PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Ear Culture Procedure

Department of Microbiology

Effective date: 01/06/2015

Last Revision: 01/06/2015

Last reviewed: 01/06/2015

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1.0 Clinical Significance

Otitis media is an infection of the middle ear that commonly affects children between the ages of 2 and 6 years of age. Hearing loss and deficits in learning are a few of the complications that may occur. The most common agents of otitis media are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis. Streptococcus pyogenes* may be encountered on a seasonal basis. Amoxicillin and amoxicillin-clavulanate are the drugs of choice for initial treatment. Treatment generally resolves the infection, but treatment failures occur and surgical intervention may be necessary. Tympanocentesis and culture of the middle ear fluid constitute a valuable tool for definitive diagnosis, to guide therapy, and to evaluate treatment failures. However, the diagnosis is usually made on clinical grounds, because of the invasive nature of tympanocentesis.

Otitis externa is an infection of the external auditory canal. Unique problems occur with this infection because of the narrow and tortuous nature of the canal and its tendency to trap foreign objects, wax, and water. Infections are classified as acute and chronic. Acute infections are often referred to as "swimmer's ear." *Pseudomonas aeruginosa* is a frequent cause of freshwater otitis. Localized infection with *Staphylococcus aureus* and *S. pyogenes* can also occur. Colonizing skin bacteria, such as corynebacteria and staphylococci, may be present in cultures and do not represent significant isolates. More invasive infections are caused by extension into the adjacent soft tissues and bone. Chronic otitis is usually caused by bacterial infection, and although *P. aeruginosa* may be predominant, a variety of anaerobes may also be present.

2.0 Principle

Middle ear fluid is obtained by passing a needle through the eardrum and aspirating fluid (tympanocentesis). If the eardrum has ruptured, draining fluid may be collected with a swab. Swabs are used to collect material from the outer ear. Ear infections are typically caused by a single organism. However, skin flora is frequently recovered in culture along with potential pathogens. A combination of nutrient and selective media are used to aid in the recovery and isolation of potential pathogens.

3.0 Scope

This procedure is classified under CLIA as highly complex. It should be carried out by technical personnel familiarized and trained to differentiate and identify potential pathogens. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and antimicrobial susceptibility testing.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used for isolate identification may be hazardous to your health if handled incorrectly. Information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Airborne and bloodborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves must be worn when handling specimens.
- A laboratory coat must be worn when handling specimens, cultures, and reagents.
- A biological safety cabinet must be used when processing specimens.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling, and Storage

5.1 External Ear

1. Insert a sterile swab into the ear canal until resistance is met.
2. Rotate swab and allow fluid to collect on swab and place swab in transport medium.

5.2 Tympanocentesis Fluid

Note: Because of the invasive nature of the collection process, these specimens are usually submitted primarily to diagnose middle ear infections only if previous therapy has failed.

1. Clean external canal with mild detergent.
2. Using a syringe aspiration technique, the physician will obtain the fluid from the ear drum.
3. Send the specimen in a sterile container or in the syringe capped with a Luer-Lok and with the needle removed.
4. If the eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum. Place swab in transport medium.

5.3 Storage and Transport

Ideally, aspirates should be delivered to the laboratory within 30 min for best recovery of microorganisms. If there is a delay, keep the aspirates at room temperature, because at lower temperatures there is likely to be more dissolved oxygen, which could be detrimental to anaerobes. Swab samples submitted in transport medium can be sent at room temperature or refrigerated for up to 1 d.

5.4 Rejection Criteria

1. Do not accept any specimen submitted in formalin.
2. Syringes with the needle attached should not be accepted due to the sharps/biohazard risk.

6.0 Materials

6.1 Equipment

- CO_2 incubator set at 35 ± 2°C
- Anoxomat system and jar
- Automated streaking instrument
- Microscope with 10X and 100X objectives

6.2 Consumables

- Sterile disposable loops (if streaking by hand)
- Glass microscope slides

6.3 Media

- 5% Sheep Blood agar (BAP)
- Chocolate agar (CHOC)
- MacConkey agar (MAC)
- CNA agar (CNA)

7.0 Specimen Processing

7.1 Aspirates

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Prepare smear for Gram stain by placing a drop of specimen on a slide and spreading it to make a thin preparation. Place the slide on an electric slide warmer set at a temperature not exceeding 42°C.
3. Place a drop of specimen onto each piece of medium.
4. Quadrant-streak each plate for isolation.

7.2 Spun-fiber Swabs

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Prepare smear for Gram stain by pressing and rolling the swab in the center of a glass slide to express absorbed material. Place the slide on an electric slide warmer set at a temperature not exceeding 42°C.
3. Use the swab to directly inoculate the media. Press and roll the swab against the surface of the agar in the first quadrant. Inoculate the least inhibitory media first (BAP & CHOC).
4. Quadrant-streak each plate for isolation.

7.3 Flocked Swabs in Liquid Amies (ESwab)

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Vigorously shake the ESwab tube containing the swab sample between the thumb and forefinger for 5 s or mix the tube using a vortex for 5 s to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid transport medium.
3. Unscrew the ESwab cap and remove the swab applicator.
4. Roll the tip of the ESwab applicator onto the surface of the first quadrant of the culture plate to provide the primary inoculum. Return the ESwab applicator to the transport medium tube for 2 s to absorb and recharge the applicator tip before inoculating each additional plate. Alternatively, a sterile pipette can be used to transfer 1 to 3 drops of the suspension onto each culture plate.
5. Prepare a smear for Gram stain by transferring 1 or 2 drops of the suspension onto the center of a glass microscope slide, using either the swab or a transfer pipette. In case of bloody or thicker specimens, care should be taken to thinly spread the sample on the slide. Place the slide in an electric slide warmer set at a temperature not exceeding 42°C.

