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In the Jungle, Welcome to the Fungal Hologic Panther's® Got What You Need

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Background

Candida auris (*C. auris*) is an emerging resistant yeast that can cause severe infections and is easily spread, especially among healthcare facilities. The yeast was first identified in Japan in 2009 and has since displayed increased resistance to common antifungal medications at an alarming rate.

Detection Limitations

- Many traditional phenotypic methods for yeast identification misidentify *C. auris* as another yeast species.

Identification Method	Potential Misidentification
Vitek 2 YST	Candida haemulonii Candida duobushaemulonii
API 20C	Candida sake Rhodotorula glutinis
API ID 32C	Candida intermedia Candida sake Saccharomyces kluyveri
BD Phoenix Yeast ID System	Candida catenulata Candida haemulonii
MicroScan	Candida famata Candida guilliermondii Candida lusitaniae Candida parapsilosis
RapID Yeast Plus	Candida parapsilosis

Table 1. Common yeast identification methods that may misidentify C. auris.[1]

- MALDI-TOF can accurately detect *C. auris*, but this method requires subculturing an original sample before MALDI-TOF use, which increases labor, turn-around time, and is prohibitive for high-volume testing.
- There is a need for an automated, high-throughput, and accurate CLIA validated PCR test for epidemiological outbreak investigation and colonization screening.

Our Process

Here, we describe the process for developing and optimizing a laboratory-developed assay (LDA) for *C. auris* detection in axilla/groin ESwabs by PCR on the fully automated Hologic Panther Fusion® Open Access™ system.

- A risk assessment and viability study were conducted to ensure laboratory safety during protocol development.
- Various concentrations of select reagents in a primer probe mix (PPM) were evaluated and optimized following Hologic's Open Access™ training recommendations.
- Validation was accomplished using *C. auris* culture previously characterized by MALDI-TOF from the CDC & FDA AR Isolate Bank to assess accuracy, precision, sensitivity, and specificity.

Risk Assessment

Given that *C. auris* can survive for weeks on surfaces, has reduced susceptibility to common disinfectants, and can colonize individuals, a risk assessment was warranted. Table 2 exemplifies the matrix used to assess the likelihood and consequence of a potential risk within our assay protocol. Table 3 displays one of the risks identified within our assay protocol as well controls implemented to mitigate the risk.

Risk Assessment Matrix		Consequence			
		Insignificant	Minor	Moderate	Major
Likelihood	Highly likely	Medium	Medium	High	High
	Likely	Low	Medium	High	High
	Possible	Low	Medium	Medium	High
	Unlikely	Low	Low	Medium	Medium

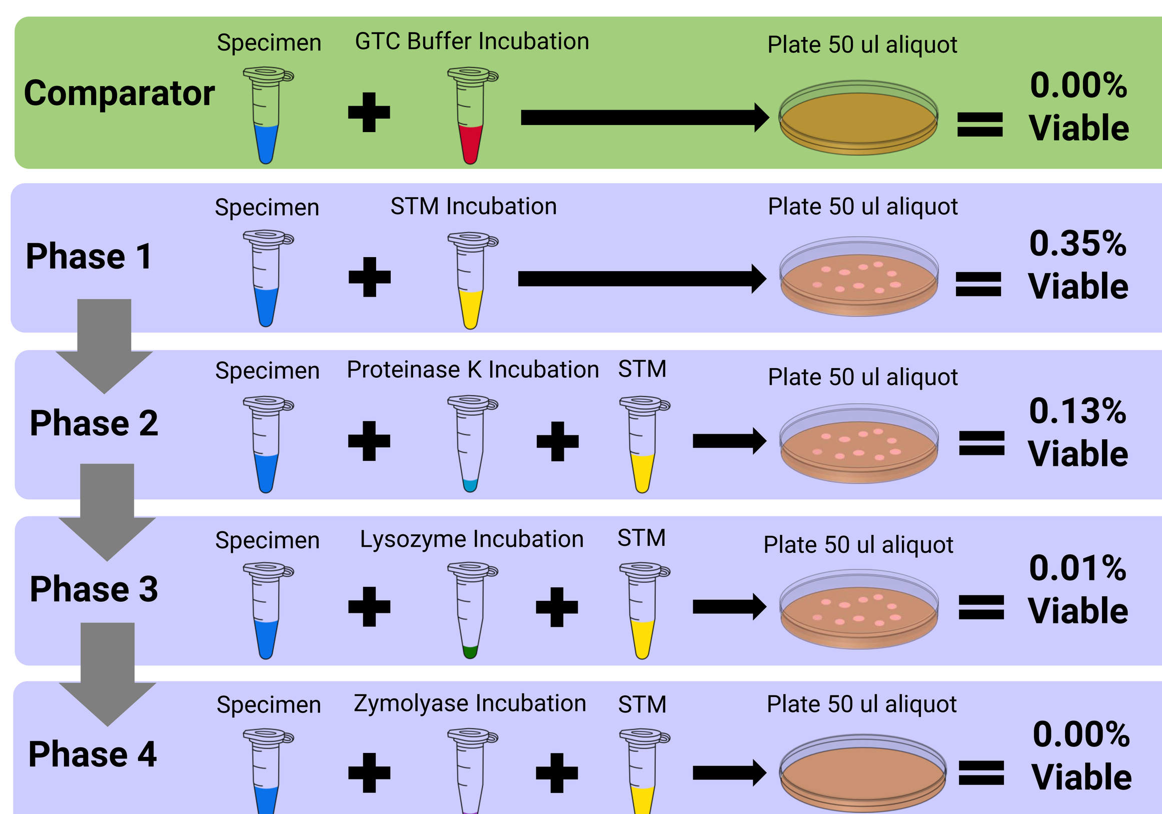
Table 2. Risk assessment matrix utilized at the State Hygienic Laboratory.

TASK	HAZARD	INITIAL RISK LEVEL WITHOUT CONTROLS	CONTROL MEASURES TO BE IMPLEMENTED	RESIDUAL RISK LEVEL WITH CONTROLS
Transferring Specimen in BSC.	Biological Hazard: Potential pathogen exposure through splash splatter or leakage/drips.	Likelihood: Likely Consequence: Moderate/Major Initial Risk Level: High	Engineering controls: Process specimens in a BSC. Administrative Controls: Ensure the BSC is functioning properly, visually inspect specimens for leakage, dispose pipette tips and other materials appropriately, complete lysis protocol before removing specimens from the BSC, decontaminate the BSC with 10% bleach solution followed by 70% ethanol solution, change gloves and gown when leaving the BSC. PPE: Face shield, double gloves, disposable back closing gown.	Likelihood: Unlikely Consequence: Moderate/Minor Risk Residual Level: Medium/Low

Table 3. Example of a risk identified within our protocol and how controls were implemented.

Viability Study

Due to a lack of *C. auris* viability studies and to ensure staff safety and prevent contamination outside of the BSC, we conducted a viability study comparing several different enzymes. Zymolyase® at 37°C for 10 minutes followed by inactivation at 95°C for 10 min effectively lysed the *C. auris* cell wall and produced 0% viability.



Optimization

The sequences and target used for *C. auris* detection as well as the final optimized PPM concentrations are shown in Table 4 and 5, respectively.

