PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC32400 – Ear Culture	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
Otitis media is an infection of the middle ear. Complications include hearing loss and learning difficulties. Common causes of otitis media are *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Alloiococcus otitis. Streptococcus pyogenes* is found on a seasonal basis. Treatment failure can occur, and surgical intervention may be necessary. Otitis externa is an infection of the external auditory canal. This narrow canal tends to trap foreign objects, wax, and water. Infections are classified as acute and chronic. Acute infections (often called swimmer's ear) are frequently caused by *Pseudomonas aeruginosa*, although other aerobic organisms can be involved. *Vibrio alginolyticus* is a cause in oceanic swimmers. Localized infections with *Staphylococcus aureus* or *Streptococcus pyogenes* can also occur. Contaminating skin microbiota such as coryneform organisms (diphtheroids) and coagulase-negative *Staphylococci* may be present but are not significant. Chronic infections are usually caused by fungi, Mycobacteria, *Nocardia* and underlying diseases such as syphilis.

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

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for ear culture.

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

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
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- 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.

- Ensure that appropriate hang hygiene practices be used.
- 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. Routine Practices 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 BA, CHO and MAC with the swab • Ensure all surfaces of swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • Prepare smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
2	Incubate the media: • Place BA and CHO in the CO_2 incubator • Place MAC in the O_2 incubator
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram stain procedure.

Probable Pathogens	
• *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
Commensal Flora	
• 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	Ensure growth on culture media correlates with gram stain results. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen
2	• Observe BA and CHO plates at 24 hours and 48 hours • Observe MAC plate at 24 hours • Examine for growth of enteric Gram-negative bacilli, *Pseudomonas aeruginosa*, Yeasts, *Vibrio alginolyticus*, ß-hemolytic *Streptococci, Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and Fungi

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 INSTRUCTIONS:

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 pathogen(s)	• Report organisms(s) identification • List quantitation • Report susceptibility results as per ASTM

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL
- Refer to MIC36400-Referral of Category B Specimens to DL for sending isolates to *Dyna*LIFE.

LIMITATIONS:

1. 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.
2. Historically, the nasopharynx was cultured in order to predict the pathogens in the middle ear. This practice is no longer recommended.

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

CROSS-REFERENCES:

- LQM70620-Laboratory Critical Results List-Microbiology
- MIC20115-Gram stain procedure
- MIC34100-Body Fluid Culture for typanocentesis fluid
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC36400-Referral of Category B Specimens to DL

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) Washington, D.C.: ASM Press
2. 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.* Washington, D.C: ASM Press

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	06 Nov 17	Initial Release	L. Steven
2.0	04 Dec 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	26 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven