incubation, respectively. All 16 (100%) *C. auris* strains were recovered from blood, ear, nasal, and axilla-groin specimens with expected reactions.

Conclusions: HC Candida + auris can easily differentiate *C. auris* from other fungal species at low concentrations in various specimen types within 48-72 hours. This chromogenic screening tool, used in conjunction with confirmatory tests or other identification methods, will enhance the detection of *C. auris* and therefore improve patient outcome.

Introduction

HC Candida + auris is a selective and differential medium developed for the isolation of *C. auris*. On the media, *C. auris* develops a white colony with a teal to teal-green center, or "bullseye" isolated colony morphology, after 48-72 hours of aerobic incubation at 35°C. Colonies also fluoresce under UV light. Most strains show the characteristic color and fluorescence after 48 hours of incubation. Within 48-72 hours of incubation, colony color will intensify. As the bullseye morphology changes to become a fully colored colony nearing 72 hours of incubation, the fluorescence may become diminished. The purpose of these studies was to challenge the HC Candida + auris media and evaluate the overall analytical performance. Analytical reactivity, cross reactivity, and contrived specimen studies were completed using the media.

Methods

For all of the analytical studies performed, HC Candida + auris plates and SabDex agar plates were incubated at 35°C and observed for growth after 48 and 72 hours of incubation. The HC Candida + auris plates were observed for color and fluorescence at each time point.

Analytical Reactivity Study: Fresh colonies of *C. auris* strains (*n* = 16) were suspended in tryptic soy broth (TSB) to prepare a suspension approximating a 0.5 McFarland turbidity standard (1.5x10^6 CFU/mL). Next, each suspension was serially diluted to achieve a concentration of 1.5x10^3 CFU/mL. After preparing the suspension, a 1µL loop was used to subculture to HC Candida + auris and SabDex agar as a control.

Cross Reactivity Study: Clinically relevant non-target organisms (located in a similar body site), or organisms that are phylogenetically related to *C. auris* (*n* = 65) were evaluated on the HC Candida + auris media at high concentrations. Bacteria (*n* = 33) were evaluated at approximately 1.5x10^8 CFU/mL and fungi (*n* = 32) were evaluated at approximately 1.5x10^6 CFU/mL. Of the fungi evaluated, 26 were yeast species and 6 were filamentous fungi species. Fresh colonies of each species evaluated were suspended in TSB to approximate a 0.5 McFarland turbidity standard. After preparing the suspension, a 10µL loop was used to subculture each organism suspension for isolation to a HC Candida + auris and a SabDex agar plate as a control.

Contrived Specimen Study: In a contrived specimen study, three body sites were sampled according to CDC's current guidance for detection of colonization of *C. auris*.(2) The three specimen types obtained were a swab sample of the external ear canal (*n* = 8), anterior nares (*n* = 8), and axilla-groin (*n* = 8). Swabs were pre-screened on HC Candida + auris by swabbing the first quadrant and streaking for isolation with a 1µL loop from the first quadrant. Each swab was subsequently placed into liquid amies transport media. The liquid amies samples of the same specimen types were pooled. Each pooled specimen type was subsequently aliquoted into 16 vials. Fresh colonies of each strain of *C. auris* were suspended in tryptic soy broth (TSB) to prepare an initial suspension approximating a 0.5 McFarland turbidity standard (1.5x10^6 CFU/mL). Serial dilutions were performed to achieve a concentration of 10^4, 10^3, and 10^2 CFU/mL per strain of *C. auris*. The 10^3 CFU/mL TSB suspension of *C. auris* was subcultured for isolation to SabDex agar using a 1µL loop for concentration verification. Each vial of liquid amies and specimen was spiked with a unique *C. auris* strain (*n* = 16) from the 10^4 CFU/mL TSB suspension to achieve a final concentration of 10^3 CFU/mL. A 1µL loop was used to streak for isolation onto HC Candida + auris.

To evaluate recovery from blood culture, human blood (5mL) was added to TSB (15mL) to simulate a blood culture. 200µL of the 1.5x10^2 CFU/mL suspension of *C. auris* was spiked into the blood-TSB and incubated aerobically at 35°C for 24 hours. After incubation, a 10µL loop was used to subculture from the enriched culture to HC Candida + auris and streak for isolation.

