Respiratory Cultures – A Review

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Agenda

RESPIRATORY CULTURE BASICS

- The Respiratory Tract
  - Upper Respiratory Tract
  - Lower Respiratory Tract
- Signs and Symptoms of Respiratory Tract Infections
- Types of Respiratory Tract Infections
- Tests for the Detection of Respiratory Tract Infection
- Respiratory Culture Collection
- Respiratory Culture Workup
  - Upper Respiratory Cultures
  - Lower Respiratory Cultures

RESPIRATORY CULTURE BASICS

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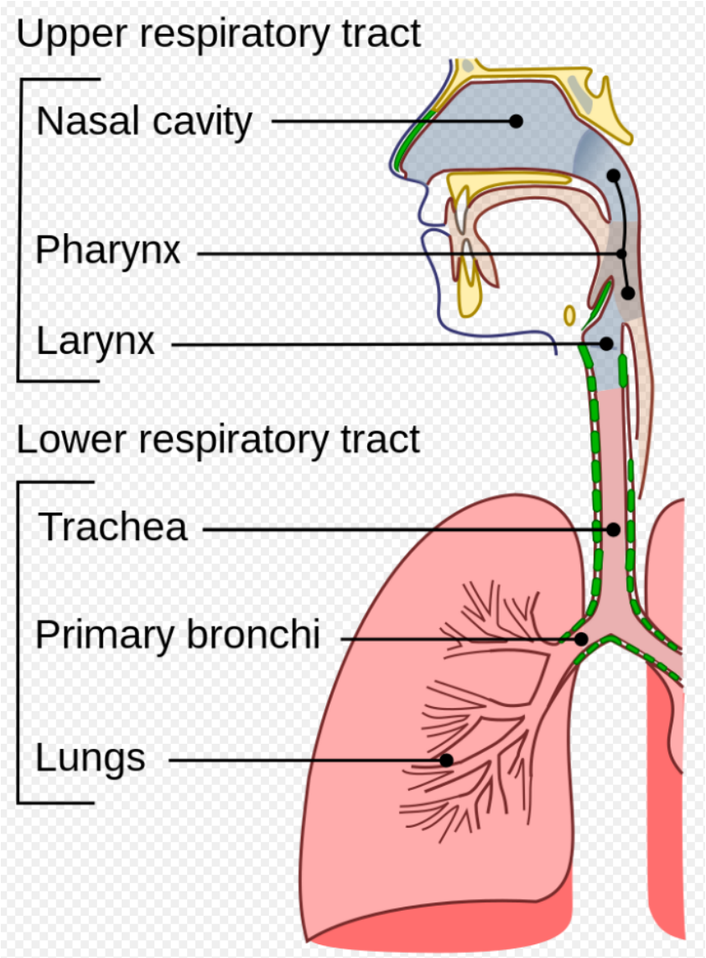
The General Respiratory Tract



