PURPOSE: To determine the presence or absence of bacterial pathogens in ear specimens.

SAMPLE INFORMATION:

Type	Swab • Amie's with or with charcoal
Source	• External auditory canal (outer ear) • Otitis media discharge swabbed from external auditory canal
Stability	If the sample is received in the laboratory and processed greater than 48 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Room temperature. If transport is > 2 hours, swabs should be refrigerated.
Criteria for rejection	1. Unlabeled/mislabeled swabs. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.

NOTE:

- **Refer to MIC34100 – Body Fluid Culture for typanocentesis fluid.**

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO) and MacConkey agar (MAC)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- 35° ambient air and 37° CO_2 incubators
- Wooden sticks
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for ear culture	
1	In the biosafety cabinet, inoculate Blood agar, Chocolate agar and MacConkey agar from the swab. Make gram stain.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place MAC plate in the O_2 incubator. Place BA and CHO plates in the CO_2 incubator.
4	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
5	Examine plates after 24 hour incubation. Record observations in the LIS.
6	Re-incubate CO_2 plates for an additional 24 hours. Discard O_2 plate.
7	At 48 hours, examine plates and record observations in the LIS.

Probable Pathogens	Commensal Flora
• *Staphylococcus aureus* • *Streptococcus pyogenes* • Other ß-hemolytic *Streptococci* • *Streptococcus pneumoniae* • *Haemophilus influenzae* • *Moraxella catarrhalis* • *Pseudomonas aeruginosa* • Non-fermentative Gram-negative bacilli • Enterobacteriaceae spp. • *Candida* spp. • Fungi	• A*erococcus* spp. • B*acillus* spp. • Coagulase negative *Staphylococcus* • *Corynebacterium* spp. • *Micrococcus* spp. • *Neisseria* spp. • Anaerobes • viridans *Streptococcus* grp.

INTERPRETATION OF RESULTS:

Step	Action
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Observe plates at 24 hours and 48 hours for growth of enteric Gram-negative bacilli, *Pseudomonas aeruginosa*, Yeasts, *Vibrio alginolyticus*, ß-hemolytic *Streptococci, Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and Fungi. Usually only one pathogen is responsible for infection. Mixed cultures should be minimally identified.
3	If specimen is discharge from otitis media infection, the most common pathogens are *Streptococcus pyogenes, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae.*
4	Skin flora such as coagulase negative *staphylococci* and coryneforms are normal in the external ear canal and should not be further evaluated.
5	Mixed cultures of Gram-negative rods should be minimally identified.

REPORTING RESULTS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth after 1 Day. Further report to follow"**
No growth after 2 days	**FINAL:** • Report: **"No Growth after 2 Days"**
Mix of skin flora	• Report: **"Mixture of skin flora"** • List quantitation.
Mix of enteric Gram-negative bacilli	• Report **"Mixture of coliform organisms"** • List quantitation.
Growth or mix of other non-pathogenic organisms	• Report **"Commensal flora"** or **"Commensal skin flora"** • List quantitation.
Growth of mixed anaerobes (if anaerobic culture was set up to identify organisms seen in gram stain)	• Report **"Mixture of anaerobic organisms"** • List quantitation.
Growth of anaerobe (if anaerobic culture was set up to identify organisms seen in gram stain)	• Report identification as gram stain result under the isolates tab. • List quantitation.
Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM. • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.

CULTURE NOTES:

- An external ear culture with a predominant Gram-negative bacillus, beta-hemolytic Streptococcus, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* generally indicates infection with that organism.
- Historically, the nasopharynx was cultured in order to predict the pathogens in the middle ear. This practice is no longer recommended.
- Treatment of otitis externa usually consists of ear drops.

LIMITATIONS:

1. False-negative cultures can result from overgrowth of the culture with normal skin flora.
2. False-positive results can be caused by over-interpretation of culture results.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	06-NOV-17	Initial Release	L. Steven
2.0	30 Nov 2018	Updated to include new Vitek 2 instrument	L. Steven