Procedure for Isolation of *Candida auris* Using Sabouraud Salt Dulcitol Broth With Chloramphenicol and Gentamicin followed by CHROMagar

1.0 Purpose

This document outlines the procedure for using Sabouraud Salt Dulcitol Broth to enrich for *Candida auris* from complex microbial communities on patient skin. *C. auris* is an emerging fungal pathogen that is present in some healthcare environments and can colonize patients' skin, often leading to bloodstream infections. Samples from non-sterile body sites contain a complex community of microorganisms. It is necessary to isolate *C. auris* from this community to obtain accurate species identification. This procedure uses broth enrichment to grow *C. auris* from patient skin, followed by culture on CHROMagar to obtain an isolate for species confirmation. This procedure will assist with determining whether a patient is colonized with *C. auris*.

2.0 Scope

This document describes the appropriate workflow and procedures when processing samples from patient skin using the Sabouraud Salt Dulcitol Broth to enrich for and CHROMagar to isolate *C. auris* from patient swabs.

3.0 Definitions

Term	Definition
CHROMagar Media	CHROMagar *Candida* Chromogenic agar
BSC	Biological Safety Cabinet
SSD	Sabouraud Salt Dulcitol
BSL	Biosafety level

4.0 Equipment

Biological Safety Cabinet
Pipets
Shaking incubator set to 40°C
Stationary incubator set to 40°C

5.0 Reagents/ Media

5.1. Sabouraud Salt Dulcitol Broth (See **Appendix A** for preparation and quality control instructions)

5.2. Sterile Cell Culture Grade Water, for example Fisher Scientific (Catalog #A1287306)

1. CHROMagar Candida Chromogenic agar, for example Hardy Diagnostics (Catalog #C9000)

5.3. 14 mL,17X100 mm, culture tubes with compression (rather than screw) caps, for example Fisher Scientific (Product# 14-959-11B)

5.4. 10 µl inoculating loops, for example Fisher Scientific (Product #22-363-600)

5.5. L-Spreaders, for example USA Scientific (Catalog #060208)
5.6. Microcentrifuge tubes, for example Fisher Scientific (catalog #05-48-129)

6.0 Safety Precautions

Perform all procedures in a BSL-2 lab. Lab coats, safety glasses, and gloves must be worn, all work must be performed in a BSC, and the BSC must be cleaned with **10% bleach** or other approved disinfectant for *C.auris* after performing the procedure.

7.0 Sample Information (Acceptability and rejection criteria, labeling, volume, handling, storage)

Specimens received for testing must be labeled according to minimum requirements of the laboratory. Patient skin swabs should be collected using BD ESwab collection and transport system (cat. 220245; Becton Dickinson and Company, Sparks, MD) or a similar collection and transport system containing 1.0 mL of Amies buffer (**See Appendix B**). After the specimen is collected, the swab should be placed into the tube containing the Amies buffer and stored at 4°C–25°C, and shipped with an ice pack to the laboratory for processing within 96 hours of specimen collection. Laboratory should develop specimen rejection criteria, such as specimens received >4 days after collection, damaged or visable leakage of transport tubes, specimens without submission forms, or tubes without specimen identifiers. Individual institutions submitting swabs are responsible for the compliance with local human subjects/IRB regulations or applying for the IRB exemption for public health outbreak surveillance and emergency response.

8.0 Quality Control

8.1. A positive and a negative control ESwab must be processed each day a new set of specimen swabs is processed.
8.2. Fill two 14 mL culture tubes with 2 mL SSD Broth for the representative sample controls. For the positive control (*C. auris*), inoculate directly from a stock plate using fungal cultures (up to one month in age) by touching a single 1 mm colony with the swab and then placing the soft end of the collection swab into the tube. Snap off the end of the swab at the marked line by bending the plastic handle against the edge of the transport media container and secure the tube cap. Be careful to keep the cap and swab of the negative control (sterile uninoculated swab) from touching any materials that may contaminate your control tube. Open the negative control ESwab, transfer the negative control swab into the collection tube, and secure the lid using a sterile BD ESwab.

8.3. There should be visible growth within 48 hours for the positive control and no growth for the negative control.

8.4. If there is growth of the negative control, a second negative control must be immediately processed.

8.5. If there is growth in the negative control a second time, discard all media from that lot, invalidate all patient specimens from that process date, switch to a new QC validated lot, and retest all invalidated specimens starting from the primary swab.

9.0 Procedure for Patient Swab Processing for *Candida auris* Using Liquid ESwabs and SSD Broth

Step	Action
9.1	The following steps should be performed in a BSC to ensure safety and sterility. Process positive and negative controls on each day a new set of specimens is processed.
9.2	Label the following items for each specimen and be sure that all information on the tube matches the submission document. a. Protocol worksheet b. 14 mL culture tube (with cap that allows gas exchange but keeps the contents sterile) c. CHROMagar *Candida* Chromogenic agar plate (store a 4°C until use) d. 1.5 mL DNA microcentrifuge tube
9.3	Prepare the positive and negative controls as outlined in **8.2** above.
9.4	Transfer 2.0 mL of SSD Broth to each 14 mL culture tube.
9.5	Vigorously shake the BD ESwab tube containing the swab sample for 5 seconds or mix the tube using a vortex mixer for 5 seconds to release the sample from the swab tip.
9.6	Unscrew the BD ESwab cap to remove the swab applicator to gain access to the liquid transport medium.
9.7	Transfer 100 µL specimen in liquid transport medium to the 14 mL culture tube containing 2.0 mL SSD Broth. Make sure the cap is not fully compressed, thus allowing for gas exchange but keeping the contents sterile. Recap and secure the BD ESwab and store all original specimens at 4°C.
9.8	Incubate culture tubes by shaking at 250 rpm at 40°C.
9.9	Check the tubes daily for up to 5 days. Once visible growth is detected (typically 48 hrs or more), use a 10 µL blue transfer loop to inoculate and streak for isolation on the pre-labeled CHROMagar *Candida* Chromogenic agar plate. Record the growth in the broth on the worksheet by the appropriate tube number. Return the tube to the shaker.

9.10	In case the initial growth in the tube does not reveal *C. auris*, continue to incubate tubes shaking at 250 rpm at 40°C for a total of 5 days.
9.11	**Incubate CHROMagar media plates at 37°C for up to 2 days.** Check the plate each day for yeast colonies. **Note:** *Candida auris* from patient and environmental samples has the tendency to display a delayed revival or slow growing phenotype.
9.12	If the CHROMagar plate has no growth, or no colonies suspicious for *C. auris* after 2 days, discard the plate and record as negative on the worksheet.
9.13	For colonies that grow, the species is determined by MALDI-TOF or other available identification platform. MALDI should be performed only on colonies that are not green (*C. albicans*) or blue (*C. tropicalis*). Choose one colony for each unique colony color (phenotype) on the plate. *C. auris* can be cream, pink, or red.
9.15	On day 5, to avoid false negatives, transfer 100µl of broth from each remaining culture tube to a CHROMagar *Candida* plate. This includes the tubes that have remained negative as well as the tubes that turned positive but from which *C. auris* was not identified in the first CHROMagar screen.
9.16	Incubate these final CHROMagar plates at 37°C and check daily for growth for up to 2 days. After 2 days, record as negative on the worksheet.
9.17	**Note:** With 5 days of growth for the tube and 2 more days of growth for the CHROMagar, a completely negative culture will take a total of 7 days to finalize.

10 Turn-around time: Reporting depends on the growth of the organism (some require lengthy incubation), the degree to which the specimen may have competing organisms, and other factors. In general, the turnaround time is approximately 4–8 days.

11 Disclaimer

The identification methods used and the results are for investigational or research purposes only and have not been validated for diagnostic purposes. The performance characteristics have been established by CDC's Mycotic Disease Branch Reference Laboratory. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This is an enrichment media, not a selective media. It is a modification of the standard Sabouraud broth exchanging dextrose with dulcitol and adding Gentamicin and Chloramphenicol. Organisms other than *C. auris* can occasionally grow.

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Appendix A

Procedure for Preparing Salt Sabouraud Dulcitol Broth With Chloramphenicol and Gentamicin

Term	Definition
SAB Media	Sabouraud's dextrose (Emmons)
Chromagar Media	CHROMagar *Candida* Chromogenic agar

Reagents/ Media (for making the broth)

1. Casein Peptone, for example Fisher Scientific (Catalog# R451102)*
2. Peptic Digest of Animal Tissue, for example Fisher Scientific (Catalog# 7181A)*
3. Dulcitol powder, for example Fisher (Catalog# AC117701000)
4. Sterile Cell Culture Grade Water, for example Fisher Scientific (Catalog #A1287306)
5. Sodium Chloride (NaCl), for example Fisher Scientific (Product# S671-500)
6. Chloramphenicol; for example SIGMA-ALDRICH (Product# R4408-10ML)
7. Gentamicin, for example SIGMA-ALDRICH (Product# G1397-10ML)

*Alternative product that combines items 1 & 2: Polypeptone peptone Animal tissue and Casein for example BD (Catalog #211910)

Other materials

2. Sabouraud dextrose agar (Emmons), for example Fisher Scientific (Catalog #OXCM0041B)
3. Blood agar plates, for example Fisher Scientific (Catalog #R01198)
4. CHROMagar Candida Chromogenic agar, for example Hardy Diagnostics (Catalog #C9000)
5. 14 mL, 17X100 mm culture tubes, for example Fisher Scientific (Product# 14-959-11B)
6. 10 µL transfer loops, for example Fisher Scientific (Catalog #22-363-608)
7. L-Spreaders, for example USA Scientific (Catalog #060208)

QC Organisms	Strain
Candida auris	AR Bank # 0387 or similar
Escherichia coli	ATCC 25922 or similar
Candida glabrata	CBS 138 or similar

Procedure Note: Perform all procedures in BSL-2 lab. Refer to the Laboratory Safety Manual when working with BSL-2 organisms. Fresh Microorganism: fresh fungal cultures (up to one month in age at 4°C on Sab dextrose plates or slants); fresh bacteria cultures (up to one month in age at 4°C on Blood agar plates or slants); storing fungal plates at room temperature for 4 weeks is acceptable.