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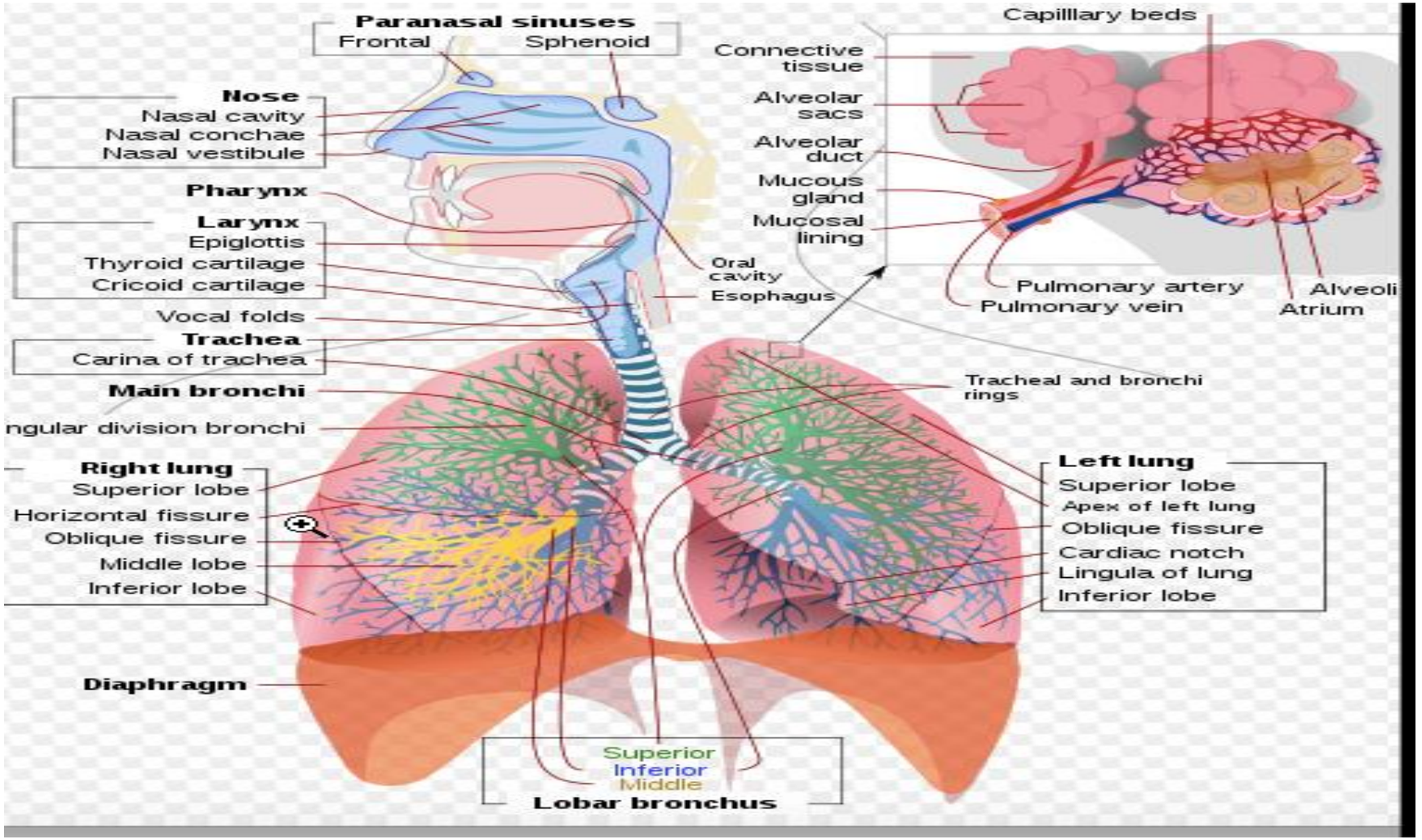
The Detailed Respiratory Tract



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General Signs and Symptoms of Respiratory Tract Infection

- Cough – Productive (mucus/phlegm) or Non-productive (Dry cough)
- Sneezing
- A stuffy or runny nose
- Sore throat
- Headaches
- Muscle aches
- Shortness of breath (SOB), tight chest or wheezing.
- High temperature (fever).

NOTE: Signs may vary based on the location of infection.

Types of Respiratory Tract Infections

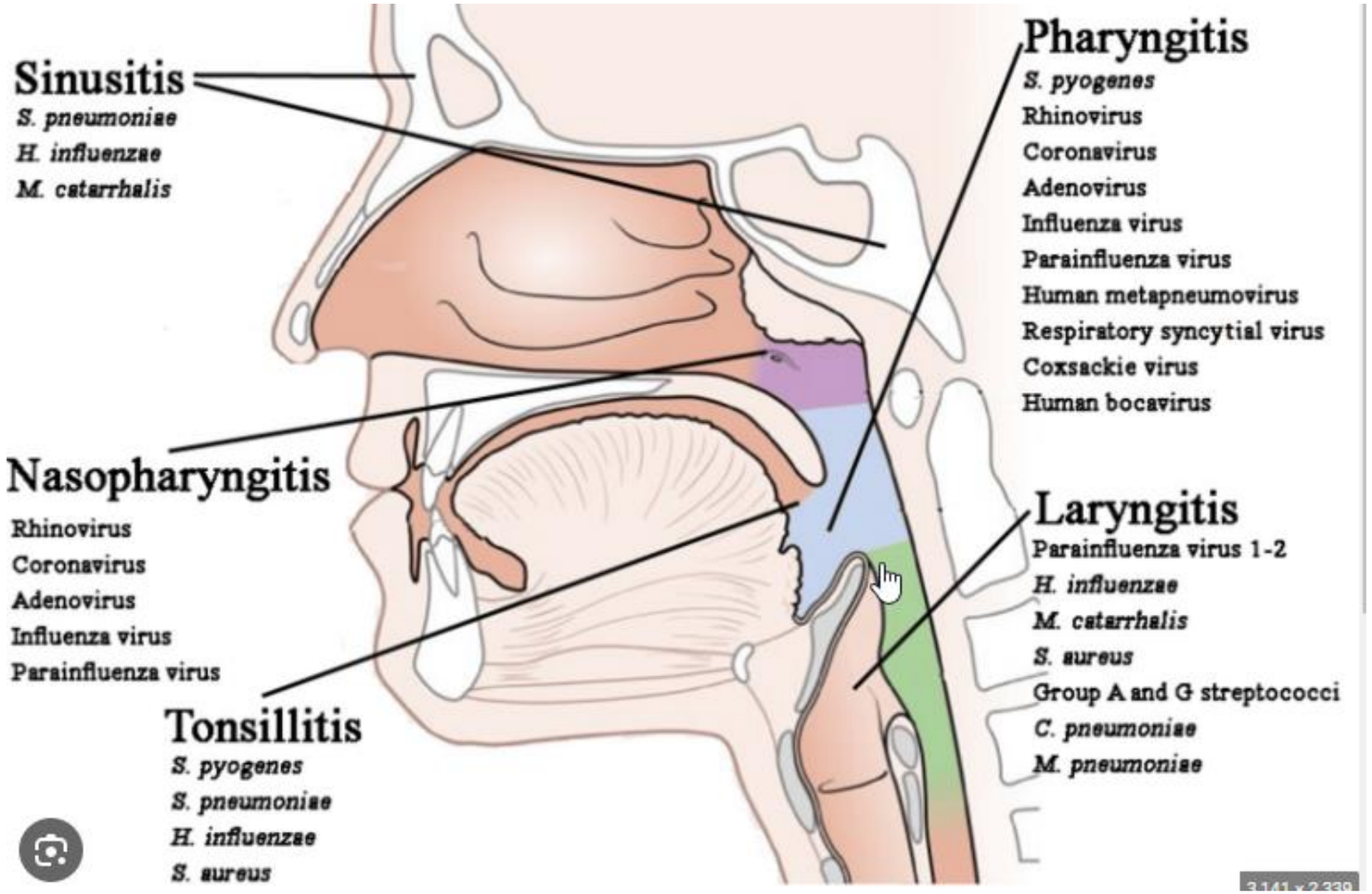
UPPER RESPIRATORY TRACT	LOWER RESPIRATORY TRACT
• Sinusitis • Nasopharyngitis • Pharyngitis • Tonsilitis • Laryngitis • Thrush*	• Bronchitis • Pneumonia • Bronchiolitis

NOTE: Yeast are part of the usual flora in the oral cavity (mouth). A diagnosis of Thrush requires invasion into the tissues of the upper respiratory tract.

Agents of Respiratory Tract Infections

- Bacteria – Some patients will be in a carrier state when conditions arise that give way to infection
- Viruses
- Fungi (Molds and Yeasts)
- Mycobacteria – M. tuberculosis and Nontuberculous mycobacteria (NTM)
- Actinomycetes – Commonly *Nocardia species*
- Parasites – i.e.,*Cryptosporidium, Entamoeba, Hookworm, Ascaris, Shistosoma, Toxocara, etc.*

Agents of Upper Respiratory Tract Infections



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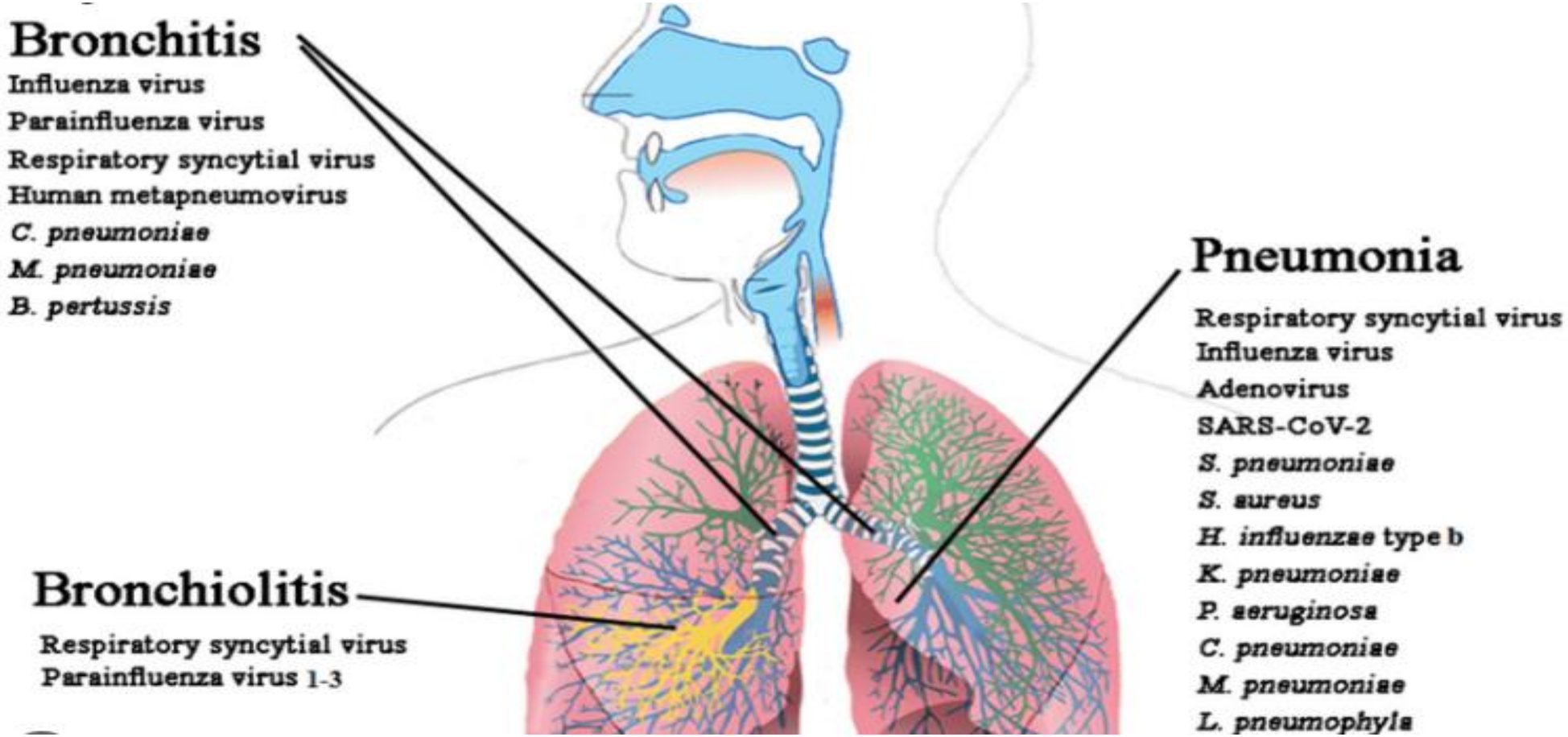
Agents of Lower Respiratory Tract Infections



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Types of Pneumonia

Community Acquired (CAP)

- Develops prior to admission to the hospital
- Usually caused by atypical pathogens
  - Streptococcus pneumoniae
  - Mycoplasma pneumoniae
  - Chlamydophila pneumoniae

Ventilator Associated (VAP)

- Usually develops due to colonization or contamination of the endotracheal attachment
- Severe pneumonia caused by hospital/healthcare acquired resistant pathogens
  - Pseudomonas
  - Acinetobacter
  - Staphylococcus aureus
  - Enterobacterales

Types of Pneumonia

Hospital Acquired (HAP)/ Healthcare Associated (HCAP)

- Develops at least 48 hours after admission
- Usually involves more resistant organisms found in the hospital
  - Gram negative rods
  - Staphylococus aureus MRSA

Aspiration

- Develops as a result of food or drink into the lung or gastrointestinal fluids into the lung
- May result in mixed anaerobic/aerobic infections

Tests for Detection of Respiratory Pathogens

- Gram Stains
- Antibody Stains
- Bacterial Culture
- Viral Culture
- Enzyme Immunoassay/ Fluorescent Immunoassay
- Viral Serology
- Single/Multiplex PCR

KNOWLEDGE CHECK

Which of the following is not found in the upper respiratory tract?

