High-throughput *C. auris* (RUO) Assay on the cobas® 5800/6800/8800 System

N. Martins Sorenson[1], J. Alexander[2], A. Moghaddasi[1], A. Hill[2], J. Harris[2], S. McCune[3], E. Gick[3], S. Blosser[4], M. Lewinski[2], K. Cady[1]

[1]Roche Molecular Systems, Santa Clara, CA, USA
[2]Roche Molecular Systems, Pleasanton, CA, USA
[3]Roche Diagnostics Corporation, Indianapolis, IN, USA
[4]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

Introduction

Candida auris is an emerging multidrug resistant pathogen that can cause a wide range of hospital-acquired infections. The prevalence of this fungal pathogen is rapidly a growing threat within the US with 1,994 clinical and 5,071 surveillance cases identified in 2022, respectively (1). Rapid identification of patients colonized or infected with *C. auris* is a key step in containing hospital spread (2). Currently, standard laboratory methods such as culture, ID, and drug susceptibility used to characterize *C. auris* can take anywhere from 4-14 days. A rapid and automated high-throughput RUO assay is critical to identify hospitalized patients colonized with *C. auris*. Here we describe the performance of a *C. auris* RUO assay on the **cobas®** omni Utility Channel (UC) using the **cobas®** 6800 system, oligos ordered from Integrated DNA Technologies (IDT), control material available from Zeptometrix, commercially available collection devices, and using no off machine pre-analytics. Inclusivity, exclusivity, and sensitivity were evaluated using contrived samples in relevant collection media. Assay limit of detection was compared to the previously published manual LDT assays (1-2). The **cobas®** x800 UC *C. auris* assay was tested with 200 remnant composite nares/axilla/groin Eswab samples (frozen/fresh) with results compared to the BD Max LDT (3) assay. The performance of this assay was 95% accuracy, 92% sensitivity, 98% specificity, 98% PPV, and 92.5% NPV when compared against a BDmax LDT (3) on frozen/fresh clinical surveillance samples. This assay will help provide a high throughput *C. auris* RUO option for labs equipped with a **cobas®** x800 family system to help support surveillance research as the number of *C. auris* cases within the United States and around the world continue to rise.This test is for research use only, and not for use in diagnostic procedures.

Methods

The **cobas®** 6800 System with the **cobas®** omni Utility Channel tool v3.0, was used for the study. To prepare the reagent cassette, 600 µL of the IDT primer/probe-mix was added to 10 mL of Roche Master mix (MMx-2). Afterwards, 10 mL of the primer/probe and MMx-2 mix were transferred to the **cobas®** omni Utility Channel Reagent Cassette. Using the "Simple Sample" or "Sample with Swab" setting, either 200 µL or 400 µL sample processing volume was utilized respectively (from a minimum loaded sample volume of 350 or 550 µL, respectively).

Assay interpretations: The **cobas®** optimization tool analysis package was utilized for results interpretation in combination with 38/2.5, and 50/2 Ct/RFI cutoffs for the 5.8S-ITS2 rRNA and Internal control channels, respectively.

Channel	Excitation-Emission (nm)	Target region	Purpose
1	435-470	--	--
2	495-521	--	--
3	540-580	5.8S-ITS2 rRNA	*C. auris* Detection
4	610-645	--	--
5	680-700	GIC	Internal Control

Table 1. cobas® x800 UC *C. auris* Assay Setup with Targets and Channel Assignments

	UNG Incubation	Pre-PCR Step	1st Measurement	2nd Measurement	Cooling
No. of Cycles	Predefined	1	5	45	Predefined
No. of Steps		3	2	2	
Temperature		55ºC; 60ºC; 65ºC	95ºC; 50ºC	91ºC; 58ºC	
Hold Time		120s; 360s; 240s	5s; 30s	5s; 25s	
Data Acquisition		None	End of Each Cycle	End of Each Cycle	

Table 2. cobas® 6800 PCR Conditions

Results

Inclusivity of the cobas® x800 UC *C. auris* assay

Currently five genetically distinct clades of *C. auris* have been identified in different geographical locations (4). *In silico* analysis predicts 100% coverage of the five *C. auris* clades, which was further confirmed by wet lab testing with two CFU concentrations.