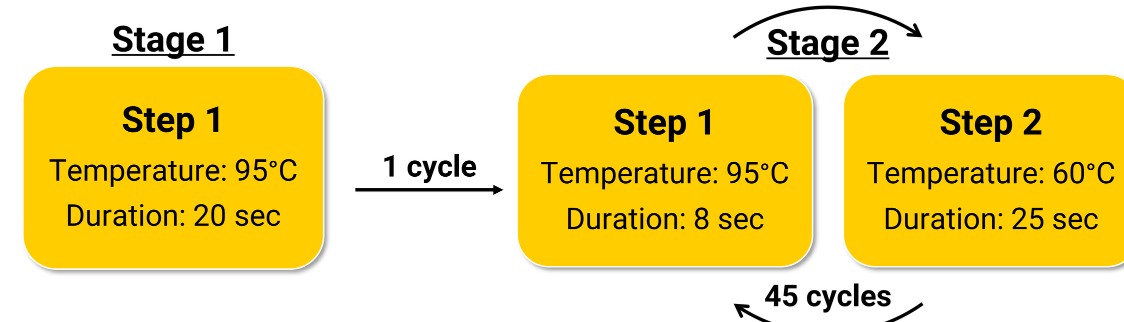
Analyte Name	Sequence	Gene Target
C. auris F Primer	CAG ACG TGA ATC ATC GAA TCT	ITS2
C. auris R Primer	TTT CGT GCA AGC TGT AAT TT	
C. auris Probe	/56-FAM/AA TCT TCG C/ZEN/G GTG GCG TTG CAT TCA /3IABkFQ/	
Panther® Fusion IC-X Primers	N/A	N/A
Panther® Fusion IC-X Probe(s)	N/A	

Table 4. Primer and probe sequences for C. auris target and internal control. [2,3]

Component	Stock Concentration	Units	Final Concentration	Concentration for 1.25X PPM	Volume (ul) for PPM
Water					903
KCl	1000	mM	0	0	0
MgCl2	1000	mM	5	6.25	6.25
Tris Buffer	1000	mM	12.5	15.5	15.5
C. auris FWD Primer	100	uM	0.75	0.95	9.5
C. auris REV Primer	100	uM	0.75	0.95	9.5
C. auris Probe	100	uM	0.5	0.65	6.25
IC-X Primers	37.5	uM	0.75	0.94	25
IC-X Probe	25	uM	0.5	0.63	25
Total					1000

Table 5. Final optimized PPM for 30 C. auris PCR tests on the Panther Fusion.[3,4]

PCR Cycling Parameters – Projected runtime: 49 min 13 sec



Validation

Limit of Detection & Sensitivity

- Plan: Accept LOD if 19/20 (95%) of dilution replicates are positive.
- Result: LOD = 93 CFU/mL
- PCR Efficiency: 98.78% (R^2: 0.9761)

Accuracy

- Plan: ≥90% specimens must agree with AR Isolate Bank.
- Result: 100% accuracy between instruments and days.

Precision

- Plan: Positive specimens must be within +/- 3 CTs.
- Result: 100% precision between instruments and days.

Specificity

- Plan: Specificity specimens must be negative for *C. auris*.
- Result: 100% specificity between instrument and days.

Interfering Substances

- Plan: To see if deodorant causes inhibition, lab staff self-collected axilla ESwabs that were then spiked with positive control. Spiked ESwabs must be within +/- 3 CTs of positive control.
- Result: No significant inhibition from axilla ESwabs.

Conclusion

- The LDA was successfully optimized and CLIA validated as a diagnostic test for epidemiological outbreak investigation and colonization screening.
- Our lysis protocol using Zymolyase® performed in the BSC prior to running on the Panther Fusion ensures lab staff safety and prevents contamination.
- The Panther Fusion's® automated extraction reduces labor and turn-around time and increases reproducibility of results, and specimens do not require additional diagnostic confirmation due to the LDA's high specificity for *C. auris*, enhancing convenience of results.
- All methods outlined in this poster for developing an LDA using the Hologic Panther Fusion® Open Access™ system are great resources to help prepare public health laboratories for increased testing volume and to improve current workflows.
- Furthermore, these methods can be easily applied to the rapid development and optimization of LDAs for other pathogens of public health significance.

Limitations

- Plating and counting CFUs to determine CFU/mL is not as precise as using DNA copies/mL to assess the LOD.

Next Steps

- Develop a g-block control to obtain a LOD in copies/mL and to use as a positive control.

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