1. Sphenoid sinus
2. Alveolar sac
3. Nasal vestibule
4. Epiglottis

You are reviewing an upper respiratory culture for a pediatric frontal sinus specimen. Which pair of pathogens should you check for?

1. Moraxella catarrhalis/E. coli
2. Haemophilus influenza/ Streptococcus pneumoniae
3. Streptococcus pneumoniae/ Kingella kingae
4. Moraxella catarrhalis/Adenovirus

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KNOWLEDGE CHECK

A NICU baby is diagnosed with bronchiolitis. Which test method can the laboratory use to detect the presence of the principal pathogens for this infection?

1. Cepheid Respiratory Combo
2. BioFire Respiratory 2.1 panel
3. Lower respiratory culture of bronchial washings
4. Upper respiratory culture of a tracheal suction

A patient develops pneumoniae 4 days after being placed on a ventilator. The MRSA screen was negative prior to ventilation. Which pair of pathogens should you check for?

1. Acinetobacter species/ Staphylococcus aureus
2. KPC producing E. coli/ Streptococcus pneumoniae
3. Chlamydia pneumoniae/ Pseudomonas aeruginosa
4. Bacteroides thetaiotamicron/ Mycoplasma pneumoniae

KNOWLEDGE CHECK

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KNOWLEDGE CHECK

Match the type of infection with the location in the respiratory tract

1. Bronchitis
2. Pneumonia
3. Pharyngitis

a. Mucosa at back of throat
b. Bronchial tubes
c. Lung

KNOWLEDGE CHECK

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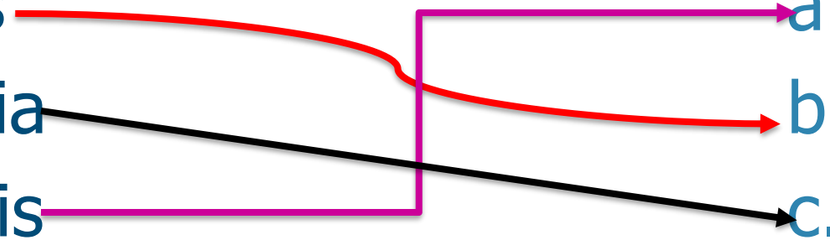

a. Mucosa at back of throat
b. Bronchial tubes
c. Lung

RESPIRATORY CULTURE COLLECTION

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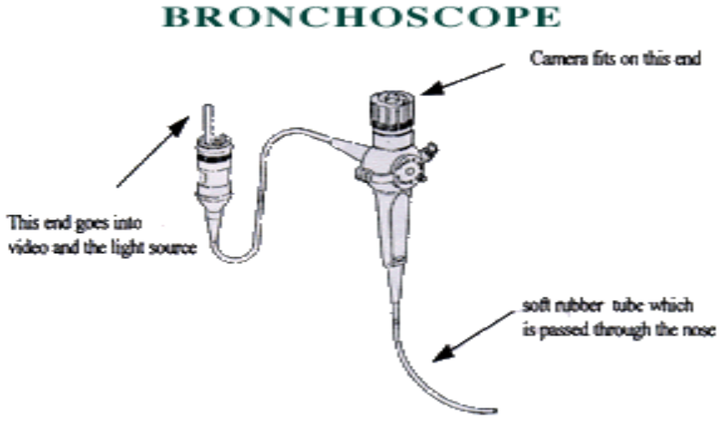
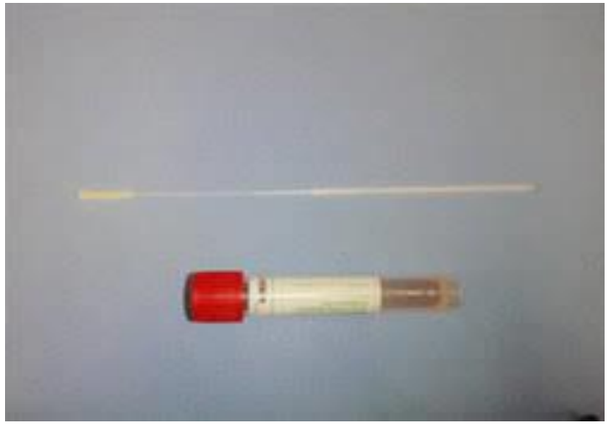
Respiratory Tract Specimen Collection





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Types of Lower Respiratory Tract Specimens

Specimen Type	Definition
Expectorated Sputum*	Sputa coughed up by the patient after instruction but without assistance
Endotracheal Aspiration*	Suction collection of secretions from the oropharynx that have collected in the endotracheal tube/site and airway
Induced Sputum*	Sputa produced by the patient after ultrasonic nebulization with 3% Saline; may appear watery in consistency
Bronchial Wash**	Bronchoscopy specimen collected from the major bronchi, including the main bronchus, at the bifurcation and the right and left bronchi – usually using a rigid bronchoscope
Bronchoalveolar Lavage	Bronchoscopy specimen collected from the distal bronchioles and alveoli in a lung segment using a flexible scope
Endobronchial Ultrasound Specimen	Fluid and/or tissue samples obtained using guided ultrasound transbronchial needle aspiration along the mediastinum (mediastinoscopy)
Protected Bronchial Brush	Brush catheter that is sheathed to protect it from contamination by flora occurring in the environment that it is passed through (i.e., normal respiratory flora in the upper respiratory tract)

*Culture usually contains normal flora organisms. **Culture may contain normal flora organisms.

Respiratory Tract Specimen Collection

Bronchial Biopsy/Bronchial Brush

- Bronchial Biopsy – Collection of actual tissue. Utensils are passed through the bronchoscope
- Bronchial Brush – Collection of cells scraped from the surface to detect cancer; can be used for culture also.

Bronchial Wash or Lavage

- Bronchial Wash or Lavage – samples the large airways (trachea, branching of tree)
- Bronchoalveolar lavage (BAL) – samples smaller airways down to the aveolar level. It may be collected using:
  - Bronchoscope
  - Catheter or canula (Mini-BAL)

https://youtu.be/HzD5F6NioXw

KNOWLEDGE CHECK

Which of the following specimens should not yield mixed flora as a source of infection?

1. Protected bronchial brush
2. Tracheal suction specimen
3. Specimen collected from an aspiration pneumonia
4. Induced sputum

An endobronchial ultrasound specimen may be a fluid or tissue sample collected from the patient's abdominal wall.

1. True
2. False

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1. True
2. **False**

RESPIRATORY CULTURE WORKUP

What Impacts Respiratory Culture Workup

- Prior Antibiotic Therapy
- Specimen Collection Method
- Specimen Preservation Method (Storage conditions or lack thereof)
- Specimen Processing – How soon and how
- Media
- Incubation
- Patient Demographics
- Colony Count
- **YOU – Skill, Commitment, Attitude, Mission Focus**

Upper Respiratory Cultures

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Upper Respiratory Tract Flora

Normal Flora Organisms	Common Carriage Organisms[1]	Common Pathogens
• Alpha and gamma hemolytic streptococcus • Beta hemolytic streptococci other than Streptococcus pyogenes • Saprophytic Neisseria species • Haemophilus species • Corynebacterium species other than *Corynebacterium diphtheriae* • Eikenella corrodens[5] • Staphylococci • Micrococci • Various anaerobic bacteria • Yeasts	• Streptococcus pneumoniae[3] • Streptococcus pyogenes • Haemophilus influenza • Moraxella catarrhalis • Neisseria meningitidis • Staphylococcus aureus • Yeasts (primarily Candida albicans)[2] Enterobacteriaceae[2]	• Streptococcus pyogenes[3, 4] • Arcanobacterium haemolyticum[4] • Beta hemolytic streptococci, large colony variants of Group C and G[4] • Haemophilus influenzae[3] • Moraxella catarrhalis[3] • Neisseria meningitidis • Corynebacterium diphtheria • Bordetella pertussis

[1]May exist as transient or “carrier” flora in normal, healthy individuals without the development of infection.
[2]More commonly found among alcoholics, diabetics and hospitalized patients (colonization with gram negative rods increases with the length of the hospital stay).
[3]Agents that cause Sinusitis
[4]Agents that cause Pharyngitis
[5]Part of normal human oral flora

Key Tests to Rule Out Upper Respiratory Bacterial Pathogens

Throat/Pharyngitis

- Gram Stain
- Rapid Group A Strep Test (EIA/FIA/PCR)
- Bacitracin
- PYR
- Beta Strep Serology

NOTE: Pharygitis can be caused by viruses and present with similar symptoms as a bacterial infection.