CFU/mL (range)	CLADE I (AR-0387)		CLADE II (AR-0381)		CLADE III (AR-0383)		CLADE IV (AR-0385)		CLADE V (AR-1097)	
	Avg. Ct ±STDEV	Hit Rate	Avg. Ct ±STDEV	Hit Rate	Avg. Ct ±STDEV	Hit Rate	Avg. Ct ±STDEV	Hit Rate	Avg. Ct ±STDEV	Hit Rate
200-900	24.5 ±0.4	100% (3/3)	24.5 ±0.1	100% (3/3)	24.6 ±0.3	100% (3/3)	24.1 ±0.1	100% (3/3)	27.2 ±0.3	100% (3/3)
20-90	28.7 ±0.6	100% (3/3)	28.6 ±0.2	100% (3/3)	28.1 ±0.2	100% (3/3)	27.5 ±0.6	100% (3/3)	31.4 ±0.4	100% (3/3)

Samples	Fungal cells (*C. auris* Clades I-V)
Input	CFU/mL (400 uL processing volume)
Background	**cobas®** PCR Media
Platform	**cobas®** 6800

Table 3. The **cobas®** x800 *C. auris* RUO assay is inclusive of all 5 clades.

Results (continued)

Comparison of cobas ® x800 UC *C. auris* assay to other LDT assays

To compare the UC assay performance to established manual LDT assays, we performed analytical sensitivity studies using spiked *C. auris* samples into Liquid Amies or **cobas®** PCR media (CPM). The limit of detection (LoD) of ~0.37 CFU/Rxn observed for Liquid Amies was similar to CPM (LoD of 0.5 CFU/Rxn) (data not shown).

The **cobas®** UC assay performed better when compared to the commonly utilized CDC (1) or Walchak (2) LDT assays when tested in a clean matrix (Table 4 and Table 5). The LoD of the **cobas®** UC *C. auris* assay was lower than both LDT assays with significantly less hands on processing and no pre-analytic step.

CFU/mL	MP96 → LC480 workflows					
	CDC			Walchak		
	CFU/RxN	Avg CT	hit rate	CFU/RxN	Avg CT	hit rate
1,480	13.5	28	**100%** (8/8)	14.8	29	**100%** (8/8)
740	6.73	29	**100%** (8/8)	7.4	29	**100%** (8/8)
370	3.36	30	**88%** (7/8)	3.7	29	**75%** (6/8)
185	1.68	32	**100%** (8/8)	1.85	30	**100%** (8/8)
93	0.84	33	**100%** (8/8)	0.93	30	**88%** (7/8)
46	0.42	33	**100%** (8/8)	0.46	30	**25%** (2/8)
23	0.21	35	**88%** (7/8)	0.23	30	**25%** (2/8)
12	0.11	35	**100%** (8/8)	0.12	30	**38%** (3/8)
6	0.05	36	**100%** (8/8)	0.06	30	**38%** (3/8)
3	0.03	37	**50%** (4/8)	0.03	30	**38%** (3/8)
1.4	N/A	N/A	**30%** (6/20)	N/A	N/A	**20%** (4/20)
0.7	N/A	N/A	**15%** (3/20)	N/A	N/A	**0%** (0/20)
	LoD by PROBIT at 95% Hit Rate **57 CFU/mL, 0.37 CFU/RxN**			LoD by PROBIT at 95% Hit Rate **1,116 CFU/mL, 11.16 CFU/RxN**		

CDC - claimes LoD = 1 cfu/rxn → *equivalent to* ***110*** *CFU/mL in the sample*
Walchak - claims LoD = 4-54 cfu/rxn → *equivalent to* ***400 - 5,400*** *CFU/mL in the sample*

Table 4. LoD performance of the reference LDT assays from CDC (1) and Walchak et al (2). Contrived samples were processed following published methods including sample pre-treatment conditions, MP96 processing and LC480 analysis. The sample was generated using clade III *C. auris* representative strain (CDC AR-0383) spiked into the diluent media and tested using all three protocols. NA: not applicable.

CFU/mL	c6800 UC Assay Results		
	Liquid Amies		
	CFU/RxN	Avg CT	hit rate (+/-)
1,480	312	22.9	**100%** (20/20)
740	160	24.2	**100%** (20/20)
370	80	25.3	**100%** (20/20)
185	40	26.7	**100%** (20/20)
93	20	27.5	**100%** (20/20)
46	10	28.6	**100%** (20/20)
23	5	29.3	**100%** (20/20)
12	2.5	30.4	**100%** (20/20)
6	1.2	31.7	**100%** (20/20)
3	0.62	33.1	**100%** (20/20)
1.4	0.31	35.4	**91%** (21/23)
0.7	0.16	35.7	**65%** (28/43)
	LoD by PROBIT at 95% Hit Rate **1.71 CFU/mL, 0.37 CFU/RxN**		

Table 5. LoD performance of the UC RUO assay. Samples were processed using the full workflow in the **cobas®** omni Utility Channel of a **cobas®** 6800 System. The contrived sample was generated using clade III *C. auris* representative strain (CDC AR-0383) spiked into the diluent media. Liquid Amies samples with a 400µL sample input.

Evaluation of Swab collection kits

Four different Swab collection devices (BD ESwab™, Copan Mswab®, **cobas®** PCR Media Uni Swab, and BD BBL™ Culture Swab) were tested and shown to be compatible with our **cobas®** x800 UC *C. auris* assay (Table 6). Given collection media was spiked with 5 different concentrations of *C. auris* AR-393 (clade III) independent of the collection volume of each device. The **cobas®** UC *C. auris* assay performed well and 10 CFU/mL was detected for all collection devices (Table 7).

Note- assay LOD and thresholds may need to be modified/updated as laboratories validate the assay with a given collection device.

Swab Collection Kits	Collection System	Type of Transport Media (volume)	Catalog number	
BD ESwab™	Flocked Swab	Liquid Amies (~1 mL)	**22-349-700**	
Copan Mswab®	FLOQ Swab	Mswab Media (~1.6 mL)	**7007248190**	
cobas® PCR Media Uni Swab Sample Kit	Woven Swab	**Cobas®** PCR Media (~4.3 mL)	**7958030190**	
BD BBL™ CultureSwab	Liquid Stuart, double swab	*N/A	**220109**	

*N/A: Not applicable due to transport vessel containing a wet sponge. BD BBL CultureSwab was used in a cobas® Collection Kit due to incompatibility with direct loading onto the **cobas®** 6800.

Table 6. Four different Swab Kits analyzed (E Swab, M Swab, **Cobas®** Uni Swab and BBL CultureSwab)

Collection Volume	Swab kits	CFU added to each collection kit									
		2x10⁵ CFU		2x10⁴ CFU		2x10³ CFU		2x10² CFU		2x10¹ CFU	
		Avg. CT (±STDEV)	Avg. RFI (±STDEV)	Avg. CT (±STDEV)	Avg. RFI (±STDEV)	Avg. CT (±STDEV)	Avg. RFI (±STDEV)	Avg. CT (±STDEV)	Avg. RFI (±STDEV)	Avg. CT (±STDEV)	Avg. RFI (±STDEV)
1.6mL	**Copan MSwab®**	15 (0.1)	13 (0.2)	18 (0.1)	12 (0.1)	22 (0.1)	12 (0.1)	26 (0.3)	11 (0.0)	29 (0.4)	11 (0.1)
4.3mL	**Cobas® UniSwab**	16 (0.3)	13 (0.1)	19 (0.1)	12 (0.3)	23 (0.2)	12 (0.1)	26 (0.4)	11 (0.1)	31 (1.2)	11 (0.1)
1mL	**BD ESwab™**	15 (0.1)	12 (0.2)	18 (0.1)	12 (0.2)	22 (0.2)	12 (0.0)	25 (0.1)	11 (0.1)	29 (0.3)	11 (0.1)
4.3mL	**BD BBL™ Culture Swab**	16 (0.3)	12 (0.2)	19 (0.1)	12 (0.3)	23 (0.2)	12 (0.1)	26 (0.6)	11 (0.1)	30 (0.5)	11 (0.1)

Table 7. Four different swabs (BD ESwab™, Copan MSwab®, **Cobas®** Uni Swab and BD BBL™ Swab) collection kits spiked with clade III *C. auris* (CDC AR-0383) (high, medium and low CFU loads)

Remnant Clinical Sample Performance

A total of 200 remnant composite nasal/axilla/groin samples collected with Eswab collection devices at the HRI/Wadsworth public health lab in 2022 were tested with our **cobas®** x800 UC *C. auris* assay and compared to their BD Max *C. auris* LDT[3] results. This study was performed in two phases: Phase I with 100 frozen samples stored at -80 ºC and Phase II with 100 freshly collected samples stored at 4 ºC for 9-22 days. Samples were blindly tested without the reference information from HRI (200µL sample processing volume was used due to limited sample volume). See Figure 1 for the *C. auris* surveillance workflow 1 and 2 with the BD Max PCR (3) with Culture/MALDI-TOF followed by the HRI/Wadsworth lab, respectively. Our UC *C. auris* assay performance compared to the BD Max LDT is shown on Table 8. The workflow 2 HRI/Wadsworth was used to to resolve the discrepant sample analysis (Table 9).

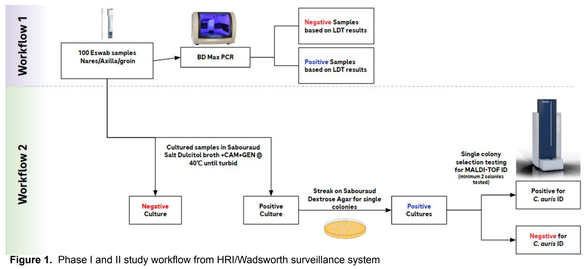

Figure 1. Phase I and II study workflow from HRI/Wadsworth surveillance system

			BD MAX Supplied Results		Accuracy %	Sensitivity %	Specificity %	PPV %	NPV %
			# Positive	# Negative					
cobas ® x800 UC Assay Results	Phase I Frozen Samples	# Positive	46	0	94	88	100	100	89
		# Negative	6	48					
	Phase II Fresh Samples	# Positive	45	2	96	96	96	96	96
		# Negative	2	50					
		# Invalid	1	-					

Table 8. Evaluation of frozen or fresh remnant clinical samples (phase I and II) with the **cobas®** x800 UC *C. auris* RUO Assay compared to LDT BD Max PCR as the reference method.

	Phase study	Culture/MALDI-TOF	BD MAX Ct	cobas ® Ct	Notes
Sample #12	I (frozen)	no growth	32.50	-	
Sample #18	I (frozen)	no growth	37.50	-	Late Ct for **BD Max**, no growth during sample enrichment
Sample # 35	I (frozen)	no growth	35.00	-	Late Ct for **BD Max**, no growth during sample enrichment
Sample #59	I (frozen)	C. albicans / E.coli	33.40	-	False Positive for **BD Max** due to Culture/MALDI-TOF results for other ***C. albicans*** and ***E. coli***.
Sample #62	I (frozen)	no growth	32.80	-	
Sample #66	I (frozen)	no growth	34.00	-	
Sample #8	II (fresh)	no growth	-	36.5	Late Ct for **RMS**, no growth observed during sample enrichment
Sample #14	II (fresh)	no growth	-	38.0	Late Ct for **RMS**, no growth observed during sample enrichment
Sample #51	II (fresh)	C. auris	30.8	*Invalid*	*Invalid result for **RMS** due to the sample being clotted.* (Insufficient volume to re-run)
Sample #95	II (fresh)	no growth	36.6	-	Late Ct for **BD Max**, no growth observed during sample enrichment
Sample #100	II (fresh)	no growth	36.9	-	Late Ct for **BD Max**, no growth observed during sample enrichment

Table 9. Discrepant samples from phase I and II study between the **cobas®** UC assay and the BD Max PCR results.

A total of 10 discrepant results were observed when comparing the **cobas®** UC Assay vs BD Max PCR results. To further evaluate the discrepant results, the two positive samples (#8 and #14) were confirmed as *C. auris* by our NGS workflow for PCR amplicon. For the negative discrepant samples, a cultured enrichment step and isolation on HardyCHROM™ Candida + auris agar plates (1) was performed and none of them were confirmed as *C. auris*.

Results (continued)

Exclusivity testing of the cobas® x800 UC *C. auris* assay

Species	Source	Source ID	Channel 3 (target)		Result
			Avg CT*	Avg RFI*	
Candida albicans	ATCC	38289	NaN	1	C. auris not detected
Candida albicans	ATCC	18804	NaN	1	C. auris not detected
Candida dubliniensis	USDA	NRRLY-17841	NaN	1	C. auris not detected
Candida duobushaemulonii	CDC	AR-0391	NaN	1	C. auris not detected
Candida duobushaemulonii	CDC	AR-0394	NaN	1	C. auris not detected
Candida duobushaemulonii	CDC	AR-0392	NaN	1	C. auris not detected
Candida famata	ATCC	201067	NaN	1	C. auris not detected
Candida famata	ATCC	60229	NaN	1	C. auris not detected
Candida glabrata	ATCC	2001	NaN	1	C. auris not detected
Candida glabrata	ATCC	MYA-2950	NaN	1	C. auris not detected
Candida guilliermondii	ATCC	14242	NaN	1	C. auris not detected
Candida haemulonii	ATCC	22991	NaN	1	C. auris not detected
Candida haemulonii	CDC	AR-0392	NaN	1	C. auris not detected
Candida haemulonii	CDC	AR-0393	NaN	1	C. auris not detected
Candida kefyr	ATCC	34137	NaN	1	C. auris not detected
Candida krusei	ATCC	14243	NaN	1	C. auris not detected
Candida krusei	CDC	AR-0397	NaN	1	C. auris not detected
Candida lusitaniae	ATCC	34449	NaN	1	C. auris not detected
Candida metapsilosis	ATCC	10232	NaN	1	C. auris not detected
Candida orthopsilosis	ATCC	96139	NaN	1	C. auris not detected
Candida parapsilosis	ATCC	22019	NaN	1	C. auris not detected
Candida parapsilosis	ATCC	90018	NaN	1	C. auris not detected
Candida sake	ATCC	28136	NaN	1	C. auris not detected
Candida sake	ATCC	22021	NaN	1	C. auris not detected
Candida tropicalis	ATCC	13803	NaN	1	C. auris not detected
Candida auris	CDC	AR-0383	24	10	C. auris detected

*Average of 2 replicates

Table 10. List of exclusivity *Candida* spp. at ≥10⁷ CFU/mL spiked into **cobas®** PCR Media diluent and tested using the full **cobas®** omni Utility Channel workflow on a **cobas®** 6800 System.

Samples	Fungal cells
Input	≥10⁷ CFU/mL (400 uL processing volume)
Background	**cobas®** PCR Media
Platform	**cobas®** 6800

Discussion

Candida auris is an emerging pathogen and has been associated with nosocomial outbreaks across the world. It has the ability to spread rapidly and is known to be multidrug resistant (5). Here, we have shown that our UC *C. auris* assay is a fully automated RUO assay capable of detecting all clades of *C. auris* while not cross reacting with other closely related *Candida* species. The low LoD and lack of an off-machine pre-analytic lysis step before sample loading can enable labs to test more samples with less hands on time (total of 3 hours) during surveillance studies. This is especially important because *C. auris* is associated with clinical outbreaks and often is resistant to multiple classes of antifungal drugs. Performance on clinical samples as compared to standard of care processes supports the usability of this assay for *C. auris* RUO. Both **cobas®** x800 UC *C. auris* assay and the BD Max PCR were more sensitive than the Culture/MALDI-TOF workup. Further NGS analysis confirmed *C. auris* results for the positive discrepant samples detected in our **cobas®** UC assay.

Conclusions

The **cobas®** UC *C. auris* assay shows equivalent or better performance to established LDT's and much higher throughput with the capability of processing between 144 and 1,056 samples per 8h shift on a **cobas®** 5800 or 8800 system, respectively. This assay will help provide a high throughput *C. auris* RUO option for labs equipped with a **cobas®** x800 family system to support surveillance research as the number of cases within the United States and around the world continue to rise. This **cobas®** x800 UC *C. auris* assay oligo pools will be posted to IDT for purchase and to be coupled with the **cobas®** Utility Channel IVD kit, **cobas** UC negative control, *C. auris* Z485 titered control material from Zeptometrix (#0804386), and commercially available collection devices (e.g. BD ESwab™, Copan MSwab®, **cobas®** PCR Media Uni Swab, and BD BBL™ Swab).

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