PURPOSE:

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

SAMPLE INFORMATION:

Type	• Swab, Amie's with or without charcoal
Source	• External auditory canal (outer ear)
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 and follow up action	1. Unlabeled/mislabeled swabs 2. Dry swabs

***Note: Tympanocentesis fluid is obtained for the diagnosis of otitis media (middle ear infection). These specimens are to be handled as sterile body fluids. (Refer to MIC34100)**

REAGENTS and/or MEDIA:

- Blood Agar (BAP), Chocolate Agar (CHOC) and MacConkey Agar (MAC)
- Identification reagents: catalase, rapid Staph, rapid Strep, oxidase, etc.

SUPPLIES:

- Wooden sticks
- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- 35° ambient air and 37° CO_2 incubators
- Vitek 2 Compact 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 Quality Control manual for reagent quality control procedures.

PROCEDURE INSTRUCTIONS:

Step	Action
Processing Swabs 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 the MAC plate in the O_2 incubator. Place BAP and CHOC plates in the CO_2 incubator.
4	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates.
5	Examine plates after 24 hour incubation. Record your observations in the LIS.
6	Re-incubate CO_2 plates for an additional 24 hours. Discard O_2 plate.

Pathogens	Normal 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	• Acinetobacter spp. • Aerococcus spp. • Bacillus spp. • Coagulase negative Staphylococcus • Corynebacterium • Micrococcus spp. • Neisseria species • Anaerobes • Viridans Streptococcus

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of Otitis externa specimens	
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*, *Streptococci*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis* and Fungi. Usually only one pathogen is responsible for otitis externa. Mixed cultures should be minimally identified.
3	Skin flora such as coagulase negative staphylococci and coryneforms are normal in the external ear canal and should not be further evaluated.
4	Mixed cultures of Gram-negative rods should be minimally identified.

Otitis externa pathogens identification			
ORGANISM	**GRAM STAIN**	**IDENTIFICATION**	**SUSCEPTIBILITY**
Staphylococcus aureus	Gram-positive cocci, in clusters	• Catalase: Positive • Slide coagulase: Positive • Tube coagulase: Positive	Vitek AST-GP67
Streptococcus pyogenes	Gram-positive cocci, in chains	• Catalase: Negative • PYR: Positive • Step grouping: A positive	KB as per ASTM
Other beta-hemolytic Streptococci	Gram-positive cocci, in chains	• Catalase: Negative • PYR: Negative • Strep grouping: B, C or G positive	KB as per ASTM
Streptococcus pneumoniae	Gram-positive cocci, in pairs	• Alpha hemolytic • Catalase: Negative • Optochin: Sensitive • Vitek GPI	KB and E-test as per ASTM
Haemophilus influenza	Gram-negative coccobacilli	• Gram-negative coccobacilli • Growth on CHOC, no growth on BA except as satelitism • ALA: Negative	Not performed. Add isolate comment: **&HAEM**
Moraxella catarrhalis	Gram-negative diplococci	• Gram-negative diplococci • Oxidase: Positive • Catarrhalis disk: Positive	Beta-lactamase (only report if positive)
Pseudomonas aeruginosa	Gram-negative bacilli	• Oxidase: Positive • Characteristic odor and pigment	Vitek AST-N213
Other Gram-negative bacilli	Gram-negative bacilli	• Vitek GN or API	Vitek AST-N213
Vibrio alginolyticus	Gram-negative bacilli	• Oxidase: Positive • Indole: Positive • Grows well on MAC • Vitek GNI	Vitek AST-N213
Candida species	Yeast	• Wet prep: Yeast • Vitek YST ID	Not performed

REPORTING RESULTS:

No growth after 2 days	• Report **"No Growth after 2 days"**
Mix of skin flora (CNS, dipthroids, Viridans strep, Bacillus spp., Micrococcus spp.)	• 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"** • List quantitation.
Growth of anaerobe	• Report **"Gram Negative Bacilli Anaerobic"** or "**Gram Positive Cocci Anaerobic"** • List quantitation.
Growth of mixed anaerobe	• Report **"Mixture of anaerobic organisms"** • List quantitation.
Growth of fungus	• Report **"Fungal species"** with isolate comment **&REF2** to state: **"This organism has been referred for further identification."** • List quantitation. • Add test ?REFE. Send to Prov. Lab for identification as per MIC10500 – Referral of Category B specimens to Provincial Laboratory. • Freeze specimen and enter into specimen isolate log.
Predominant or pure growth of yeast (if not predominant or pure report as part of commensal flora)	• If Vitek identified as Candida albicans, report. • If not Candida albicans, report as **YSTNOT** (Yeast, not Candida albicans). • List quantitation.
Growth of pathogens	• Report organism identification. • List quantitation. • Report susceptibility results as per DynaLIFE ASTM.

PROCEDURE 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:

- False-negative cultures can result from overgrowth of the culture with normal skin flora.
- False-positive results can be cause 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