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Media Preparation

1	Dissolve 5g Casein, 5g of Peptic Digest of Animal Tissue, 20g of Dulcitol powder and 100g sodium chloride (NaCl) in 900 mL of cell culture water and bring up to 1 liter with cell culture water.
2	Mix well, adjust pH to 5.6 ±0.2, then filter sterilize through 0.2µm filter or autoclave and cool to RT. Add Gentamicin and Chloramphenicol (50mg/ L final concentration each).
3	Store the media at 4°C for up to 3 months.

Quality Control

1	Run quality control on each lot of freshly made media. The following steps should be performed in a biological safety cabinet to ensure safety and sterility.
2	The four quality control cultures to be used are: a.) *C. auris*, inoculate using fresh fungal cultures (up to one month in age), b.) *C. glabrata*, inoculate using fresh fungal cultures (up to one month in age), c.) *Escherichia coli*, inoculate using fresh bacteria cultures (up to one month in age), and d.) negative control only containing media.
3	Using a sterile loop, scrape and resuspend *C. auris* or *C. glabrata* cells from stock culture in 5 mL of sterile water and adjust to a concentration of ~1–5 x 10^6 cells/mL. This is generally five 1 mm colonies or one large 3 mm colony. (This is approximately a 0.5 McFarland). For *E. coli*, use three 2–4 mm colonies. It is not essential to quantitate the cells since there should be no growth at the end of the validation. The concentration of each organism does not have to be exact; this is a qualitative validation.
4	Place 2.0 mL of enrichment broth into four 14 mL culture tubes. Inoculate one tube each with 20 ul of the *C. auris, C. glabrata,* and *E. coli*. The last tube receives no inoculum.
5	Vortex each tube and plate 100µl from the *C. auris, C. glabrata,* and blank tubes on individual Sabouraud Dextrose Agar plates and 100µl of the *E. coli* culture tube on an LB agar plate. Spread the inoculum using an L-spreader. These are the T0 plates.
6	Incubate T0 plates at 40°C for 48 hours, then store at 4°C.
7	Incubate tubes at 40°C shaking at 250 rpm for 72 hours.
8	At 72 hours, plate 100µl from the *C. auris, C. glabrata,* and blank tubes on individual Sabouraud Dextrose Agar plates and 100µl of the *E. coli* culture tube on an LB agar plate. These are the T72 plates.
9	Incubate T72 plates at 40°C for 48 hours.
10	Assess selectivity of media by comparing the growth on the plate at T0 to growth at T72. Expected results are shown in Table 1.
11	Record results. If unexpected results are obtained, do not use the media.

Table 1. Expected results after 72 hours of incubation of quality control organisms.

Organism	**Expected result**
Candida auris	Greater than 2x as much growth for T72 as T0
Escherichia coli	Growth at T0, no growth at T72

Candida glabrata	Equal or slightly less growth at T72 than T0

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Appendix B

Procedure for collection of the specimen

Before collecting a specimen, make sure that proper PPE is used, including gown and gloves. The most sensitive results have been generated using a single ESwab to swab both axilla and groin. There is also a report that nasal swabs can detect skin colonization with high sensitivity, but this method has not been studied as well as composite axilla/groin ESwabs.

1. Remove the ESwab from the packaging; take care not to touch the tip.
2. Rub the ESwab on the axilla using all sides of the swab. Target the crease where the arm meets the body. Run the ESwab back and forth approximately five times. Repeat the procedure with the same ESwab on the other axilla.
3. Using the same ESwab as for the axilla, rub the swab over the groin in the inguinal crease where the leg meets the pelvic region. Run the swab back and forth approximately five times. Repeat the procedure with the same ESwab on the other side of the groin.
4. Place the ESwab in the transport media and break off the end as indicated in the directions that accompany the swabs.
5. Replace the cap, making sure that the end of the swab slides into the cap as it is tightened.
6. Place the tube on ice or at 4°C until it can be processed.
7. Manufacturers generally suggest that ESwabs be processed within 72 hours. For *C. auris*, internal validation has shown no decrease in positivity rates up to four days after collection (CDC, unpublished results). Any deviation from the manufacturer's directions must be validated.