7.4 Incubation

1. Incubate the BAP, CHOC, CNA, and MAC in a humidified incubator at 35 ± 2°C with 5% CO_2.
2. Incubate the BAP-ANA plate in an Anoxomat jar using the anaerobic recipe on the processor.

8.0 Procedure

8.1 Potential Pathogens

The most common pathogenic organisms associated with ear infections are:

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Staphylococcus aureus*
- Beta-hemolytic streptococci
- Gram-negative rods
- Anaerobic bacteria
- Yeasts
- Molds (commonly *Aspergillus* spp.)
- Unusual and infrequently encountered potential pathogens include *Turicella otitidis* (a long coryneform rod implicated in otidis media), *Nocardia* spp., and rapidly-growing mycobacteria (associated with chronic infections).

8.2 Mixed Flora

Mixed flora includes any of the following organisms. Unless seen in the direct Gram stain, these organisms are reported as "mixed flora" with no additional workup or AST.

- *Bacillus* spp.
- Coagulase-negative *Staphylococcus* spp.
- *Corynebacterium* spp.
- *Streptococcus* spp. (other than beta-hemolytic strep)
- *Micrococcus* spp.

8.3 Day 1

After at least 18 to 24 h of incubation, examine the plates for growth. Anaerobic plates that do not have growth distinctive from the aerobic plates should be immediately returned to anaerobic conditions. Note the quality of the specimen (Q score) and the organisms that were reported in the Gram stain. Determine the number of potential pathogens growing in the culture (exclude bacteria included as mixed flora described above).

8.3.1 Sterile Cultures

1. Report: **No growth to date**.
2. Re-incubate the plates.

8.3.2 Potential Pathogens ≤ Q Score

1. Speciate and report the presumptive isolate(s) according to the identification charts, and document workup in the computer.
2. Perform susceptibility testing on organisms, if appropriate.
3. Re-incubate the plates.

8.3.3 Potential Pathogens > Q Score, but Correlating Pathogens are < Q Score

1. If the correlating potential pathogens do not exceed the score, speciate and report the presumptive isolate(s) according to the identification charts, and document workup in the computer.
2. Perform susceptibility testing on correlating organisms.
3. Report non-correlating isolates generically by reporting as: **Mixed flora including (list non-correlating potential pathogens)**.
4. Attach the comment: **This is a mixed culture of potential pathogens. Correlation of the culture results with the gram stained direct smear indicates one or more isolate is more significant than others. The organisms seen only in culture may not relate to infection and may represent colonization or contamination. [MXSIG]**
5. Re-incubate the plates.

8.3.4 Potential Pathogens that Correlate > Q Score

1. Report the isolates generically: **Mixed flora including (list potential pathogens)**.
2. Attach the comment: **This is a mixed culture of potential pathogens. Correlation of culture results with the gram stained direct smear does not identify any isolate as more significant than another. Bacteria may not relate to infection and may represent colonization or contamination. [MXNSIG]**

8.3.5 Q Score is 0

1. Identify and report *P. aeruginosa* if present in pure culture or with mixed flora. Perform AST. However, if *P. aeruginosa* is present with other potential pathogens, include in generic list and do not perform AST.
2. Report potential pathogens generically: **Mixed flora including (list potential pathogens)**. Identification testing should be limited to tests that can be completed on that same day (e.g., gram stain, motility, spot tests, etc.).

3. After listing potential pathogens, add the comment: **This is a mixed culture suggesting the probability of contamination. Collection of another specimen is suggested, avoiding superficial sources of contamination. [SWCONT]**

8.3.6 Cultures with Mold

If mold is observed growing on the culture plates, check to see if a fungus culture was ordered.

- If a fungus culture is in progress, report "**Mold present. Refer to fungus culture for identification**." Tape the plates and give them to the Mycology lab.
- If a fungus culture was not ordered, tape the plates and consult during Rounds. The occurrence of confluent growth of many colonies is suggestive of otomycosis.

8.4 Day 2

After 2 d of incubation, examine the plates for growth.

8.4.1 Sterile Cultures

1. If the plates are sterile, report: **No growth.**
2. Discard the culture plates unless specimen was tympanocentesis fluid or surgically obtained.
3. Continue to incubate the anaerobic BAP and the CHOC plate for tympanocentesis and surgical specimens for 5 d. Enter the following comment: **Plates will be incubated for 5 days. [PL5]**

8.4.2 Culture with Growth on Day 2

Refer to the workup protocol listed under Day1.

9.0 Retention of Cultures

All plates with growth should be held in the storage cupboard for 7 d. Cultures are retained in case the clinician requests additional workup or antimicrobial susceptibility testing.

10.0 Quality Control & Quality Assurance

10.1 Quality Control

1. Verify that media meet expiration date and QC parameters. Each new lot/shipment of CHOC should be tested with control strains (refer to the Quality Control Reference Guide).
2. Temperatures of all controlled instruments and environments must be checked daily and recorded in LIS. This includes refrigerators, freezers, incubators, water baths and heating blocks. The thermometers used for measuring temperatures are checked against a NIST certified thermometer prior to being placed into use and must not vary more than $\pm$ 1° C.
3. Each anaerobic jar should be checked with a methylene blue strip. Before opening each jar, verify that the strip turned white. Document the acceptability of QC for all opened jars in LIS once per shift. CO_2 incubators must be checked daily by the digital reading and weekly using a Fyrite device. Document CO_2 readings in LIS.

11.0 Limitations

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

12.0 References

1. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

13.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 01/06/2015

Microbiology Supervisor Reviews: Jerry Claridge 01/07/2015

Revisions & Updates: