Lower Respiratory Cultures: Best Practices

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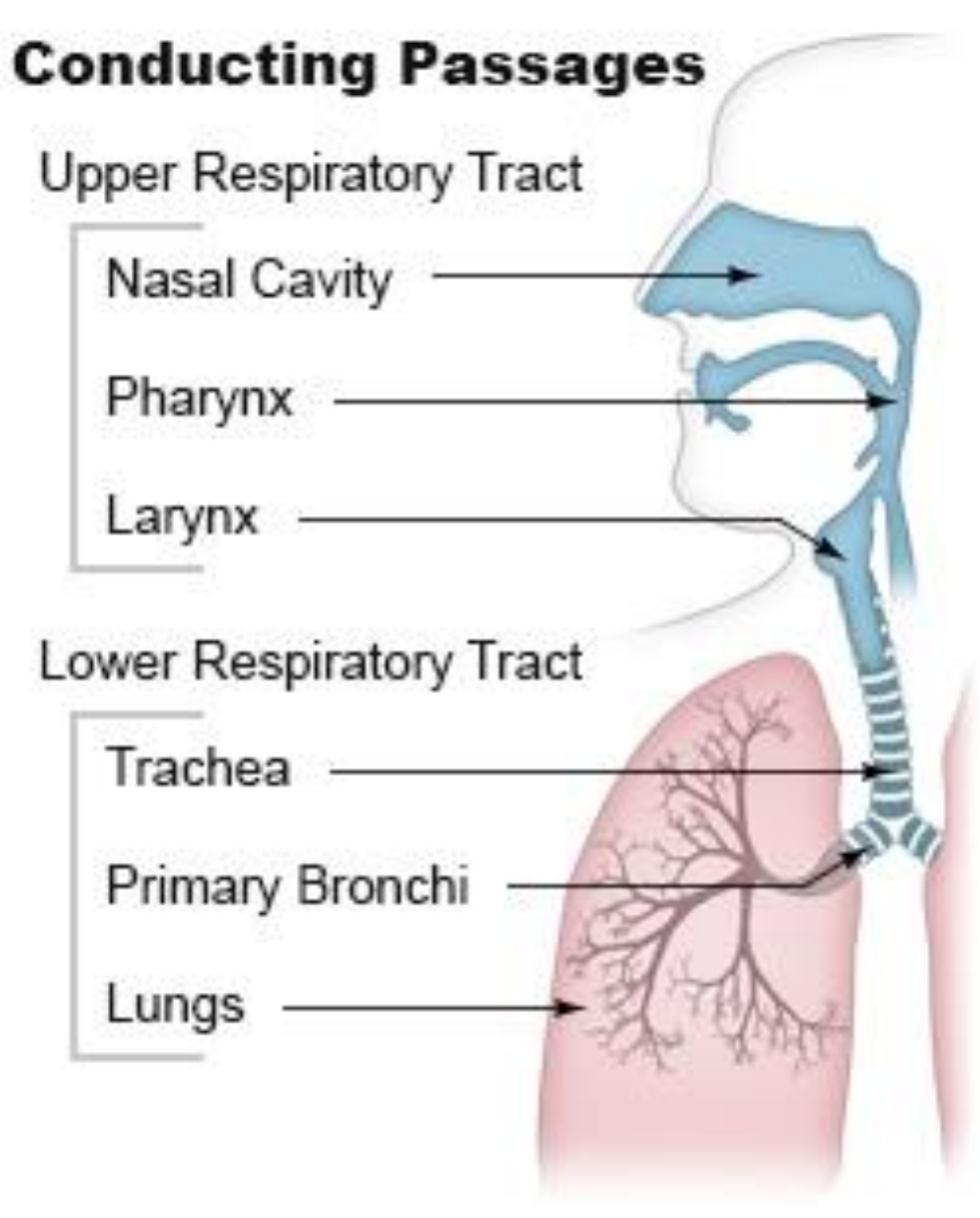
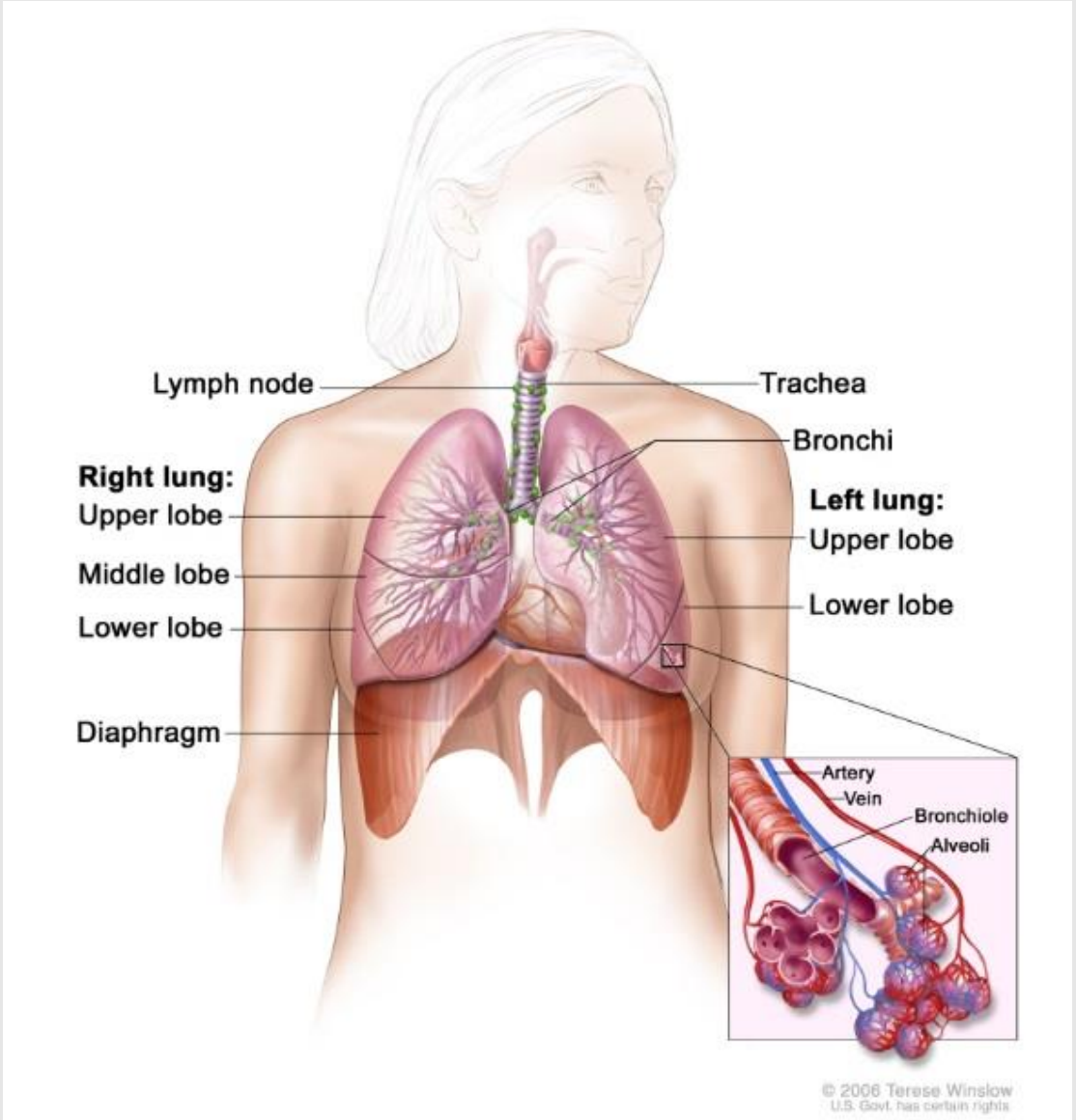
Objectives

- Introduce common Lower Respiratory Tract diseases
- Review briefly specimen collection of LRT specimens
- Discuss the process and importance of the Gram stain
- Review culture workups
- Discuss less frequently encountered organisms
- Discuss new/emerging diagnostics and special circumstances

Lower Respiratory Tract Anatomy





Diseases of the LRT

Diseases

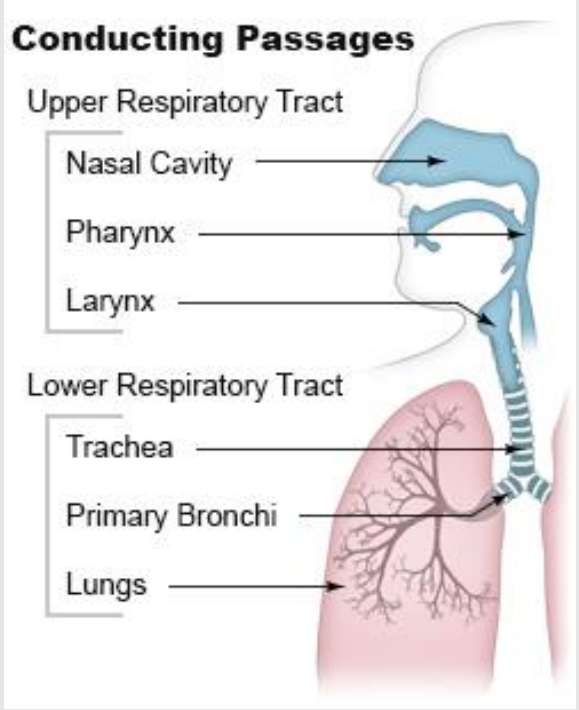
- Acute bronchitis
- Bronchiolitis
- Acute pneumonia
- Chronic pneumonia

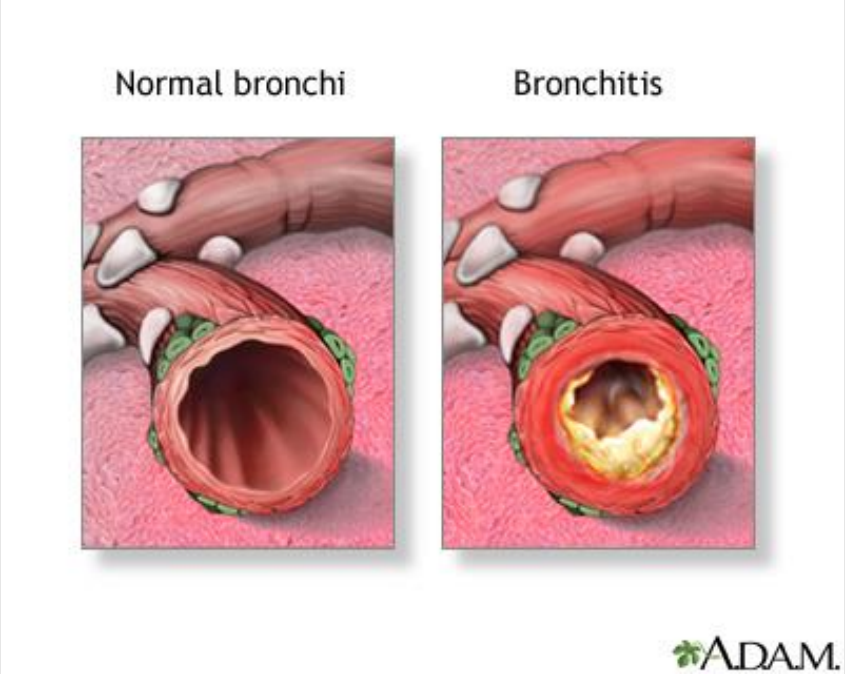
Etiologies

- Viral
- *Streptococcus pneumoniae*
- *Klebsiella pneumoniae*
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Legionella pneumophila*
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*

Acute Bronchitis

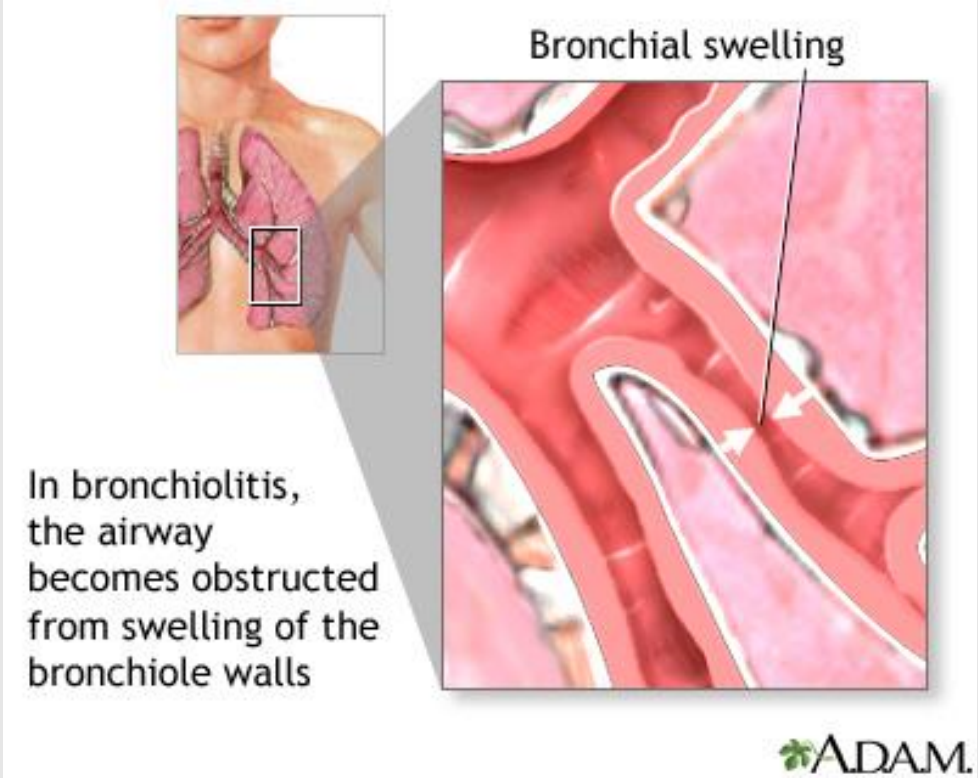
- Self-limited syndrome
- Inflammatory process of large and mid-sized airways
- Characterized by cough, w/wo sputum
- Negative for signs of pneumonia
- Primarily caused by viruses, rhinovirus, influenza, RSV, hMPV
- < 10% caused by bacteria; *M. pneumoniae*, *C. pneumoniae*, *B. pertussis*





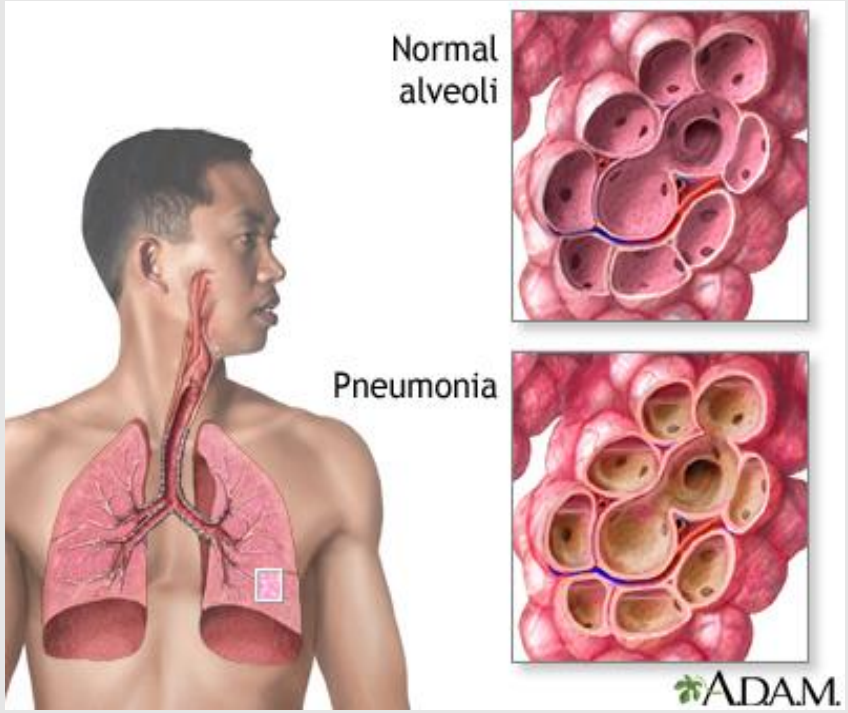
Bronchiolitis

- Small airway inflammation/obstruction
- Inflammatory changes in small bronchi and bronchioles
- Characterized by prominent cough, mild fever, increased respiratory rate, nonspecific systemic symptoms
- Most common acute viral LRT infection during the first 2 years of life
- Caused by viruses; RSV most frequently, others hMPV, influenza, parainfluenza



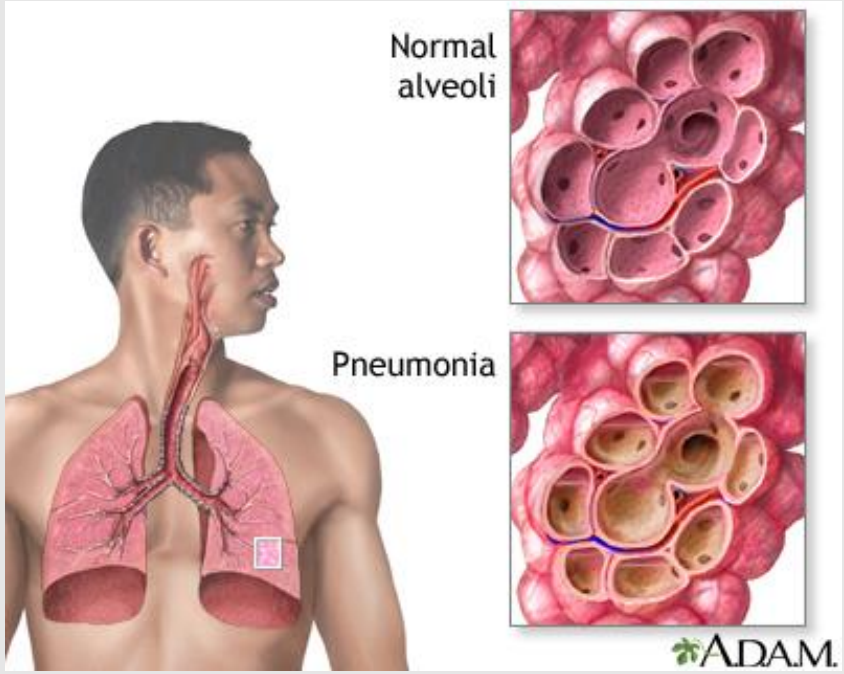
Acute Pneumonia

- Infection that causes inflammation of the alveoli
- Characterized by prominent cough, fever, chest pain, dyspnea, thick blood-tinged or yellow/green sputum, abnormal chest x-ray is common
- Top 10 most common cause of death among all age groups in the US
- Single most common cause of infection-related mortality in the US
- Many syndromes, CAP, HAP, HCAP, VAP, BAP, bacterial pneumonia coinfection in influenza
- Ever expanding number of infectious agents can cause acute pneumonia, bacteria, fungi, viruses, parasites, and noninfectious causes



Chronic Pneumonia

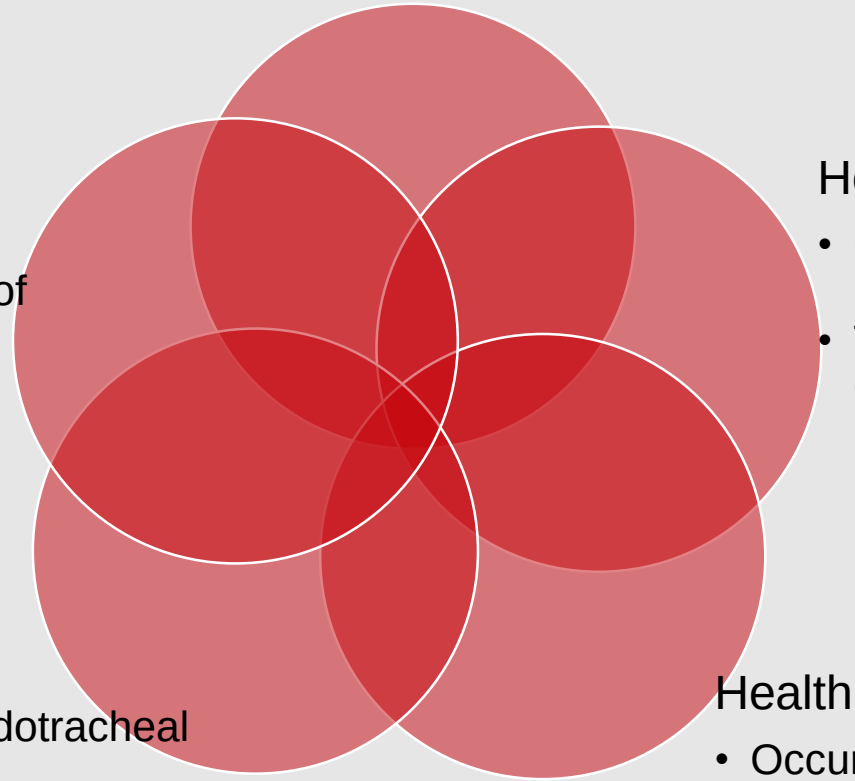
- Infection that causes persistent inflammation of the alveoli
- Characterized by persistent or progressive cough, dyspnea, chronic sputum production, w/wo fever, lasting weeks or months rather than days
- Always associated with an abnormal chest x-ray
- Many bacterial, fungal, parasitic, and noninfectious causes
- Viruses rarely progress to chronic pneumonia



The Pneumonia's

Community Acquired Pneumonia

- S. Pneumonia is 60% of bacterial cases
- S. Pneumonia cases declining due to use of 13-valent vaccine



Bacterial Aspiration Pneumonia

- Follows aspiration of microbiota of URT
- Anaerobic bacteria involved in majority of cases

Hospital Acquired Pneumonia

- Occurs 48 hours or more after hospital admission
- Typically resistant to one or more commonly used antimicrobial agent

Ventilated Acquired Pneumonia

- Develops more than 48-72 hours after endotracheal intubation
- Frequency of 9-40% of intubated patients
- Increase prevalence of Gram Negative pathogens

Health Care Acquired Pneumonia

- Occurring in non-hospitalized patient with extensive health care contact, recent hospitalization, nursing home, wound care, etc.

What Specimens are Acceptable?

- Expectorated sputum
- Induced sputum – not routinely used for bacterial culture
- Suctioned sputum – common for neonates, infants, children
- Endotracheal aspirates
- Tracheal aspirates/vented patient – clinically misleading due to bronchus colonization
- Bronchial washings
- Bronchoalveolar lavage (BAL)
- Transbronchial biopsy specimens

Sputum

Expectorated (Deep Cough)

- NO rinsing with nonsterile water prior to collection
- NO saliva or postnasal discharge
- Collect PRIOR to antibiotic therapy whenever possible

Induced (Mechanical)

- Involves toothbrush
- NO toothpaste
- DO rinse mouth with sterile water
- Use of ultrasonic nebulizer

Bronchoscopy Samples

Bronchial Wash Samples

- Bronch wash samples are from major bronchi, at bifurcation, and right and left bronchi
- Commonly done to diagnose cancer in patients with bronchial lesions or masses
- Wash samples from different lung locations should not be pooled

BAL

- BAL are collected from distal bronchioles and alveoli, bronchoscope is wedged into distal airway lumen in lung segment (RUL, RML, RLL)
- Aliquots from same site may be combined for cultures and smears, should be discussed with local stakeholders

Transport & Processing

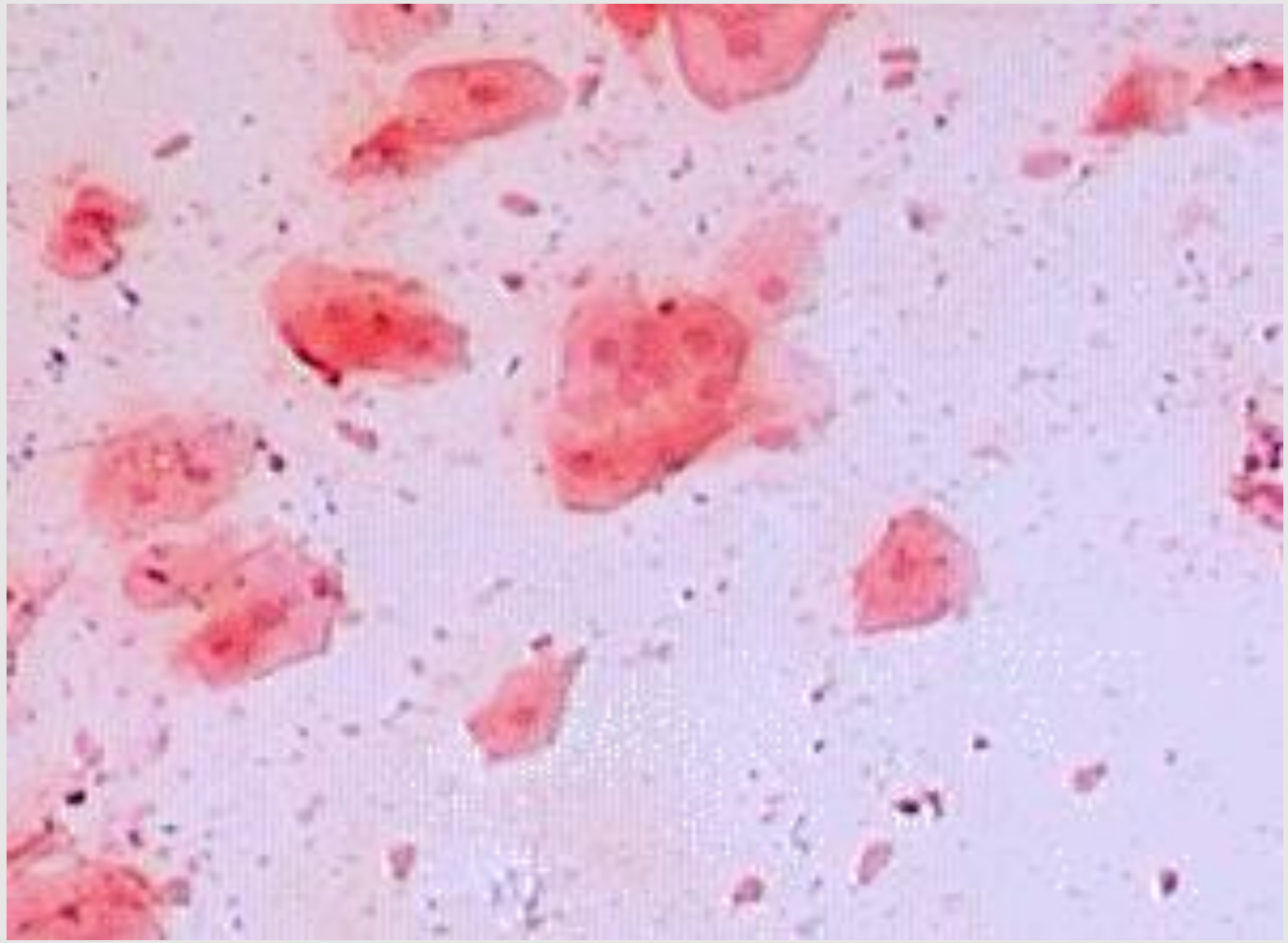
- Delaying transport & processing of more than 4 hours may result in decreased ability to recover fastidious pathogens and/or overgrowth of URT bacteria
  - *S. pneumonia*
  - *H. influenzae*
- BAL Samples may be concentrated prior to inoculation and gram stain

Sputum Acceptability Criteria

- Poorly collected sputum specimens are wasteful and can lead to erroneous reporting and treatment!
- Qualification:
  - Sputum for *Legionella, Nocardia*, AFB, or from cystic fibrosis patients.
- Sputum cultures are already insensitive, don’t dilute their diagnostic sensitivity further by accepting poor samples

What Quantity Epithelial Cells Do You Reject?

