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Disclosures

Ashley Emmons is an employee of Roche Diagnostics Corporation functioning as a **Molecular Scientific Liaison** within the non-commercial division of Medical & Scientific Affairs.

Data presented is intended for educational use to provide the participant with scientific, evidence-based information in compliance with FDA guidelines.

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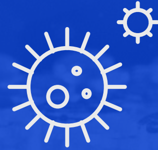
Learning Objectives



Describe the *Candida auris* emerging health threat

Recognize the challenges associated with addressing emerging health threats

Understand how the cobas omni Utility Channel can be used by laboratorians to respond to evolving testing needs and pathogens

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Candida auris Background

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Candida auris

Overview

***C. auris* is an emerging fungus of concern for 3 main reasons**

It is often multi-drug resistant with some strains resistant to all three available classes of antifungals used to treat *Candida* species

It is challenging to identify with standard laboratory methods and misidentification can lead to inappropriate management

Outbreaks in healthcare settings have occurred and it is critical to rapidly identify *C. auris* so special precautions can be taken to stop it's spread

CDC https://www.cdc.gov/fungal/candida-auris/index.html (Accessed 03/13/2024)

Candida auris

Basic facts

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- First described as a novel *Candida* species in 2009
    - Isolated from the external ear canal of a patient in a Japanese hospital
- Rapid global spread observed after 2009
- Budding yeast with cells that may be single, in pairs, or in groups
    - Ovoid, ellipsoidal to elongate – 2.5-5 microns – rarely forms hyphae or pseudohyphae, does not form germ tubes
- Growth varies depending on culture medium
    - Can grow at 40°C
- Genetic analysis demonstrates 4 distinct geographical clades with a potential 5th
    - Genetic differences suggest independent emergence of different clades
- May survive on moist or dry surfaces for 7 days and might remain viable up to 4 weeks
- Thrives on skin and forms a multi-layer biofilm

https://www.ncbi.nlm.nih.gov/books/NBK56329/

Candida auris
Identification

C. auris can be misidentified

as a number of different organisms including other *Candida* species when using traditional phenotypic methods for yeast identification

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)

can differentiate *C. auris* from other *Candida* species, but not all the included device reference databases allow for detection

Molecular Methods

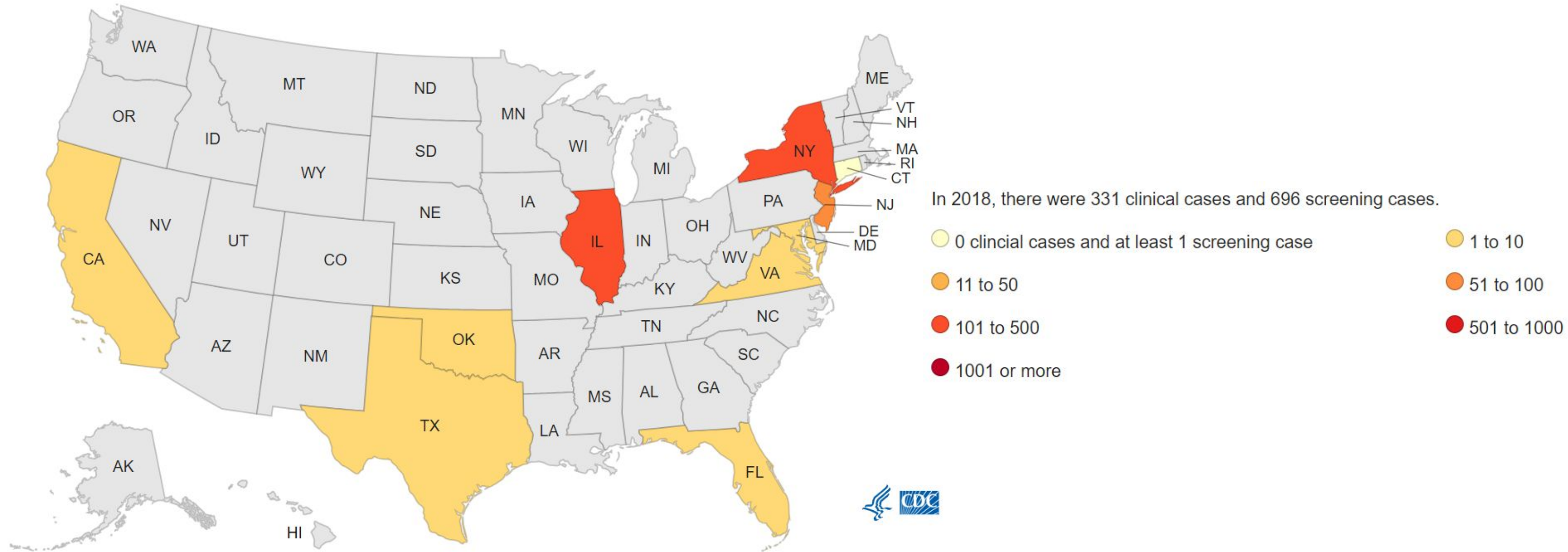
- Sequencing (D1-D2 region of the 28s rDNA or the Internal Transcribed Region of rDNA)
- FDA-approved pathogen panels for positive blood culture identification (GenMark and BioFire)
- Currently **no FDA-approved tests** for colonization swabs but various PCR methods have been developed and are available in the scientific literature including a CDC protocol

Note:
Healthcare facilities or laboratories that suspect they have a patient with *C. auris* infection should contact state or local public health authorities and CDC (candidaauris@cdc.gov) immediately for guidance

https://www.cdc.gov/fungal/candida-auris/identification.html
https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.html

Candida auris tracking data - 2018

Made nationally notifiable in 2018

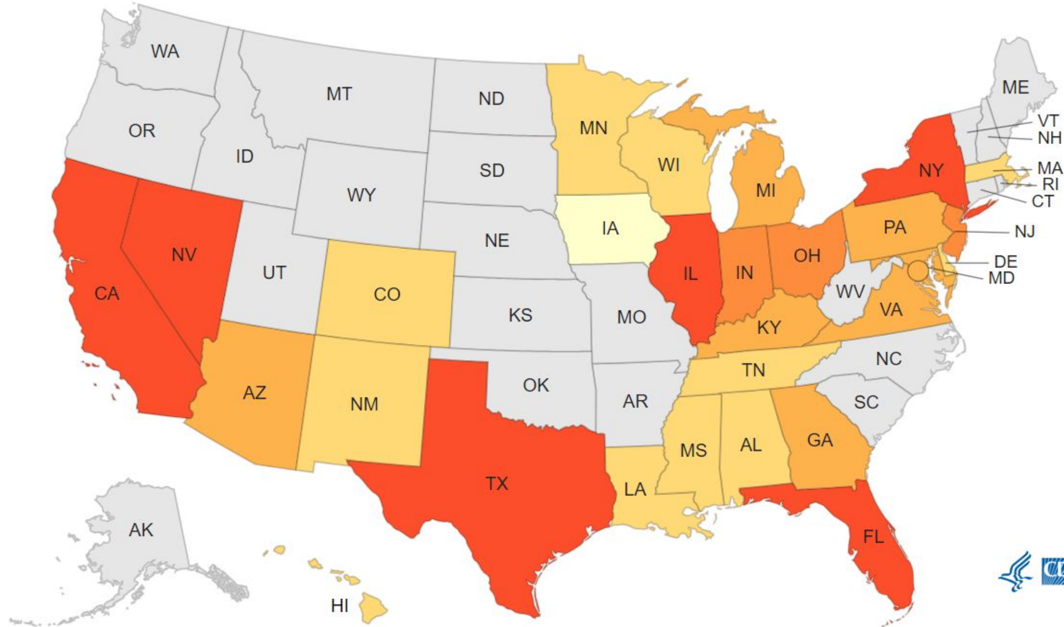


C. auris is reportable in 22 states as of September 2023

https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html
https://www.cdc.gov/fungal/fungal-disease-reporting-table.html

Candida auris tracking data - 2022

Made nationally notifiable in 2018



In 2022, there were 2,377 clinical cases and 5,754 screening cases.

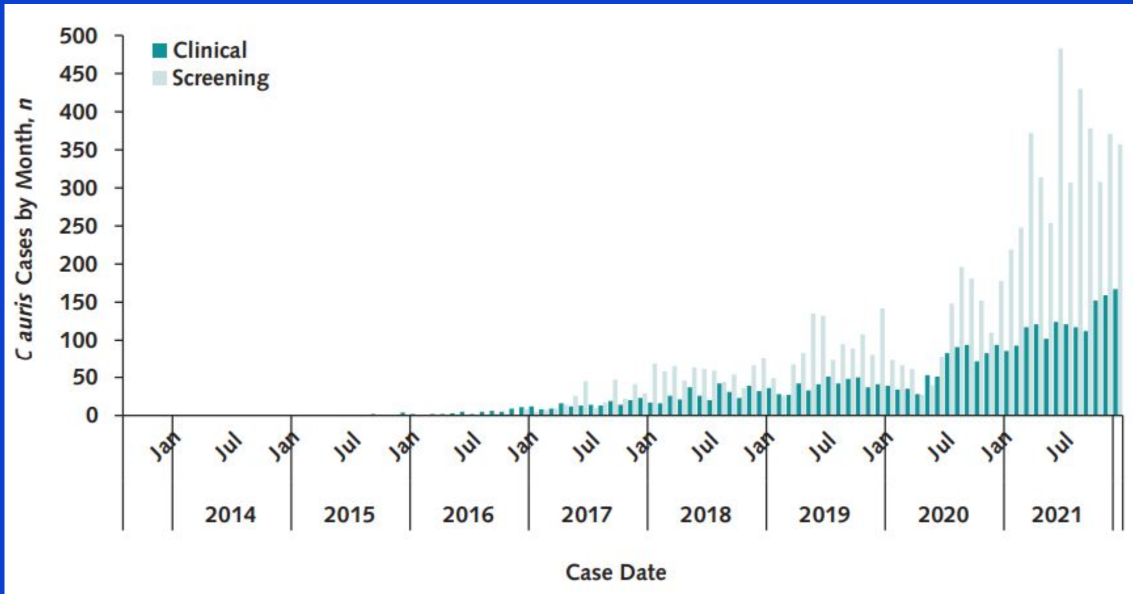
- 0 clincial cases and at least 1 screening case
- 1 to 10
- 11 to 50
- 51 to 100
- 101 to 500
- 501 to 1000
- 1001 or more

C. auris is reportable in 22 states as of September 2023

https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html
https://www.cdc.gov/fungal/fungal-disease-reporting-table.html

Worsening spread of *Candida auris* in the United States

2019 to 2021



- Year over year increase in clinical cases in **2019** was **44%**

  (from 330-476)

- Year over year increase in clinical cases in **2020** was **59%**

  (from 476-756)

- Year over year increase in clinical cases in **2021** was **95%**
  (756-1,471)

Lyman et al (2023) Annals of Int Med: 76(4) 489-495. https://www.acpjournals.org/doi/10.7326/M22-3469

Worsening spread of *Candida auris* in the United States

2019 to 2021

Table. Percentage Resistance of *Candida auris* Isolates Tested by the Antimicrobial Resistance Laboratory Network, 2018 to 2020*

Year or Region	Azoles†	Amphotericin B‡	Echinocandins§
Year			
2018 (*n* = 463)	372 (80.3)	151 (32.6)	2 (0.4)
2019 (*n* = 1006)	787 (78.2)	242 (24.1)	14 (1.4)
2020 (*n* = 1294)	1109 (85.7)	331 (25.6)	15 (1.2)
Region‖			
Mid-Atlantic (*n* = 135)	133 (98.5)	115 (85.2)	4 (3.0)
Midwest (*n* = 156)	17 (10.9)	2 (1.4)	0 (0.0)
Mountain (*n* = 25)	24 (96.0)	1 (4.0)	0 (0.0)
Northeast (*n* = 1051)	1046 (99.5)	468 (44.5)	22 (2.1)
Southeast (*n* = 172)	170 (99.4)	9 (5.2)	0 (0.0)
West (*n* = 556)	553 (99.5)	17 (3.1)	1 (0.2)

* Data are numbers (percentages). Numbers are based on records with any minimum inhibitory concentrations (MICs). About 1% of all records for all times were missing MICs for 1 or 2 drug classes.
† The tentative MIC breakpoint for fluconazole was ≥32 mcg/mL.
‡ The tentative MIC breakpoint for amphotericin B was ≥2 mcg/mL.
§ The tentative MIC breakpoint for echinocandins was ≥4 mcg/mL (anidulafungin or micafungin).
‖ The Central region is excluded because of the small number of isolates.

Susceptibility patterns vary by geography due to local circulation of specific clades

In ***2021***, **7** patients with pan-resistant isolates and **19** other patients with echinocandin-resistant isolates were detected compared with **6** and **3**, respectively, in 2020

Lyman et al (2023) Annals of Int Med: 76(4) 489-495. https://www.acpjournals.org/doi/10.7326/M22-3469

Treatment/management of invasive infections

Echinocandins are recommended for initial therapy

Dose information for adults & children ≥ 2 months of age

Echinocandin drug	Adult dosing	Pediatric dosing
Anidulafungin	Loading dose 200 mg IV, then 100 mg IV daily	Not approved for use in children
Caspofungin	Loading dose 70 mg IV, then 50 mg IV daily	Loading dose 70 mg/m^2/day IV, then 50 mg/m^2/day IV (based on body surface)
Micafungin	100 mg IV daily	2 mg/kg/day IV with option to increase to 4 mg/kg/day IV in children at least 40 kg

Even after invasive infection treatment, patients generally remain colonized with *C. auris* for long periods, possibly indefinitely

https://www.cdc.gov/fungal/candida-auris/c-auris-treatment.html

Management: non-invasive, non sterile body sites

Urine, external ear wounds, respiratory specimens, skin colonization, etc.

- If there is no evidence of infection, treatment is ***not recommended***
- Infection control measures should be used for all patients with *C. auris* ***regardless*** of specimen source
- Colonized patients are at risk for developing invasive infections and require additional prevention measures
  - Appropriate medical device care *(such as strict adherence to recommended central venous catheter and urinary catheter insertion and maintenance practices and meticulous care of tracheostomy sites)*
  - *Surgical Site Proced*ures *(meticulous skin preparation procedures should be followed to prevent infection)*
  - Antibiotic Stewardship

https://www.cdc.gov/fungal/candida-auris/c-auris-treatment.html

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Infection & prevention control

Primary methods in healthcare settings

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- Hand hygiene
  - Alcohol-based hand sanitizer, etc.
- Transmission
  - Based precautions *(contact precautions or enhanced barrier precautions similar to those taken for other MDROs)*
- Room/patient placement
  - Consideration for private rooms or cohorting in shared rooms *(consider dedicated healthcare personnel)*
- Follow recommended practices to reduce transmission in shared rooms
- CDC recommends continued contact precautions or barrier precautions for length of stay
- Routine reassessment of colonization is not recommended
- No specific decolonization procedure is known at this time

https://www.cdc.gov/fungal/candida-auris/c-auris-infection-control.html

The emerging health threat challenge

Challenges addressing emerging infectious diseases

Limited understanding of pathogen

Limited or ***lack*** of accepted, well-characterized samples

Limited access to positive sample material

Lack of publications describing identification approaches (primer/probe designs)

No standards, controls, reagents

No FDA-approved/cleared diagnostic tests

No high-throughput testing methods to facilitate high testing demand

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6816404/

Candida auris as an example

Candida auris
Identification

C. *auris* can be misidentified
as a number of different organisms including other *Candida* species when using traditional phenotypic methods for yeast identification

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)
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https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.html

Candida auris

CDC assay design

9.0 Primer/Probe Sequences

9.1 C. auris Primers/Probe:

V2424F (CAURF), 5′-CAG ACG TGA ATC ATC GAA TCT-3′

V2426 (CAURR), 5′-TTT CGT GCA AGC TGT AAT TT-3′

V2425P (CAURP), 5′-/56-carboxyfluorescein (FAM)/AAT CTT CGC /ZEN /GGT GGC GTT GCA TTC A /3IABkFQ/-3′

3IABkFQ: Iowa Black® FQ

ZEN/Iowa Black FQ is a Double-Quenched Probe, which provides superior performance compared to traditional single-quenched probes

- Assay requires manual steps (pipetting, plate setup, etc)
- Low/Med throughput option
- Can the CDC assay be modified to fit the cobas omni Utility Channel for a more automated, higher throughput option?

https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.h

cobas omni Utility Channel

cobas omni Utility Channel

Allows labs to run optimized LDTs with the same technology used to run IVDs

- ***Same*** master mix components (except primers/probes)
- ***Same*** automated extraction technology
- ***Same*** automated amplification technology

The cobas® x800 family

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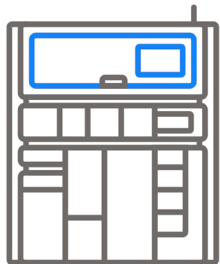
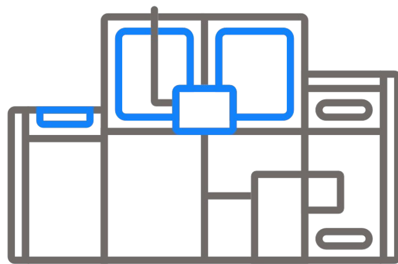


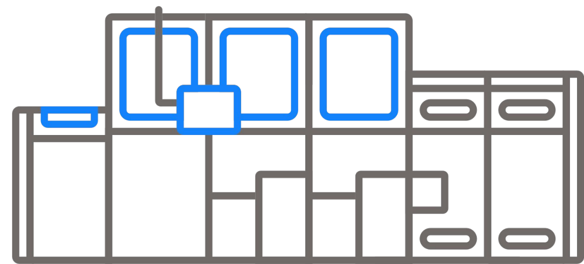
cobas® 5800 system

Up to 144 tests/shift*



cobas® 6800 system

Up to 384 tests/shift*



cobas® 8800 system

Up to 1056 tests/shift*

*8 hour shift

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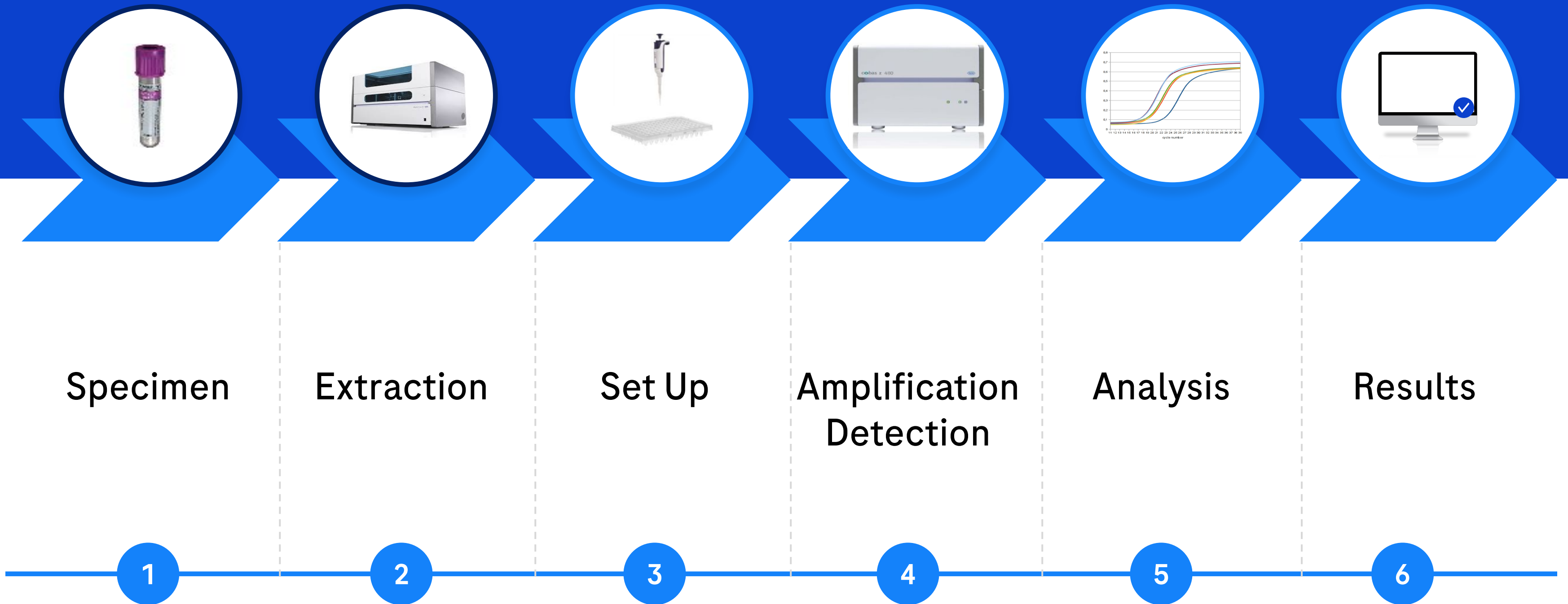
Example traditional workflow



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Example cobas omni Utility Channel workflow

Specimen

Extraction
Set-up
Amplification
Detection
Analysis

Results

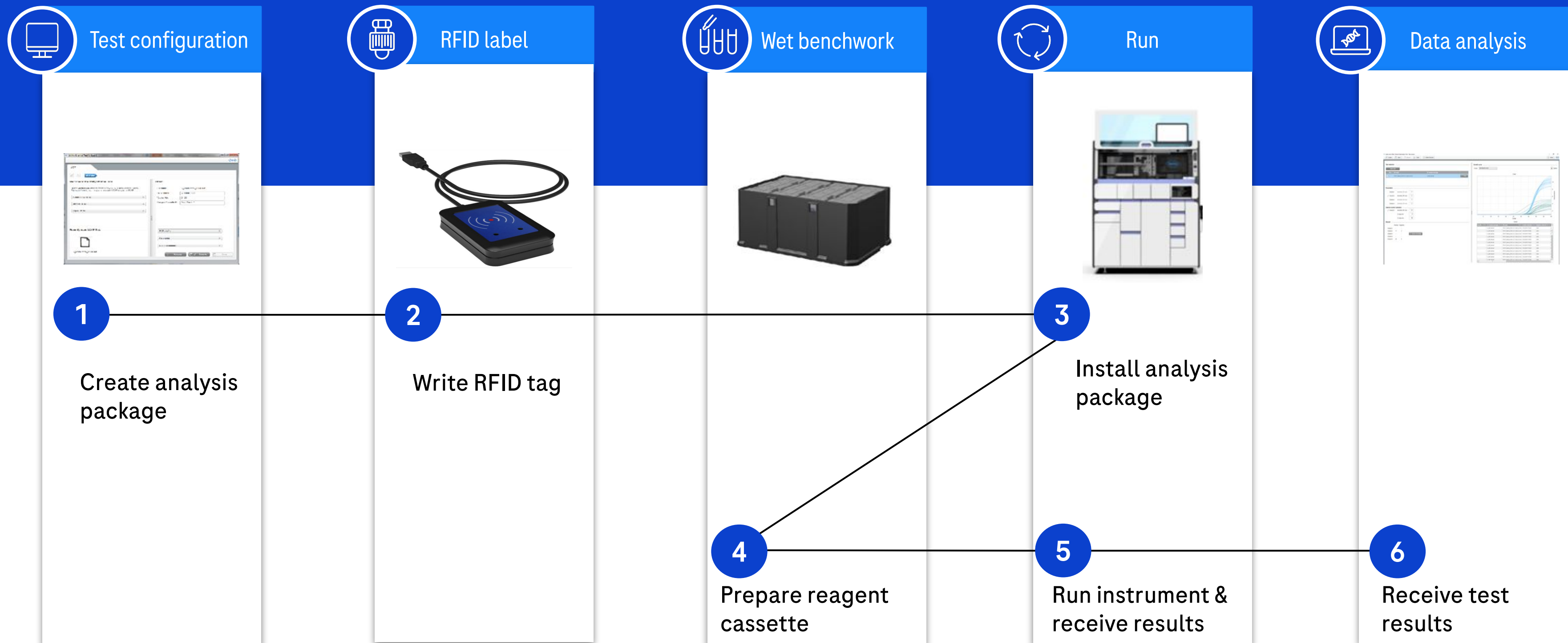
1

2

3

cobas omni Utility Channel

Workflow



cobas omni Utility Channel

Requirements

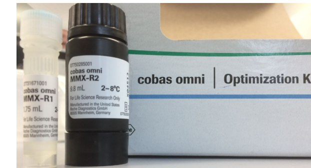
- Computer for omni software

- RFID reader/writer device (TWN3 Legic NFC USB)

- **cobas omni** Utility Channel Reagent Kit, 192T + Primer/Probes

- **cobas**® Buffer Negative Control Kit

- **cobas omni** Optimization Kit



Reagent cassette

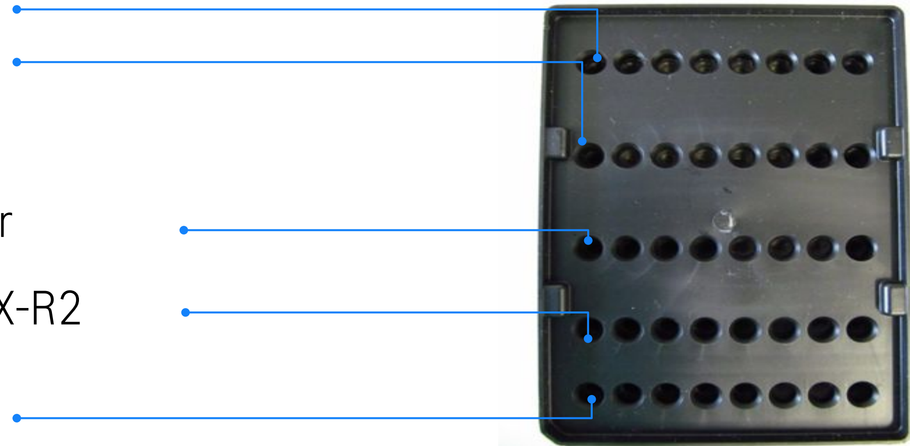
Proteinase

Internal Control

Elution Buffer (EB) - Tris base buffer

LDT primers/probes mixed with MMX-R2

Master Mix Reagent 1 (MMX-R1)



cobas omni Optimization Kit

Kit Configuration

Components:

Master Mix Reagent 1
Manganese acetate
Potassium hydroxide
< 0.1% Sodium azide

Master Mix Reagent 2
Buffer
Polymerase
AmpErase
Nucleotides
IC primer/probes

Benefit: Optimize on a generic thermal cycler during primary test development using the same chemistries you will use on the family of cobas® x800 instruments.

Design primers & probes

Note: Only the default master mix can be used for PCR reactions with the utility channel. Therefore, the majority of assay design is dependent on identifying primers and probes that are compatible with the master mix, or that can be optimized to become compatible.

cobas omni Utility Channel basics

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Internal control

The internal control of cobas omni Utility Channel is intended to be used as process control. Its non-human RNA-sequence was selected to minimize interference with primers and probes specific for human, viral or bacterial targets

Channels	Common fluorophores	Common dark quenchers	Excitation wavelength/ width [nm]	Emission wavelength/ width [nm]
1	• Atto 425/Cyan 500	• ZEN™/Iowa Black® FQ • BHQ®-1	435/25	470/20
2	• 6-FAM • FAM-dT	• ZEN™/Iowa Black® FQ • BHQ®-1 • BHQ®-2	495/10	521/10
3	• HEX • VIC • Cal Fluor® Orange 560 • SIMA-dT	• ZEN™/Iowa Black® FQ • BHQ®-1 • BHQ®-2	540/10	580/20
4	• Cal Fluor® Red 635 • LC Red® 640	• TAO™/Iowa Black® FQ • BHQ®-1 • BHQ®-2	610/20	645/20
5	Reserved for internal control (included in the reagent cassette)[a]			

General primer sequence characteristics

Amplicon size
50 to 250 bp

Primer length
18 to 25 bp

Those which do not contain internal secondary structure, are not complementary to each other at their 3' ends and are not self-complementary

Avoid mismatches close to the 3' end
Avoid having a "T" at the 3' end

3' methylation (last base or second to the last base) can help minimize primer-dimer formation

Have a GC content of 35%-65%
Avoid runs of identical nucleotides (i.e. 4+ consecutive residues such as CCCC or GGGG)

Have a melting temperature of around 60˚C to 70˚C that is at least 3˚C to 5˚C higher than the annealing temperature allowing annealing to occur between 55˚C and 65˚C

Candida auris CDC primer assessment

https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.html

Category	Specificiation	Details	Ok?
Amplicon size	50 to 250 bp	135 bp	✔
Primer Length	18 to 25 bp	21/20 bp	✔
Composition	GC content is 35-65%	43%/35%	✔
	Primers end on A or C	no/yes	?
	No complementarity to each other at their 3' ends	yes	✔
	No internal secondary structures	yes	✔
	No runs of 4+ identical nucleotides	yes	✔
	60-70°C and ≥ 3-5°C higher than annealing temperature	52.4/50.8	☐

Fwd: 5'-CAGACGTGAATCATCGAATCT-3'OH
Rev: 5'-TTTCGTGCAAGCTGTAATTT-3'OH

Candida auris CDC primer assessment

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Table 1: Comparison between methylated and unmethylated primers - **cobas**® 6800

1000 CFU/mL						
Input Vol. (uL)	Positive Replicates	Average Ct Unmethylated	Positive Replicates	Average Ct Methylated	ΔCt	Average ΔCt
150	2/2	36.69	2/2	35.05	1.64	1.38
200	2/2	35.09	2/2	34.5	0.59	
350	2/2	34.43	2/2	32.1	2.33	
500	2/2	34.08	2/2	33.1	0.98	
100 CFU/mL						
Input Vol. (uL)	Positive Replicates	Average Ct Unmethylated	Positive Replicates	Average Ct Methylated	ΔCt	Average ΔCt
150	2/2	40.49	2/2	40.57	-0.08	1.105
200	2/2	40.47	2/2	38.19	2.28	
350	2/2	38.09	2/2	36.96	1.13	
500	2/2	37.50	2/2	36.4	1.10	

McCune et al (2023) High-Throughput, Automated Detection of Candida auris on the cobas® x800 with the cobas® omni Utility Channel – APHL ID Con

General probe sequence characteristics

Probe length 18 to 30 bp

- Have a GC content of 45% to 65%
- Avoid runs of identical nucleotides *(i.e. 4+ consecutive residues such as CCCC or GGGG)*
  Avoid having a "G" at the 5' end
- The melting temperature of the probe should always be higher (5˚C to 10˚C) than the primer
- Dark quencher dyes result in better fluorescent signals
- Hydrolysis probes with an internal, rather than a 3'- terminal quencher

Candida auris CDC probe assessment

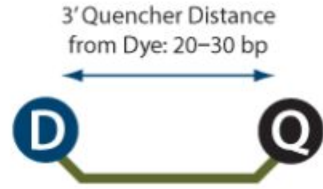
Category	Specification	Details	Ok?
Amplicon size	50 to 250 bp	135 bp	✔
Primer Length	18 to 30 bp	25 bp	✔
Composition	GC content is 45-65%	52%	✔
	Avoid having a "G" at the 5'-end	yes	✔
	No complementarity to each other at their 3' ends	yes	✔
	No internal secondary structures	yes	✔
	No runs of 4+ identical nucleotides	yes	✔
	5-10°C higher than the primer Tm	63.6°C	✔

5’-AATCTTCGCGGTGGCGTTGCATTCA-3’OH

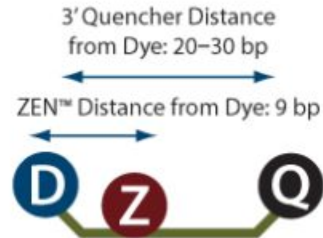
https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.html

Optimization

Probe modification



A. Traditional 5′ Nuclease Probes.



B. ZEN™ Dual-Quenched Probes.

Candida auris **probe**
5'-FAM-AATCTTCGC-ZEN-GGTGGCGTTGCATTCA-3IABkFQ-3'OH

CDC already had an internal quencher in their design

cobas omni Utility Channel: Guidelines for Establishing a PCR-based LDT – User Guide Version 2.0
https://www.cdc.gov/fungal/lab-professionals/Real-time-PCR-based-Id-C-auris.html

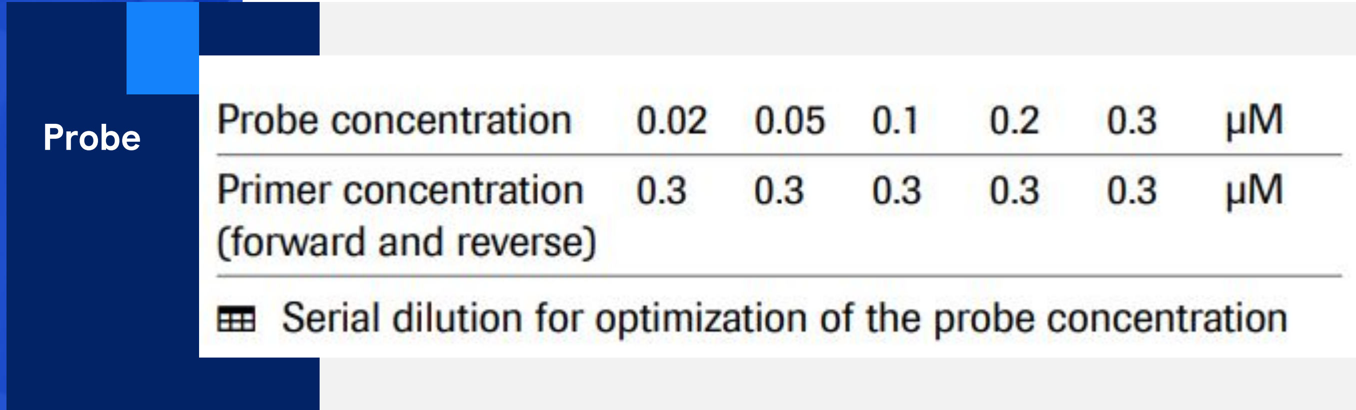
Titration of primers & probes

Note: Only the default master mix can be used for PCR reactions with the utility channel. Therefore, the majority of assay design is dependent on identifying primers and probes that are compatible with the master mix, or that can be optimized to become compatible.

Optimization

Primer/probe titration

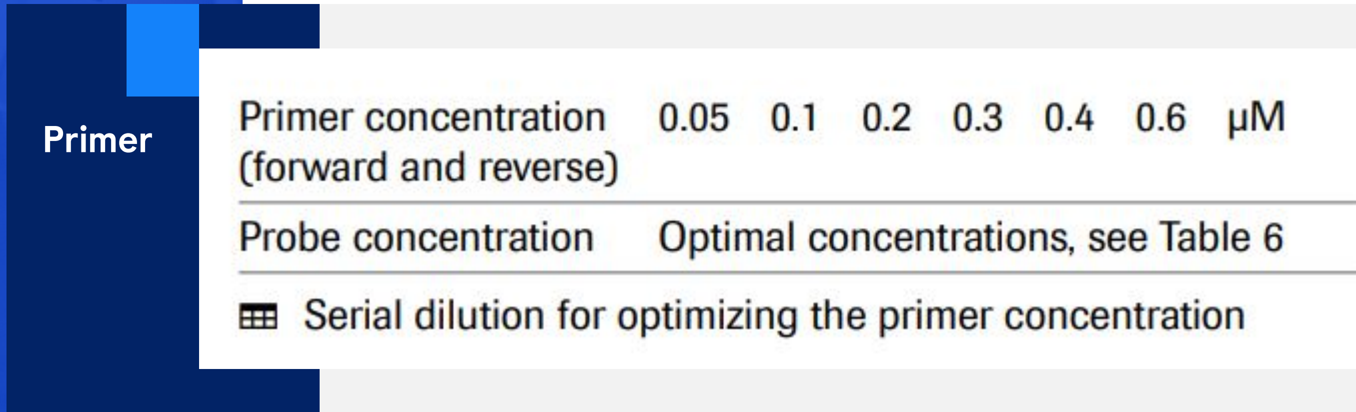
Probe



Probe concentration	0.02	0.05	0.1	0.2	0.3	µM
Primer concentration (forward and reverse)	0.3	0.3	0.3	0.3	0.3	µM

Serial dilution for optimization of the probe concentration

Primer

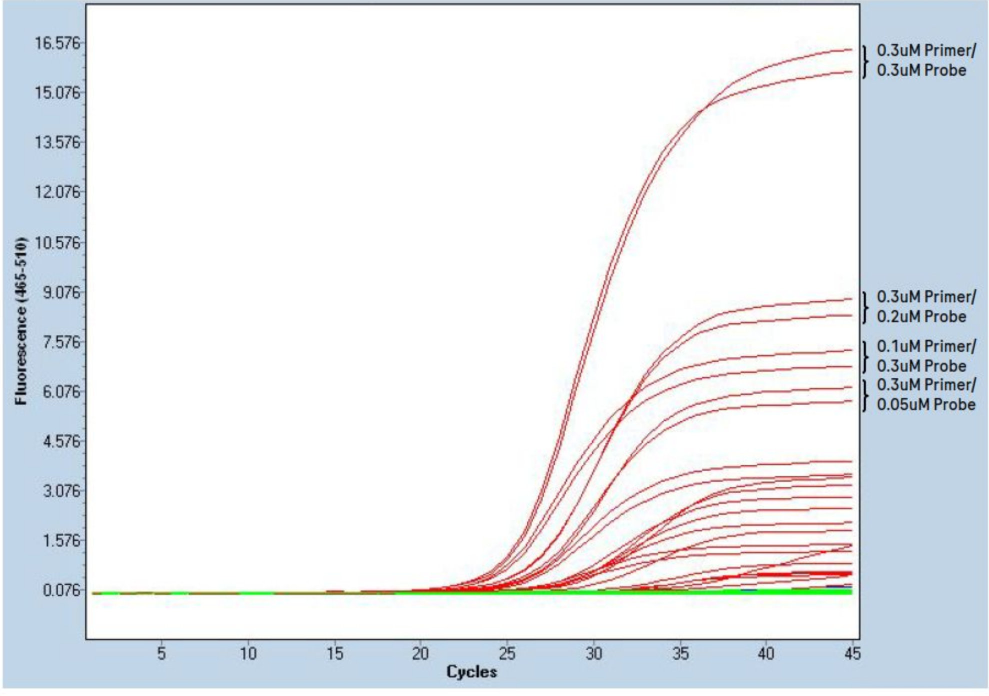


Primer concentration (forward and reverse)	0.05	0.1	0.2	0.3	0.4	0.6	µM
Probe concentration	Optimal concentrations, see Table 6						

Serial dilution for optimizing the primer concentration

Optimization

Primer/probe titration



McCune et al (2023) High-Throughput, Automated Detection of *Candida auris* on the cobas® x800 with the cobas® omni Utility Channel – APHL ID Con

High-Throughput, Automated Detection of *Candida auris* on the **cobas**® x800 with the **cobas**® omni Utility Channel

Stephen McCune, Erin Gick, Robin Thomas, Sara J. Blosser
Medical & Scientific Affairs, Roche Diagnostics Corporation, Indianapolis, IN

APHL ID Con, March 13-15, 2023

Background:

The US Centers for Disease Control and Prevention (CDC) has recognized *Candida auris* as an emerging fungal pathogen associated with multiple-drug resistance and healthcare-associated outbreaks. Despite the public health relevance, few high throughput diagnostic or screening tests for this organism are available in the US.

The CDC's Antimicrobial Resistance (AR) Lab Network, established in 2016, "provides nationwide lab capacity to rapidly detect antimicrobial resistance and inform local responses to prevent spread", including testing for *Candida auris*. The AR Lab Network utilizes both a low- and medium- throughput, laboratory-developed test for *C. auris* patient-colonization screening and isolate identification.

This study demonstrates the utilization of the **cobas**® omni Utility Channel on the **cobas**® 6800/8800 Systems to assess the feasibility of conducting fully automated, higher throughput *C. auris* testing, using the same primer/probe design currently employed by the AR Lab Network, as well as steps that can be taken to optimize the assay.

Image Credits: CDC AR Lab Network

cobas® omni Utility Channel

Condensing this...

To this...

*Run includes the extraction, set-up, amplification, detection, and analysis steps in a fully automated, walk-away format.

cobas® omni Utility Channel Workflow

Methods:

Primers and probes from the *C. auris* AR Lab protocol[1] were evaluated for system compatibility on the **cobas**® z480 analyzer using the **cobas**® omni Optimization Kit, then concentrations titrated to be most effective with the **cobas**® omni master mix. Once the optimal primer/probe concentration was found, the assay was evaluated on a **cobas**® 6800 instrument using the **cobas**® omni Utility Channel with pure, titered, whole *C. auris* isolate from Zeptometrix® (Cat.#0804386) spiked at varying concentrations into Liquid Amies medium. During testing on the **cobas**® 6800, input volume, primer methylation, RFI sensitivity adjustment, and pre-extraction incubation[2] were evaluated for impact on assay performance.

RFI, or Relative Fluorescence Intensity, is used to establish the LOD of your assay

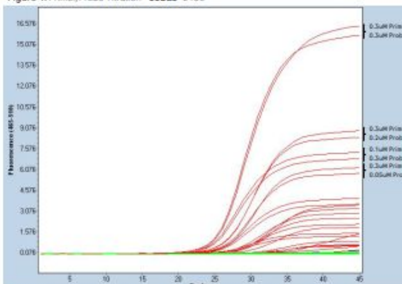
RFI = plateau fluorescence/baseline fluorescence

- **Baseline** average of first 5 cycles
- **Plateau** average of last 5 cycles

Establishing the RFI works best when you evaluate at least 5 specimens with Ct values 20-37 in duplicate. Ex. 25, 28, 31, 34, 37

Results:

Figure 1: Primer/Probe Titration - **cobas**® z480



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1000 CFU/mL						
Input Vol. (uL)	Positive Replicates	Average Ct Unmethylated	Positive Replicates	Average Ct Methylated	ΔCt	Average ΔCt
150	2/2	36.69	2/2	35.05	1.64	1.38
200	2/2	35.09	2/2	34.5	0.59	
350	2/2	34.43	2/2	32.1	2.33	
500	2/2	34.08	2/2	33.1	0.98	
100 CFU/mL						
Input Vol. (uL)	Positive Replicates	Average Ct Unmethylated	Positive Replicates	Average Ct Methylated	ΔCt	Average ΔCt
150	2/2	40.49	2/2	40.57	-0.08	1.105
200	2/2	40.47	2/2	38.19	2.28	
350	2/2	38.09	2/2	36.96	1.13	
500	2/2	37.50	2/2	36.4	1.10	

Results:

Table 2: Replicate positivity rate after RFI sensitivity and input volume adjustment - **cobas**® 6800

CFU/mL	200 uL Sample Input				500 uL Sample Input			
	2.5 RFI Cutoff Value		1.5 RFI Cutoff Value		2.5 RFI Cutoff Value		1.5 RFI Cutoff Value	
	Hit Rate (n=3)	Avg. Ct	Hit Rate (n=3)	Avg. Ct	Hit Rate (n=3)	Avg. Ct	Hit Rate (n=3)	Avg. Ct
100	3/3	34.45	3/3	34.45	3/3	32.64	3/3	32.64
50	2/3	35.69	3/3	36.30	3/3	34.30	3/3	34.30
40	3/3	35.70	3/3	35.70	3/3	34.59	3/3	34.59
30	3/3	36.06	3/3	36.06	3/3	35.51	3/3	35.51
20	1/3	36.41	3/3	37.54	2/3	36.72	3/3	36.85
10	0/3	NEG	3/3	38.76	2/3	36.51	3/3	37.58
1	0/3	NEG	0/3	NEG	0/3	NEG	2/3	40.01

Table 3: Replicate positivity rate at 1.5 RFI sensitivity after pre-extraction incubation - **cobas**® 6800

CFU/mL	200 uL Sample Input		500 uL Sample Input	
	Hit Rate N=3	Avg. Ct	Hit Rate N=3	Avg. Ct
100	3/3	32.29	3/3	30.69
50	3/3	33.46	3/3	32.13
40	3/3	34.12	3/3	32.07
30	3/3	34.28	3/3	32.62
20	3/3	35.95	3/3	33.88
10	3/3	37.96	3/3	34.49
1	2/3	41.22	3/3	38.73

Primers and probes from the AR Lab protocol were titrated from 0.3 to 0.05μM and run on the **cobas**® z480 using the Utility Channel Optimization kit (Figure 1). The primer/probe set that showed the most robust fluorescence was chosen. Primer modification in some cases has been demonstrated to enhance PCR specificity, and increase sensitivity, by reducing competition with non-productive reactions[3]. Methylation of the AR Lab primers showed a reduction in Ct value by an average of 1.1 to 1.3 (Table 2). An adjustment that can be used to modify assay sensitivity using the **cobas**® Omni Utility channel, is the RFI (relative fluorescence index). Methylated AR Lab primer/probes were able to detect three replicates down to 30 CFU/mL with an RFI sensitivity of 2.5, and down to 10 CFU/mL with an RFI sensitivity of 1.5 (Table 2). Finally, a pre-extraction incubation using Proteinase K and Bacterial Lysis Buffer (used in the CDC protocol[2]) was evaluated for contribution to assay sensitivity. With the pre-extraction incubation, the assay was able to detect three replicates down to 10 CFU/ml at 2.5 RFI (not shown), and 1 CFU/mL at 1.5 RFI using a 500 μL input volume (Table 3). Pre-extraction did not impact the 200 μL input volume appreciably.

Discussion: This study demonstrated proof-of-concept application of the **cobas**® omni Utility Channel using a previously published AR Lab protocol for *Candida auris*. Primer/probe design of the AR Lab protocol was compatible with the **cobas**® omni Utility Channel. Methylation of primers decreased the Ct value for samples compared to unmodified primers under the same conditions. The assay was able to detect down to 1-10 CFU/mL depending on input volume, pre-incubation steps, and RFI modification. In the CDC protocol a 200 μL sample input volume is used[2], however based on our data, a 500 μL sample input volume may be able to further enhance assay detection. Studies evaluating the performance of this assay with previously characterized clinical specimens are ongoing.

References/Disclosures

[1] Leach, L, Zhu, Y, & Chaturvedi, S. (2018). JCM. 56(2), e01223-17. https://doi.org/10.1128/JCM.01223-17
[2] https://www.cdc.gov/fungal/candida-auris/pdf/Real-time-PCR-based-Id-C-auris-508.pdf
[3] Schoenbrunner, NJ et al., (2017). *Biol Meth Protoc*, 1-10. https://doi.org/10.1093/biomethods/bpx011

Stephen McCune, Erin Gick, and Robin Thomas are employees of Roche Diagnostics Corporation. Sara J. Blosser was an employee of Roche Diagnostics Corporation during the study, but currently is an employee of CDC.

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Journal of Virological Methods
Volume 269, July 2019, Pages 49-54

Clinical evaluation of multiplex RT-PCR assays for the detection of influenza A/B and respiratory syncytial virus using a high throughput system

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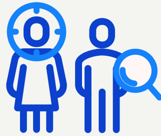
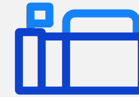
Summary

Roche



- *Candida auris* is an emerging multidrug resistant pathogen that can cause a wide range of nosocomial infections and it's prevalence is rapidly growing in the US

- Emerging health threats are challenging for laboratories to address as IVD approved/cleared tests are often not available and initial LDT solutions *(if they exist)* are often low throughput and manual



- The cobas omni Utility Channel provides a pathway to develop high-throughput, automated LDT assays that can be used to address emerging health threats

Doing now what patients need next

P.A.C.E # 279-014-24

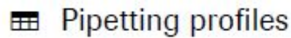
Contact hours: 0.5

Level of instruction: Intermediate

Doing now what patients need next

Sample types

Sample material	Processing volume [μL]
Plasma	200, 350, 500, 850
PreservCyt®	400
RCCM	400
Serum	200, 500, 850
Swab	400
Urine	850
VTM	400
Whole blood	500
U_Simple sample	150, 200, 350, 500, 850
U_Sample with swab	400
U_Alcohol-based sample	400



Notes from the Field

- Predilute whole blood (ex. 1:7 in cobas® PCR Media)
- Dissolve stool samples (ex. in 2 mL PCR Media or PBS)

Tip: When using sample types outside of the above list, extra caution should be used to monitor performance of the system and confirm results. Special attention should be taken with sample types that could impact pipetting such as viscous or clotty samples.

Sample types

Target	Sample type	Reference
FluA/B/RSV	Contrived swabs/simulated specimen	Hein, R et. al. AMP 2018 poster.
T.vag	PreservCyt	Hein, R et. al. CVS 2018 Poster.
HSV 1/2 & VZV	Contrived swabs/simulated specimen	Hein, R et. al. CVS 2018 Poster.
Factor II/V	EDTA-whole blood	Neumann et. al. Poster
BKV	Serum	Hasan et. al. Journal of Virological Methods. 2016
C.diff	Stool	Eigner et. al. Journal of Microbiological Methods. 2020
BKV	EDTA-plasma, serum and urine	Fritzsche et. al. Journal of Virological Methods. 2021
HDV	Serum	Pfluger et. al. JHEP reports. 2021
FluA/B/RSV	Spiked UTM	Eigner et. al. Journal of Virological Methods. 2019
SARS-CoV-2	Oropharyngeal and nasopharyngeal swabs in UTM	Norz et.al. Diagnostics.2021
HSV/VZV	EDTA-plasma and CSF	Lutgehetmann et.al. Poster
Zika virus	Plasma and urine	Boujnan et.al. Donor Infectious Disease Testing. 2018.