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Culture, Upper Respiratory

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The document describes the work up and reporting of cultures for the detection and isolation of pathogenic microorganisms in patient specimens from upper respiratory sources. The procedure is to be performed by trained qualified Microbiology Laboratory staff.

II. CLINICAL SIGNIFICANCE:

The most frequent bacterial agent implicated in pharyngitis is *Streptococcus pyogenes* (Group A Streptococcus [GAS]). Other pathogens that may be involved are beta-hemolytic Streptococcus Groups C & G and *Arcanobacterium haemolyticum*. Pathogens from the nasopharynx include *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The most common pathogens worked up in ear and sinus contents are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*.

III. PRINCIPLE:

This test is used to recover and identify aerobic bacteria that may be involved in human infections within the upper respiratory tract. Upper respiratory sources include Throat/Pharynx, Nasopharynx (NP), Sinus Contents, and Ear. A gram stain, aerobic culture, identification, and susceptibility testing will be performed (if indicated).

IV. SPECIMEN COLLECTION AND HANDLING:

A. Refer to the Laboratory Test Directory (LTD) for detailed collection information.

B. Collect and submit acceptable upper respiratory specimens using aseptic technique.

C. **Specimen**

1. Culture swab submitted in a transport system with Amies media, e.g. Culturette, ESwab, etc.

D. **Shipping and Handling**

1. Specimens must be transported at room temperature (20-26°C or 68-78.8°F).

E. **Rejection Criteria**

1. Specimens submitted in containers with formalin or that previously contained formalin.
2. Specimens delayed in transit (greater than 48 hours from time of collection).

F. **Storage**

1. Refrigerated or room temperature (2-8°C or 20-26°C) for 7 days.
2. Specimens are discarded in the biohazard waste containers after 7 days.

V. REAGENTS:

A. 5% sheep blood agar plate (BAP)
B. Chocolate agar plate (CHOC)
C. MacConkey agar plate (MAC)
D. Columbia-CNA Agar (CNA)
E. Selective Strep agar plate (SSA)
F. Gram stain reagents
G. Biochemical identification reagents
H. Commercial identification system
I. Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) reagents

VI. EQUIPMENT:

A. Vortex mixer
B. Forceps
C. Inoculating loops, 0.01mL
D. Frosted glass slides
E. Sterile cotton-tipped swabs
F. Sterile plastic transfer pipettes
G. Laminar Flow Biological Safety Cabinet (BSC), Type IIB
H. Incubator 35-37°C with 5% CO2 atmosphere
I. Light microscope,10X, 40X, 100x oil immersion objectives

J. MALDI-TOF MS

K. Identification system

L. Antimicrobial susceptibility testing system

VII. QUALITY CONTROL (QC):

A. Refer to Quality Control procedures for commercially prepared reagents. The laboratory participates in the appropriate required proficiency testing (PT)/external quality assessment (EQA) program accepted by College of American Pathologists (CAP).

VIII. PROCEDURE:

A. **Culture Setup**

1. Throat/Pharynx:
    a. Inoculated onto BAP and Selective Strep Agar (SSA) plates.
2. Nasopharynx (NP):
    a. Inoculated onto BAP and CHOC agar plates.
3. Sinus:
    a. Inoculated onto BAP and CHOC agar plates.
    b. A slide is prepared for a Gram Stain (GS).
    c. NOTE: If specimen is received in another collection device besides a swab, order Culture, Wound Deep with source, sinus contents.
4. Ear:
    a. Inoculated onto BAP, CHOC, and MAC agar plates.
    b. A slide is prepared for a Gram Stain (GS).

B. **Incubation**

1. Culture plates are incubated at 35-37°C with 5% CO2.
2. Cultures are re-incubated daily after examination.

C. **Plate Reading**

1. Examine plates after 18-24 hours and again at 48 hours of incubation.
2. Use Appendix A for workup guidelines.
3. For suspicious colonies, describe colony types and quantitate isolate(s) as rare, few, moderate, or many in the work card.
4. Document testing performed and results in the work card.
5. Reconcile plate examination with gram stain report. Refer to Gram Stain Reading and Reporting.
6. Plates with representative growth as reported are held at room temperature for 7 days.

IX. INTERPRETATIONS:

Refer to Appendix A: Upper Respiratory Workup Guidelines for identification and workup guidelines of potential pathogens and commensal flora.