A. 5 SECs/LPF
B. ≥10 SECs/LPF
C. ≥ 25 SECs/LPF
D. We culture everything!



Leber, Amy L. *Clinical Microbiology Procedures Handbook 5th Ed.* Volume 1. Aerobic Bacteriology 3.2.1.18 Rejection Criteria for Sputum Culture. ASM 2023.

Gram Stain Setup

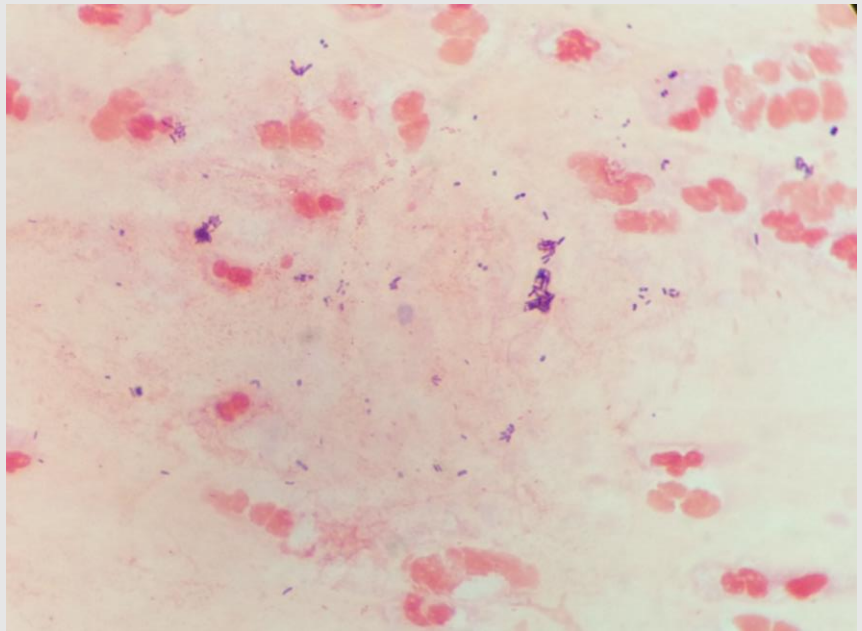
- Perform slide prep and fixation in BSC
- Purulent part of the sputum is taken, and smear is made on a clean grease-free glass slide.
- BAL/Bronch Wash place 5 drops of sample into cytocentrifuge chamber
- Use of sterile loop or applicator stick to spread thin layer of material onto slide
- Smear is left to air dry and heat fix in BSC
  - Alternatively, air dry and methanol fix for direct specimens
- Slide is stained either manually or by automated stainer
  - Automated staining can introduce more stain variation

Gram Stain Assessment

Assess quality of slide under low power (10x objective)

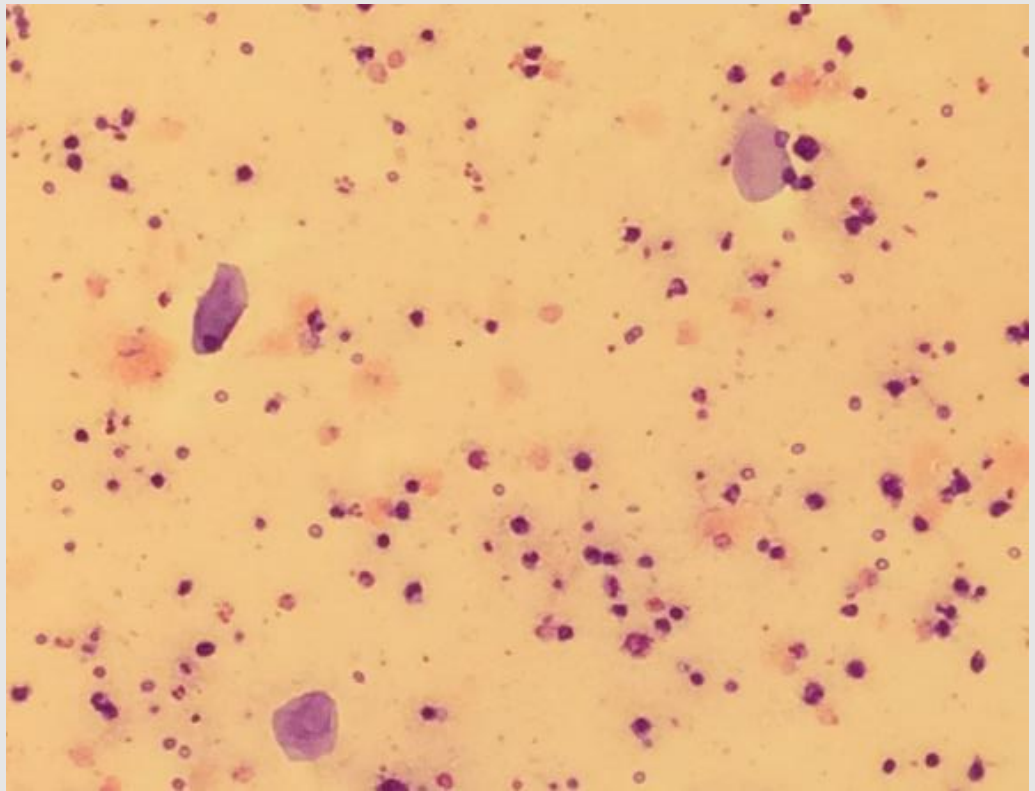
Good Indicators

- Decolorized elements
  - SECs
  - WBCs
  - RBCs



Poor Indicators

- Stain precipitate crystals
- Overly thick layer



Gram Stain Interpretation

- Examine 20-40 fields under 10x objective
  - Determine area representative of inflammation or purulence
  - Average number of cells in representative fields that contain cells
  - Reject specimens more representative of saliva (≥10 SECs/LPF)

PMN's and SECs		
# Observed 10x objective	**PMN's**	**SECs**
0	None	None
0-3	Very Few	Few
3-10	Few	
10-25	Moderate	Many
>25	Many*	

Gram Stain Interpretation

- Examine 20-40 fields under Oil Immersion
  - Note staining characteristics, predominant shapes and arrangements
  - Large umbers of a single type, especially if associated with PMNs, is likely to indicate infection
- Specimens from neutropenic patients may have few PMNs
- Fungal hyphae, *Actinomyces* and *Nocardia* may only be detected on low power due to low numbers and aggregation

Microorganisms	
# Observed 100x objective	**Bacteria**
0	None
1-2	Rare
2-3	Few
3-10	Moderate
>10	Many

Gram Stain Reporting

Report semi-qualitative “Few/Mod/Many” for SECs, PMNs, and bacteria as appropriate

Special Considerations:

1. Accuracy is highly dependent on training and skill of microscopist
2. Reporting of “Normal Flora” in gram stain report is based upon local policy determination
3. The most important role of the Gram stain is as a determinant of specimen acceptability

What percentage of sputum specimens exhibit a predominate bacterial morphology?

