IFU

Instructions for Use

AURIS ENRICHMENT BROTH

Cat. no. R12	Auris Enrichment Broth, 13x100mm tube, 2ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Auris Enrichment Broth is recommended for the selective enrichment of *Candida auris* from patient skin and environmental samples. The medium is based on the CDC formulation for Sabouraud Salt Dulcitol (SSD) Broth with Chloramphenicol and Gentamicin.[5]

SUMMARY

Candida auris is an emerging fungal healthcare-associated pathogen that represents a serious multi-drug resistant (MDR) global threat. Though the organism often presents fewer virulence factors than *Candida albicans*, *C. auris* may persistently colonize the skin and hospital environment, making its transmission within and between healthcare settings more difficult to control.[6,7] *C. auris* fungemia results in a wide range of mortality rates (from 32%-66%), depending upon the patient's overall condition, underlying disease, geographic region, access to medical care, and age.[7] A review of the organism's genome demonstrates it harbors genes well-characterized as virulence factors in other *Candida* species, as well as genes for biofilm production and MDR transcription factors.[7]

Hardy Diagnostics Auris Enrichment Broth is based on the CDC formulation for Sabouraud Salt Dulcitol (SSD) Broth with Chloramphenicol and Gentamicin as outlined by Welsh et al.[5,6] The medium is sometimes referred to as Salt SAB Broth, and maintains a lower pH to encourage the growth of fungal isolates. The broth contains dulcitol as the sole carbon source, and chloramphenicol and gentamicin as selective agents against unwanted bacterial growth. Peptones provide nitrogen and amino acids. The high salinity (10% wt/vol), along with incubation at high temperatures (40°C), increase selection for *C. auris* isolates. Enrichment under these conditions are at the upper limits the organism can tolerate, so recovery may require longer incubation periods to rule out false negatives. In a study by Welsh et al., only specimens processed through high salt and high temperature enrichment broth procedures demonstrated 100% recovery of *C. auris* isolates compared to direct plating.[6]

The CDC protocol is recommended for surveillance of *C. auris* from skin swabs and environmental samples.[10] Positive broth samples may be subcultured to chromogenic media, such as HardyCHROM™ Candida + auris (Cat. no. G343), for further identification. Suspect colonies recovered on chromogenic media can be identified to the species level using MALDI-TOF or other test methods.

C. auris may persist on environmental surfaces in healthcare settings and has been cultured from multiple locations in patient rooms. The organism exhibits some degree of tolerance to disinfectants. It can develop low-level resistance, allowing it to survive on surfaces for greater than 3 weeks.[13] *C. auris* can be found on high-touch surfaces located close to the patient's bedside, such as tables, rails, and carts; mobile or reusable equipment shared between patients such as blood pressure cuffs, glucometers, temperature probes, and ultrasound machines; and even on surfaces further away from the bedside such as windowsills.[5] Environmental disinfection procedures should be performed through routine cleaning and disinfection of patient rooms, as well as on mobile and reusable equipment. Fungicidal disinfectants should be used following the manufacturer's directions for the correct contact time. Environmental sampling may be useful in instances of outbreak investigations, surveillance, or to determine the root cause of reservoirs of ongoing transmission.

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	100g
Dulcitol	20g
Casein Peptone	5g
Peptic Digest of Animal Tissue	5g
Chloramphenicol	50mg
Gentamicin	50mg

Final pH 5.6 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Consult listed references for information on collection.[2,5,8,10,11,12] Collect specimens and environmental samples using proper PPE, including gown and gloves. The CDC recommends processing swabs in a BSL-2 lab.[5]

Patient Specimen Collection: Patient specimens should be collected using a sterile, regular tipped polyester swab from the axilla and groin areas on both sides of the patient for best results. Alternatively, a nasal swab may be used to detect skin colonization. Collect the specimen in an appropriate transport medium such as Liquid Amies.

Environmental Sample Collection: Collect the environmental sample using a sterile, regular tipped polyester swab and transport medium such as Liquid Amies. For moist surfaces, like sinks or shower drains, a sterile, dry swab with an 80mm (Cat. no. 552C) or 100mm (Cat. no. 553C) breakpoint may be used for collection. Sample a 5x10cm area for large surfaces, or the entire surface of the object for small surfaces.[13,14] Sampling multiple areas of the same surface aids in recovery. Dry swabs used to sample moist environments can be placed directly into the Auris Enrichment Broth tube. After sampling, break the swab off in the broth and cap the tube. Begin at step 5 under Method of Use.

For either swab source, deliver swabs to the laboratory as soon as possible after collection. For processing delays, the swab may be stored at 2-6°C for brief periods. Follow the transport media manufacturer's recommendations for processing specimen times for best recovery.

Method of Use:

1. Ensure the cap is secure and tight on the specimen transport tube.

2. Vigorously mix the transport tube for 5 seconds, or vortex the tube for 5 seconds, to release the sample from the swab.

3. Open the transport tube and aseptically remove the patient swab using sterile forceps.

4. Using a sterile pipet tip, transfer 100µL of the transport medium containing the specimen to the Auris Enrichment Broth tube. Cap the tube loosely to allow for aerobic gas exchange during incubation. Re-cap the patient transport tube and store or discard as dictated per laboratory procedure.

5. Incubate Auris Enrichment Broth aerobically (with loosened caps) at 40°C. *C. auris* can grow at temperatures up to 42°C, but 37-40°C is optimal to account for temperature fluctuations in the incubator. If a 40°C incubator is not available, incubate at 37°C.

6. Check tubes for turbidity indicative of growth daily for up to 5 days. Visible growth in the form of turbidity in the medium typically occurs at 48 hours or more. Continue incubating negative tubes for a full 5 days. If no growth is detected after 5 days, discard tubes.

7. For positive (turbid) broth tubes, subculture the broth using a 10µL loop to a HardyCHROM™ Candida + auris (Cat. no. G343) plate, or other mycology media intended for the differential and selective isolation of *C. auris*, and streak for isolation.

8. See the Instructions for Use (IFU) for HardyCHROM™ Candida + auris (Cat. no. G343) for incubation and interpretation procedures.

INTERPRETATION OF RESULTS

Examine tubes for turbidity indicative of growth after 48-72 hours, or check tubes daily for signs of growth for up to five days. Tubes with no growth may be discarded after 5 days.

For positive tubes, see the Instructions for Use (IFU) for HardyCHROM™ Candida + auris (Cat. no. G343) for incubation and interpretation procedures.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The broth contains 10% sodium chloride, which inhibits the growth of most, but not all, yeast species. *Candida*

glabrata is one of the species that may grow in media with a high salinity content.[5,8] Therefore, dulcitol is used as a carbon source, rather than glucose, as *C. glabrata* cannot assimilate or ferment dulcitol.

Auris Enrichment Broth is a selective enrichment medium. It is not intended for differential analysis. Subculture to a differential chromogenic agar, such as HardyCHROM™ Candida + auris (Cat. no. G343) is recommended to differentiate *C. auris* from other *Candida* species. Suspect colonies can be identified using MALDI-TOF, DNA sequencing for detection of marker genes D1/D2, or the ITS (internal transcribed spacer) region, or whole genome sequencing to the species level.

Alternatively, broth enrichment samples can be used to conduct molecular identification using MALDI-TOF or rDNA analysis.[9]

Failure to incubate Auris Enrichment Broth at elevated temperatures may result in the overgrowth of non-target commensal microorganisms from specimens. Subculture to a differential and selective agar is needed and confirmatory testing is required to identify suspect isolates to the species level. Failure to incubate tubes with loose caps may result in false negative results.

If performing molecular tests directly from the broth, polyester (Dacron®), rayon, or flocked nyon tipped swabs with plastic shafts are recommended. Calcium alginate, cotton swabs, or swabs with wooden shafts should not be used.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as inoculating loops, swabs, applicator sticks, other culture media, transport media (such as Liquid Amies), and HardyCHROM Candida + auris (Cat. no. G343), incinerators, refrigerators, incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Candida auris CDC B11903	B	48-72hr	40°C	Aerobic	Growth; turbidity
Candida albicans ATCC® 10231	B	72hr	40°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 25923	B	72hr	40°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis

(CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Auris Enrichment Broth should appear translucent and light amber in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory,* Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

3. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA.

4. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

5. The Centers for Disease Control and Prevention (CDC) . *Candida auris* Colonization Screening: https://www.cdc.gov/fungal/lab-professionals/c-auris-colonization-screening.html

6. Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, Litvintseva AP. 2017. Survival, persistence, and isolation of the emerging multi-drug resistant pathogenic yeast *Candida auris* on a plastic health care surface. *J Clin Microbiol* 55:2996–3005.

7. Rossato, L. and A.L. Colombo. 2018. *Candida auris*: What Have we Learned About its Mechanisms of Pathogenicity? *Frontiers in Micro*.

8. The Centers for Disease Control and Prevention (CDC). Guidance for Detection of Colonization of *Candida auris*: https://www.cdc.gov/fungal/candida-auris/c-auris-guidance.html

9. Kordalewska, M. and D.S. Perlin. Molecular Diagnostics in the Times of Surveillance for *Candida auris*. 2019. *J. Fungi*. 5:77.

10. The Centers for Disease Control and Prevention (CDC). Screening for *Candida auris* colonization in healthcare settings: https://www.cdc.gov/fungal/candida-auris/c-auris-screening.html

11. Jorgensen et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

12. Centers for Medicare & Medicaid Services (CMS). Individualized Quality Control Plan (IQCP).

13. Dire O., A. Ahmad, S. Duze, M. Patel. Survival of *Candida auris* on environmental surface materials and low-level resistance to disinfectant. 2023. *J. Hosp. Infect.* 137:17-23.

14. Piedrahits, C.T., J.L. Cadnum, A.L. Jencson, A.A. Shaikh, M.A. Ghannoum, and C.J. Donskey. Environmental Surfaces in Healthcare Facilities are a Potential Source for Transmission of *Candida auris* and Other *Candida* Species. Infection Control & Hospital Epidemiology. 2017. Cambridge University Press.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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