Results

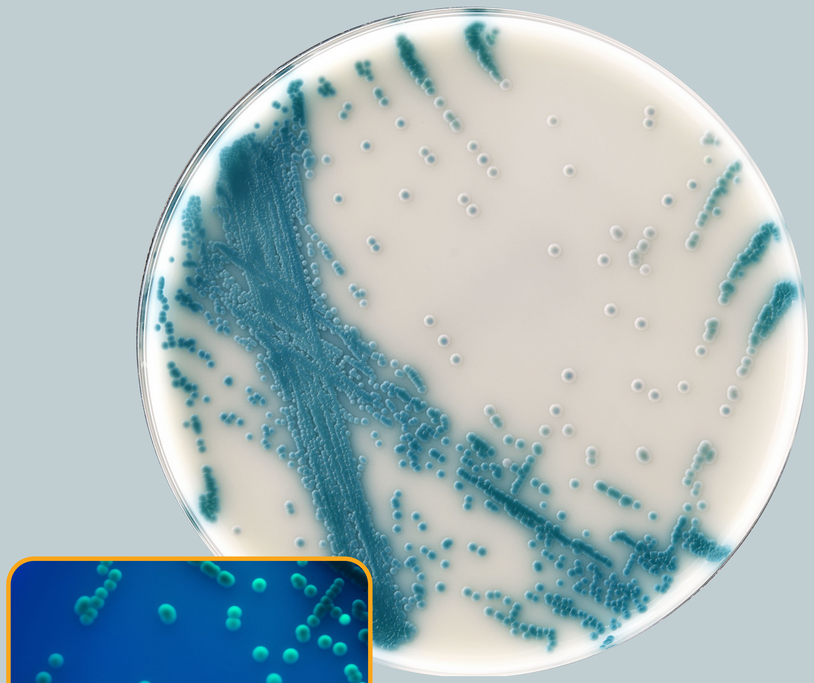
Analytical Reactivity: 13/16 (81.3%) strains of *C. auris* were recovered at the LoD with a teal or teal-green "bullseye" morphology and fluorescence in the isolated colonies by 48 hours of incubation (Table 1). After 72 hours of incubation, 16/16 (100%) were recovered at the LoD with the "bullseye" morphology and fluorescence.

Table 1. Analytical Reactivity of *Candida auris* strains (*n* = 16) on HardyCHROM™ Candida + auris

Source	Strain	48 Hour Color	48 Hour Fluorescence	72 Hour Color	72 Hour Fluorescence
CDC	0381	Teal Bullseye	+	Teal Bullseye	+
CDC	0382	Teal Bullseye	+	Teal Bullseye	+
CDC	0383	White	+	Teal Bullseye	+
CDC	0384	White	+	Teal Bullseye	+
CDC	0385	Teal Bullseye	+	Teal Bullseye	+
CDC	0386	Teal Bullseye	+	Teal Bullseye	+
CDC	0387	Teal-green Bullseye	+	Teal Bullseye	+
CDC	0388	Teal-green Bullseye	+	Teal Bullseye	+
CDC	0389	Teal Bullseye	+	Teal Bullseye	+
CDC	0390	Teal Bullseye	+	Teal Bullseye	+
ATCC	B11903	Teal Bullseye	+	Teal Bullseye	+
BEI	NR-52713	Teal Bullseye	+	Teal Bullseye	+
BEI	NR-52714	Teal Bullseye	+	Teal Bullseye	+
BEI	NR-52715	Teal Bullseye	+	Teal Bullseye	+
BEI	NR-52716	White	+	Teal-green Bullseye	+
BEI	NR-52717	Teal Bullseye	+	Teal Bullseye	+

Cross Reactivity: All bacterial species (*n* = 33) and one yeast species were inhibited on HC Candida + auris (Table 2). Twenty-five yeast strains and six filamentous fungi grew on HC Candida + auris (Table 3). Three organisms were blue in the first or second quadrant, but not in isolated colonies: *Candida lusitaniae*, *Candida metapsilosis*, and *Candida guillermondii*. Of the yeast species evaluated, two produced a similar morphology to *C. auris* after 72 hours of incubation. *Candida duobushaemulonii* and *Candida parapsilosis* exhibit pink to white colonies after 48 hours, and teal or teal to mauve bullseye pattern after 72 hours. Both species fluoresced under UV light after 48 and 72 hours of incubation.

Contrived Specimen Study: 14/16 (87.5%) of *C. auris* strains tested in external ear canal specimens were recovered with the correct morphology and fluorescence by 48 hours. 16/16 (100%) of *C. auris* strains tested in anterior nares specimens were recovered with the correct morphology and fluorescence by 48 hours. 15/16 (93.8%) of *C. auris* strains tested in spiked axilla-groin specimens were recovered with the correct morphology and fluorescence by 48 hours. All *C. auris* strains (100%) were recovered from a contrived blood culture enrichment with the proper color morphology and fluorescence after 48 hours of incubation. Any isolated colonies that did not have the bullseye morphology at 48 hours developed the bullseye morphology at 72 hours of incubation.