A. 10-20%
B. 21-30%
C. 31-40%
D. 41-50%

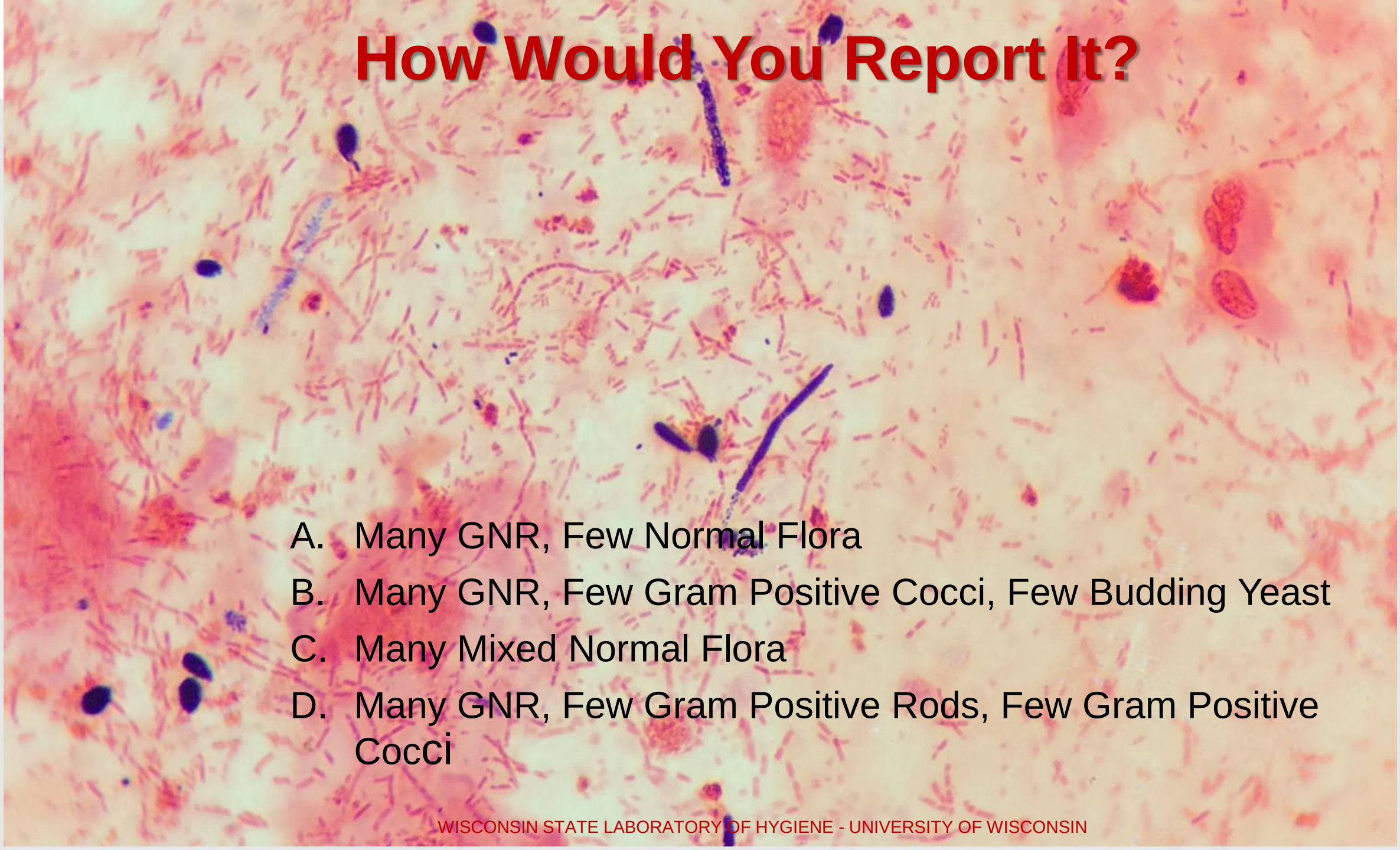
IDSA/ATS Guidelines:

Utility of Gram stain and culture

- Only 14% of adequate specimens had a predominant morphotype
- Yield of S. pneumoniae from culture 40-50%
- Positive Gram stain correlates well with positive culture
- Gram stain results can discover less common pathogens (S. aureus or GNR)

CID 2007:44 (Suppl 2)

How Would You Report It?



A. Many GNR, Few Normal Flora
B. Many GNR, Few Gram Positive Cocci, Few Budding Yeast
C. Many Mixed Normal Flora
D. Many GNR, Few Gram Positive Rods, Few Gram Positive Cocci


what's
next
?

Routine Culture Work Up

Resources

- Infectious Diseases Society of America/American Thoracic Society

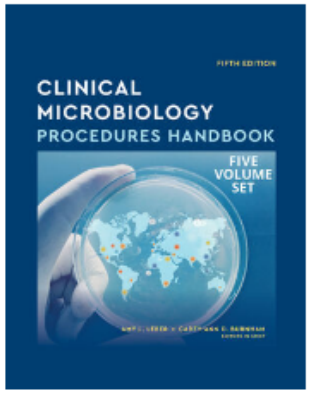
Diagnosis and Treatment of Adults with Community-acquired Pneumonia

An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America

Joshua P. Metlay*, Grant W. Waterer*, Ann C. Long, Antonio Anzueto, Jan Brozek, Kristina Crothers, Laura A. Cooley, Nathan C. Dean, Michael J. Fine, Scott A. Flanders, Marie R. Griffin, Mark L. Metersky, Daniel M. Musher, Marcos I. Restrepo, and Cynthia G. Whitney; on behalf of the American Thoracic Society and Infectious Diseases Society of America

THIS OFFICIAL CLINICAL PRACTICE GUIDELINE WAS APPROVED BY THE AMERICAN THORACIC SOCIETY MAY 2019 AND THE INFECTIOUS DISEASES SOCIETY OF AMERICA AUGUST 2019

- American Society of Microbiology



Clinical Microbiology Procedures Handbook, 5th Edition

Last Updated: May 2023

The Usual Suspects

Which character was eventually identified as Keyser Söze?

A. Kevin
B. McManus
C. Fenster
D. Keaton
E. Verbal

A B C D E

The Usual Suspects

Community Acquired Pneumonia

- *Streptococcus pneumonia*
- *Haemophilus influenza*
- *Staphylococcus aureus*
- *Moraxella catarrhalis*
- *Klebsiella pneumoniae*
- *Mycoplasma pneumonia*
- *Legionella species*
- *Chlamydia pneumoniae*

Clicker

IDSA guidelines include recommendations to routinely perform diagnostic testing, including culture, on patients with CAP?

A. True
B. False

ISDA Guidelines for Culture

- “We recommend NOT obtaining sputum Gram stain and culture routinely in adults with CAP managed in the outpatient setting”
  - What percent of lower respiratory tract cultures DO NOT identify a pathogenic agent?
    A. 0-20%
    B. 20-40%
    C. 40-60%
    D. 60-80%

ISDA Guidelines for Culture

- “We recommend NOT obtaining sputum Gram stain and culture routinely in adults with CAP managed in the outpatient setting”
  - Low recovery
    - 40-60% of cultures do not recover a pathogen
  - Radiology may help with diagnosis
  - Treat empirically
  - Viral pathogens


Diagnosis and Treatment of Adults with Community-acquired Pneumonia
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ISDA Guidelines for Culture

- When to culture?
  - Patients being managed in an inpatient setting, AND
    - Classified as severe CAP, or
    - Empirically treated for MRSA or *P. aeruginosa*, or
    - Previously infected with MRSA or *P. aeruginosa*, or
    - Were previously hospitalized and received antibiotic treatment in last 90 day
- Why not routinely culture?
  - Lack of evidence for better patient outcomes.

Table 1. 2007 Infectious Diseases Society of America/American Thoracic Society Criteria for Defining Severe Community-acquired Pneumonia

Validated definition includes either one major criterion or three or more minor criteria

Minor criteria

- Respiratory rate $\geq$ 30 breaths/min
- Pa_{O2}/F_{IO2} ratio $\leq$ 250
- Multilobar infiltrates
- Confusion/disorientation
- Uremia (blood urea nitrogen level $\geq$ 20 mg/dl)
- Leukopenia* (white blood cell count $<$ 4,000 cells/μl)
- Thrombocytopenia (platelet count $<$ 100,000/μl)
- Hypothermia (core temperature $<$ 36°C)
- Hypotension requiring aggressive fluid resuscitation

Major criteria

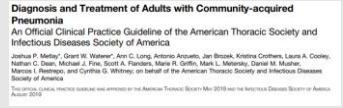
- Septic shock with need for vasopressors
- Respiratory failure requiring mechanical ventilation

*Due to infection alone (i.e., not chemotherapy induced).


Diagnosis and Treatment of Adults with Community-acquired Pneumonia
An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America

ISDA Guidelines for Other Diagnostic Tests

- Blood cultures
  - Follow same recommendations as LRT culture
- Legionella urine antigen
  - When outbreak related
  - Severe CAP
- *S. pneumonia* urine antigen
  - Severe CAP
- Influenza testing
  - If prevalence of influenza is high in the community


Diagnosis and Treatment of Adults with Community-acquired Pneumonia
An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America

Culture Work Up

- ASM Clinical Microbiology Procedure Handbook, 5th Ed.

Report in Any Amount
Streptococcus pyogenes
Group B streptococci (pediatric population)
Neisseria gonorrhoeae
Non-*Candida* yeasts(Cryptococcus)
Molds (not saprophytic contaminants)
Francisella tularensis
Bordetella species
Yersinia pestis
Nocardia
Bacillus anthracis

Always report (but don't look too hard)
Streptococcus pneumoniae
Haemophilus influenzae

Culture Work Up

- ASM Clinical Microbiology Procedure Handbook, 5th Ed.

Report in significant amounts, even if not predominant organism
Moraxella catarrhalis
Staphylococcus aureus
Pseudomonas aeruginosa
Stenotrophomonas maltophilia
Acinetobacter spp.
Burkholderia spp.

Report in significant amounts, AND the predominant organism
Neisseria meningitidis
Non-*pyogenes* beta-hemolytic streptococci (e.g., *S. agalactiae*)
Single species of Gram negative bacilli
Corynebacterium spp (Non-*diphtheria)*

Significant amounts
Qualitative culture: >3+ growth, or 90% pure
Quantitative culture: $>10^4$ CFU/ml for BAL
$>10^3$ CFU/ml for protected brush

Culture Work Up

- ASM Clinical Microbiology Procedure Handbook, 5th Ed.

Include in Normal Flora
Viridans streptococci and/or nonpathogenic Neisseria; other coryneform bacilli, coagulase-negative staphylococci; Rothia; anaerobes; Haemophilus species (not H. influenzae); Eikenella; Aggregatibacter; Capnocytophaga; Moraxella (not M. catarrhalis); enterococci; Candida spp.; and insignificant numbers of S. aureus, Gram-negative bacilli, and N. meningitidis

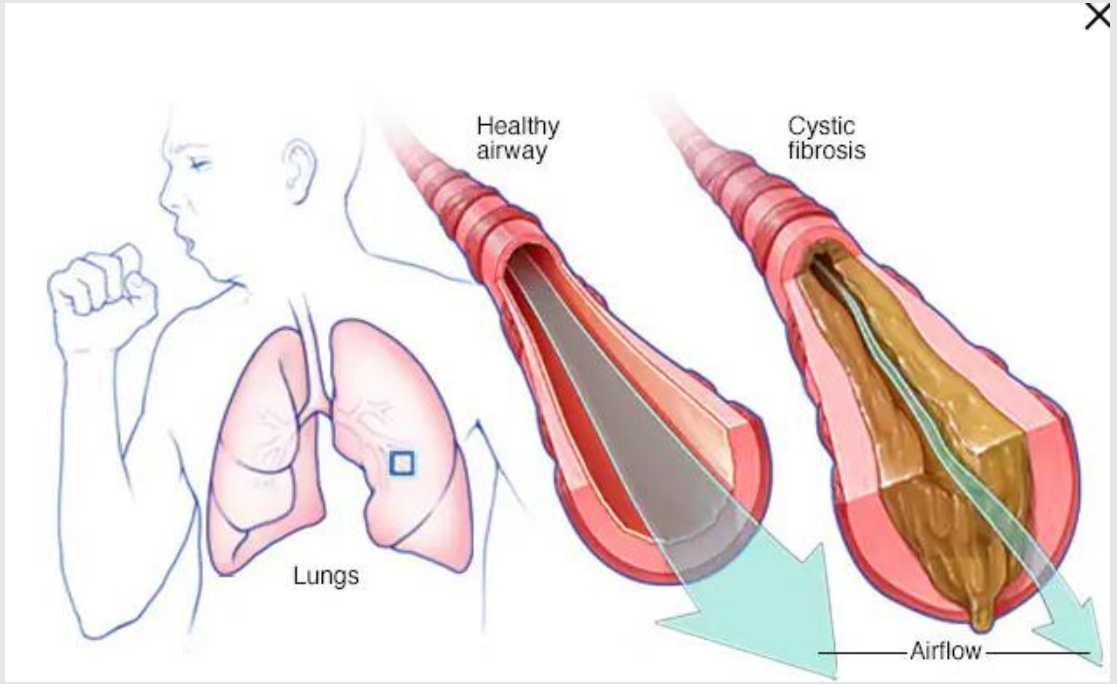
Culture Work Up

- ASM Clinical Microbiology Procedure Handbook - Changes

4th Ed	5th Ed
Neisseria meningitidis	
Report if significant, even if not predominating	Report in significant amounts, AND the predominant organism
Staphylococcus aureus	
Report in significant amounts, AND the predominant organism	Report if significant, even if not predominating
P. aeruginosa, S. maltophilia, Acinetobacter, Burkholderia	
Removal of “inpatient only” designation	