A. **Gram Stain**

1. Refer to the Gram Stain Procedure to report Gram stain results for upper respiratory sources.

B. **Culture Results**

1. Day 1 (18-24 hours incubation)
   If suspect pathogen(s) are present, describe the colony types and quantitate as rare, few, moderate or many in the electronic work card.
   a. Evaluate and workup suspect pathogen(s) recovered as indicated in Appendix A: Upper Respiratory Workup Guidelines.
   b. When a pathogen is being reported and commensal flora is present, the commensal flora is quantified and reported with the following mnemonics, "with (quantity) Commensal Flora" or "with No Commensal Flora."
   c. If no pathogens are being reported and only commensal flora is present, select the Culture Growth Comment, "Commensal Flora Present."
2. Day 2
   a. All media is reviewed again for any additional suspect pathogens.
   b. Evaluate and work up any additional organism(s) as indicated in Appendix A.
   c. Commensal flora in the human oropharynx may be present and include:
      i. Alpha Streptococcus, including *Streptococcus pneumoniae*
      ii. *Neisseria spp*. other than *N. gonorrhoeae or N. meningitidis*
      iii. *Staphylococcus spp*. including *S. aureus*
      iv. *Haemophilus spp*. including *H. influenzae*
      v. Diphtheroids
      vi. Gram negative bacilli
      vii. Beta-hemolytic Streptococcus other than *S. pyogenes* (group A)
      viii. *Candida spp.*

X. REPORTING:

A. **Culture Results**

1. Negative
   a. Throat/Pharynx:
      i. Preliminary report on day 1: "No Group A, C or G Streptococcus

or Arcanobacterium sp. Recovered" or "Culture in Progress."

ii. Finalize report on day 2: "No Group A, C or G Streptococcus or Arcanobacterium sp. Recovered."

b. Nasopharynx (NP), Sinus Contents, Ear:

i. Preliminary report on day 1: "No growth at 1 day"

ii. Finalize report on day 2: "No growth at 2 days"

2. Commensal Flora

a. Nasopharynx (NP), Sinus Contents, Ear:

i. Preliminary report on day 1: "Commensal Flora Present"

ii. Finalize report on day 2: "Commensal Flora Present.

3. Pathogen(s) Recovered

a. Throat/Pharynx:

i. Report Group A, C or G Streptococcus or *Arcanobacterium sp*. with quantity.

ii. Do not report commensal flora.

b. Nasopharynx (NP), Sinus Contents, and Ear:

i. Report the quantity, identification, and susceptibility of pathogens when applicable.

ii. Report the presence / absence of commensal flora, using the following mnemonics: "with (quantity) Commensal Flora" or "with No Commensal Flora."

XI. LIMITATIONS:

A. Many debilitated patients are often benignly colonized with potential pathogens.

B. Isolation of a pathogen from a respiratory specimen may or may not be indicative of pneumonia.

C. Administration of antibiotics prior to obtaining respiratory tract specimens may result in false negative cultures.

XII. REFERENCES:

A. Gilligan, P.H., K. Alby, and M.K. York, 2016, Lower Respiratory Tract Cultures, In Leber, A.L., Editor in Chief, Clinical Microbiology Procedures Handbook, 4th ed, ASM Press, Washington, DC

B. Vandamme, P.A.R., 2015, Chapter 16, Specimen Collection, Transport, and Processing: Bacteriology, In Jorgensen, J.H. et al, Manual of Clinical Microbiology, 11th ed, ASM Press, Washington, DC

C. Introduction to Microbiology Part II: Guidelines for the Collection, Transport, Processing, Analysis, and Reporting of Cultures from Specific Specimen Sources, Chapter 2, pp 68-78, In Winn, Washington C., et al, Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition, 2006, Lippincott Williams & Wilkins

D. CLSI. 2004 Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard – Third Ed., CLSI M22-A3, Wayne, PA.

Attachments

Appendix A Upper Respiratory Work Up Chart.pdf

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	10/12/2022
	Ann Marie Blenc: System Med Dir, Hematopath	10/5/2022
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	10/4/2022
Policy and Forms Steering Committee Approval (if needed)	Corey Webber: Mgr, Division Laboratory	9/30/2022
	Daniel Ortiz: Technical Dir, Microbiology	9/30/2022
	Benjamin Von Bredow: Assoc Tech Dir, Micro-Path	9/28/2022
	Joyce Mitchell: Mgr Laboratory	9/23/2022
	Corey Webber: Mgr, Division Laboratory	9/23/2022
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