Sinusitis

- Gram Stain
- Bacitracin
- Optochin
- Bile Solubility
- Catarrhalis Test
- Oxidase
- Indole
- ALA
- RapID Panels (NH/STR)

Other Upper Respiratory Infections:
Epiglottis

Primary Pathogens:

- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*

NOTE: The epiglottis is cartilage flap that closes the larynx during swallowing to prevent the passage of food and drink into the lungs (Source of aspiration pneumonia) .

Other Upper Respiratory Infections:
Ear

Outer Ear (Otitis Externa)

- Gram negative rods – fermenters and nonfermenters (i.e., Pseudomonas aeruginosa)
- Vibrio species
- Beta hemolytic streptococci
- Fungi (yeast and molds)
- Staphylococcus aureus

NOTE: Also known as 'Swimmers Ear'. Some pathogens may occur as normal skin flora.

Inner Ear (Otitis media)

- Haemophilus influenzae
- Beta hemolytic streptococci
- Moraxella catarrhalis
- Corynebacterium species
- Streptococcus pneumoniae
- Alloiococcus otitis
- Turicella otitidis
- Nocardia species

NOTE: Tympanocentesis method used to collect fluid from the inner ear

Other Upper Respiratory Infections:
Paranasal Sinuses

The paranasal sinuses are:

- Ethmoid
- Frontal
- Maxillary
- Sphenoid

Invasive procedures are used to collect culture material (fluid/pus) from the sinuses

Common Pathogens isolated from these specimens are:

- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Moraxella catarrhalis*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Gram negative rods (i.e., Enterobacterales, Pseudomonas aeruginosa, etc.)*
- *Mold*

Lower Respiratory Cultures

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Contaminating Upper Respiratory Flora Found in the Lower Respiratory Tract Flora

- Viridans group streptococci
- Nonpathogenic Neisseria species
- Coagulase negative staphylococci
- Group F streptococci
- Haemophilus species (not H. influenzae)
- Actinobacillus species
- Capnocytophyga species
- Streptococcus pneumoniae
- Rothia species
- Diphtheroids
- Anaerobes
- Enterococci
- Gram Negative Bacilli
- Eikenella species
- Yeasts (except Cryptococcus neoformans)
- Moraxella species
- Neisseria meningitidis
- Staphylococcus aureus < Normal Flora

Lower Respiratory Tract Pathogens – **All Specimen Types**

Workup Any Quantity

- Bacillus anthracis
- Brucella species
- Cryptococcus neoformans
- Francisella tularensis
- Group B Streptococci (Pediatric patients < 13 months)
- Molds (non-media contaminants)
- Nocardia species
- Pseudomonas aeruginosa
- Rapidly growing mycobacteria
- Rhodococcus equi
- Streptococcus pyogenes
- Streptomyces species
- Yersinia pestis

Lower Respiratory Tract Pathogens – **Sputa Group**

Workup Moderate or Greater Quantity IF GREATER THAN THE NORMAL FLORA

- Corynebacterium species
  - **If urea positive, or**
  - **An intensive care unit patient**
- Enterobacterales
- Group B, C or G streptococci
- Haemophilus influenzae
- Moraxella catarrhalis
- Neisseria meningitidis
- Pasturella species
- Staphylococcus aureus – ***rule out MRSA***
- Streptococcus pneumoniae
- Nocardia species
- Pseudomonas aeruginosa
- Rapidly growing mycobacteria
- Rhodococcus equi
- Streptococcus pyogenes
- Streptomyces species
- Yersinia pestis
- **For Inpatients ONLY**
  - Stenotrophomonas maltophilia
  - Acinetobacter species
  - Burkholderia species