Candida auris colonies under UV light

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Results

Table 2. Cross Reactivity Organisms Inhibited on HardyCHROM™ Candida + auris (*n*=34)

Organisms Inhibited		
Aeromonas hydrophila	*Klebsiella aerogenes*	*Shigella flexneri*
Bacillus cereus	*Klebsiella pneumoniae*	*Shigella sonnei*
Bacillus subtilis	*Lactobacillus fermentum*	*Staphylococcus aureus*
Bacillus thuringiensis	*Lactobacillus leichmannii*	*Staphylococcus aureus (MRSA)*
Citrobacter freundii	*Listeria monocytogenes*	*Staphylococcus epidermidis*
Corynebacterium jeikeium	*Micrococcus luteus*	*Staphylococcus intermedius*
Cryptococcus laurentii	*Proteus mirabilis*	*Staphylococcus lugdenensis*
Enterobacter cloacae	*Proteus vulgaris*	*Staphylococcus saprophyticus*
Enterococcus faecalis	*Pseudomonas aeruginosa*	*Streptococcus agalactiae*
Escherichia coli	*Pseudomonas luteola*	*Streptococcus mitis*
Eschericia coli O157	*Salmonella enterica*	*Streptococcus pneumoniae*
		Streptococcus pyogenes

Table 3. Cross Reactivity Organisms Recovered on HardyCHROM™ Candida + auris (*n*=31)

Strain	48 Hour Color	48 Hour Fluorescence	72 Hour Color	72 Hour Fluorescence
Aspergillus brasiliensis	3+ blue	weak	3+ blue	weak
Aspergillus flavus	2+ white	-	2+ white	-
Aspergillus fumigatus	2+ white	-	2+ white	-
Aspergillus niger	2+ yellow	-	2+ brown	-
Aspergillus oryzae	1 blue CFU	-	1 blue CFU	-
Aspergillus terreus	3+ brown	-	1+ brown	-
Candida boidinii	2+ light pink	+	2+ light pink	+
Candida dubliniensis	2+ green	-	2+ green	-
Candida duobushaemulonii	3+ white[1]	+	3+ teal bullseye[1]	+
Candida duobushaemulonii	4+ white[1]	+	4+ mauve bullseye[1]	+
Candida duobushaemulonii	4+ white[1]	+	3+ teal and mauve bullseye[1]	+
Candida guilliermondii	4+ pink[1]	weak	4+ purple with blue center[1]	-
Candida haemulonii	3+ white	+	3+ purple bullseye[1]	weak
Candida inconspicua	2+ white	-	2+ white	weak
Candida kefyr	3+ mauve	+	3+ mauve	weak
Candida lambica	2+ pink	+	2+ purple	+
Candida lusitaniae	4+ purple[1]	-	4+ purple[1]	-
Candida metapsilosis	3+ pink[1]	weak	3+ blue with purple center[1]	weak
Candida norvegensis	2+ white[1]	weak	2+ light pink	weak
Candida pararugosa	3+ lavender	-	4+ purple	-
Candida parapsilosis	3 + white[1]	weak	3+ mauve bullseye[1]	-
Candida parapsilosis	4+ pink bullseye[1]	+	4+ mauve bullseye[1]	+
Candida parapsilosis	4+ white[1]	+	4+ teal bullseye[1]	+
Candida utilis	4+ mauve	+	3+ purple	+
Cryptococcus albidus	3+ tan[1]	weak	3+ tan[1]	-
Cryptococcus gattii	3+ white	+	3+ gray	weak
Cryptococcus neoformans	4+ white[1]	-	4+ white[1]	-
Malassezia furfur	2+ white	-	2+ white	-
Rhodotorula mucilaginosa	2+ pink	-	2+ pink	-
Saccharomyces cerevisiae	2+ blue	+	3+ blue	weak
Wickerhamomyces anomalus	4+ purple	weak	4+ purple[1]	weak

[1]Color may vary depending on growth or colony size; therefore, isolated colonies may exhibit different colors. In this table, the isolated colony color is reported.

Conclusions

- Most strains of *C. auris* can be detected at low concentrations on HC Candida + auris in 48 hours with a unique teal to teal-green bullseye isolated colony morphology that also fluoresces.
- The unique colony morphology allows for rapid differentiation of *C. auris* from other yeasts.
- *C. duobushaemulonii* and *C. parapsilosis* are two cross-reactive species that should be considered for ruling out if the teal to teal-green bullseye morphology is observed. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) with FDA-cleared libraries that include *C. auris* may be utilized. (2) *C. duobushaemulonii* is rarely isolated in the United States, although *C. parapsilosis* is a common cause of invasive candidiasis.(3)
- HC Candida + auris can be used as a screening tool prior to another confirmatory test or identification by the public health lab.
- The HC Candida + auris plate is a novel and effective way to assist identification of this evasive pathogen.

References:

(1) Centers for Disease Control and Prevention. Information for laboratory staff. *https://www.cdc.gov/fungal/candida-auris/fact-sheets/fact-sheet-lab-staff.html*

(2) Centers for Disease Control and Prevention. Identification of *Candida auris*. *https://www.cdc.gov/fungal/candida-auris/identification.html*

(3) Centers for Disease Control and Prevention. Invasive Candidiasis Statistics. *https://www.cdc.gov/fungal/diseases/candidiasis/invasive/statistics.html*