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Lower Respiratory Tract Pathogens – **Protected Brush/Bronchoalveolar Lavage**

Workup In Pure Culture ONLY

- Corynebacterium species
  - **If urea positive, or**
  - **An intensive care unit patient**
- Enterobacterales
  - **Immunocompromised patient, or**
  - **An intensive care unit patient**
- Group B, C or G streptococci
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Neisseria meningitidis*
- *Staphylococcus aureus* – ***rule out MRSA***
- *Streptococcus pneumoniae*

Workup Any Quantity

- *Pasturella species*
- *Stenotrophomonas maltophilia*
- *Acinetobacter species*
- *Burkholderia species*
- **Workup Moderate or Greater Quantity**
- *Anaerobes – Possible aspiration pneumonia*

Lower Respiratory Tract Specimen Workup – Is It **Normal Flora** or a **Pathogen**?

Consult the physician for guidance IF…

- Normal Respiratory Flora is isolated from:
  - Lung – Tissue/Aspirate/Biopsy
  - Transtracheal Aspirate
  - Endobronchial Ultrasound

KNOWLEDGE CHECK

Which of the following is **not** a paranasal sinus?

1. Maxillary
2. Ethnoid
3. Sigmoid
4. Sphenoid

Some pathogens may occur as normal skin flora in some cases of 'Swimmers Ear'.

1. True
2. False

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1. Maxillary
2. Ethnoid
3. **Sigmoid**
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1. **True**
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KNOWLEDGE CHECK

You isolate what appears to be moderate normal respiratory flora including a few gram-negative rods, with 3 colonies of beta hemolytic streptococci from the tracheal suction of a 1-year-old child with no known allergies. The gram stain is a borderline specimen. What should you do next?

1. Forward a report that the specimen is unsuitable for culture based on the gram stain quality assessment.
2. Rule out GBS and follow workup and reporting guidelines if found.
3. Consider the beta hemolytic streptococci as normal respiratory flora.
4. Review the gram stain and reject the culture if confirmed.

You are reviewing a bronchoalveolar lavage culture at 48 hours? The CO2 plates are no growth, but the gram stain shows Moderate WBCs with moderate mixed morphotypes present. What should you do next?

1. Forward a final report of 'No growth' after 48 hours incubation.
2. Reincubate the CO2 plates an additional 24 hours and forward a final report of 'No Growth' if no change after 72 hours incubation.
3. Reincubate the CO2 plates an additional 24 hours and review the anaerobic plates after 72 hours incubation.
4. Check the gram stain and antibiotic history, reincubate the CO2 plates an additional 24 hours, and consider checking the anaerobic plates for growth at 48 hours based on no antibiotic therapy and the gram stain results, if indicated.

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Critical Values

- Normal organism related critical values
- Different organisms isolated after the initial report
- Isolation of Mold

What Would You Do?

PATIENT	SCENARIO
Sputum Induction	Gram stain= equal numbers (>25) of squamous epithelial cells and WBC
Bronchial Washing	Moderate Normal Flora, Few Bacillus species
RML Tissue Aspirate	Mixed Bacterial Flora including Mixed Anaerobic Flora
Bronchial Brushing	One colony gram negative rod isolated
Expectorated Sputum	Moderate Normal Respiratory Flora, Moderate Eikenella corrodens like colonies with bleach odor

What Would You Do?

PATIENT	SCENARIO
Sputum Induction	Gram stain= equal numbers (>25) of squamous epithelial cells and WBC - **Process the specimen. Specimen quality assessment is performed for expectorated sputum and tracheal suction specimens only.**
Bronchial Washing	Moderate Normal Flora, Few Bacillus species – **You must rule out the possibility of Bacillus anthracis.**
RML Tissue Aspirate	Mixed Bacterial Flora including Mixed Anaerobic Flora – **Consult with the physician. This suggests the possibility of aspiration pneumonia or an abscess.**
Bronchial Brushing	One colony gram negative rod isolated – **Check patient type, location, whether GNR is in pure culture, and type of GNR. When in doubt, consult with the physician.**
Expectorated Sputum	Moderate Normal Respiratory Flora, Moderate Eikenella corrodens-like colonies with bleach odor – **This can be contaminating flora from the upper respiratory**