Cystic Fibrosis

- Rejection criteria
  - DO NOT reject specimen collected via bronchoscopy
  - No more than one specimen per month
  - Do not reject based on Gram stain criteria
- Culture processing
  - Add plate for *S. aureus* (mannitol salt or chromagenic agar)
  - Selective media for *Burkholderia cepacia*



Cystic Fibrosis

Report in Any Amount
S aureus
P. aeruginosa
Stenotrophomonas
Achromobacter
B. cepacia
Other non-glucose fermenting Gram negative bacilli
Mold
Aerobic actinomycetes
Mycobacteria, rapid growers

Predominant or significant
H. influenzae
Enterobacterales
S. pneumoniae

Predominant/significant growth

- At least one quantitation higher that background flora
- ≥10^4 CFU/ml for BAL specimen cultures
- ≥10^3 CFU/ml for protected brush specimen

Quantitative Cultures - BAL

- Set up
  - Serial dilution
  - Calibrated loops

- Reporting
  - Quantitative reporting
  - Thresholds established to delineate organisms as pathogens vs. oral flora (10^4 for BAL, 10^3 for protected brush)

- Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016 Sep 1;63(5):e61-e111.

Quantitative Cultures - BAL

Clinical utility

- Can increase specificity of diagnosis

Pros	Con
Significantly more antibiotic modifications/de-escalation	No significant difference in clinical outcomes (day on vent, LOS, antibiotic usage)
Potential to identify slower growing pathogens	Preanalytical variables
Rule out pneumonia	Add complexity to processing and interpretation
No consensus if quantitative cultures are better than semiquantitative cultures	

- Baselski V, Klutts JS, Baselski V, Klutts JS.2013.Point-Counterpoint: Quantitative Cultures of Bronchoscopically Obtained Specimens Should Be Performed for Optimal Management of Ventilator-Associated Pneumonia. J Clin Microbiol51:.740-744

Trivia Break

This year, the Milwaukee Brewers started the season with a four game winning streak. What is the longest winning streak in Brewers history to start a season?

A. 4
B. 7
C. 9
D. 13

2013 & 2014 – 9 games
1987 – 13 games

Molecular Testing-Multiplex panels

Viral panels

Pros	Cons
Reduction in time to result	High patient charges
Reduction in hospital length of stay	Positive result may not affect patient management
Appropriate patient isolation	Detection of colonized viruses
Appropriate patient de-isolation	
Reduction in antibiotic use	
Cost effective???	

Esposito S, Mencacci A, Cenci E, Camilloni B, Silvestri E and Principi N (2019) Multiplex Platforms for the Identification of Respiratory Pathogens: Are They Useful in Pediatric Clinical Practice? Front. Cell. Infect. Microbiol. 9:196

Medical appropriateness and economics of nucleic acid amplification testing for infectious diseases. Clin Biochem. 2023 Jul:117:48-52

Rapid multiplex PCR for respiratory viruses reduces time to result and improves clinical care: Results of a systematic review and meta-analysis. Journal of Infection 86 (2023) 462–475

Molecular Testing – Multiplex panels

Viral panels

Target	Would Results Change Clinical Management?	Situations in Which Test Results Might Be Useful
Adenovirus PCR, Qual	Unlikely	only to establish etiology in immunocompromised patients; treatment indications limited
Coronavirus HKU1 PCR	Unlikely	only to establish etiology in immunocompromised patients
Coronavirus NL63 PCR	Unlikely	
Coronavirus 229E PCR	Unlikely	
Coronavirus OC43 PCR	Unlikely	
Human metapneumovirus PCR	Unlikely	only to establish etiology in immunocompromised patients
Human Rhinovirus/Enterovirus PCR	Unlikely	only to establish etiology in immunocompromised patients
Influenza A PCR	***Possibly***	*treatment available but only indicated for early or severe disease; may be useful for public health purposes as prophylactic therapy for close contacts available*
Influenza B PCR	***Possibly***	
Parainfluenza Virus 1 PCR	Unlikely	only to establish etiology in immunocompromised patients
Parainfluenza Virus 2 PCR	Unlikely	
Parainfluenza Virus 3 PCR	Unlikely	
Parainfluenza Virus 4 PCR	Unlikely	
Respiratory Syncytial Virus	Unlikely	establish etiology; infants receiving monthly prophylaxis can stop once diagnosis is made
Bordetella pertussis	Unlikely	if diagnosis made within 3 weeks of symptom onset (rare), treatment with antibiotics may decrease disease transmission but does not alter clinical course
Chlamydophilia pneumoniae	Unlikely	treatment available, but usually included in empiric antibiotic regimens anyway
Mycoplasma pneumoniae	Unlikely	treatment available, but usually included in empiric antibiotic regimens anyway

Doron S, Horowitz G. Medical appropriateness and economics of nucleic acid amplification testing for infectious diseases. Clin Biochem. 2023 Jul;117:48-52

Molecular Testing – Syndromic panels

Table 1 Syndromic panels for the diagnosis of community-acquired respiratory infections

Diagnostic assay	Microorganisms detected	Type of sample	Turn-around-time
Verigene, Luminex	6 viruses 3 bacteria	Nasopharyngeal swab	<2h
NxTAG, Luminex	18 viruses 3 bacteria	NF, BAL, nasal aspirate, TA, sputum, FA	5-6 h
DiagCore,Quiagen	19 viruses 3 bacteria	All types of samples	1 h
Clart Pneumovir 2, Genomica	18 viruses	NF, nasopharyngeal lavage, BAL	2 h
Xpert Xpress SARS-CoV-2/Flu/RSV, Cepheid	4 viruses	NF, nasal exudate, nasal lavage/aspiration	36 min
ePlex Respiratory Pathogen 2, GenMark	16 viruses 2 bacteria	NF	90 min
Unyvero, Curetis	20 bacteria *P. jirovecii* 17 resistance markers	Sputum, TA, BAL	<5 h
Anyplex II RV16, Seegene	16 viruses	NF, nasopharyngeal aspirate, BAL	4,5 h
RespiFinder 2SMART, PathoFinder	20 viruses 4 bacteria	Sputum, BAL, NF, nasopharyngeal aspirate	2,5 h
bioFire FilmArray 2.0 Pneumonia plus, bioMerieux	18 bacteria 9 viruses 7 Resistance markers	Sputum, TA, BAL	<1 h
bioFire Respiratory Panel 2.1 Plus, bioMerieux	4 bacteria 19 viruses	NF	45 minutes

NF: nasopharyngeal exudate. TA: tracheal aspirate. FA: pharyngeal exudate. BAL: bronchoalveolar lavage

- 63.3% increase of specimen reported as positive.
- Potential of antibiotic adjustment in 70% of patients.

Burillo A, Candel FJ, Canut-Blasco A. Value of syndromic panels in the management of severe community-acquired pneumonia. Rev Esp Quimioter. 2022 Apr;35 Suppl 1(Suppl 1):15-20

Those Less Frequent…

Pneumocystis jirovecii

- Originally classified as protozoan
- Reclassified as fungus in 1988
- Found in environment and passed person to person
- Most healthy children exposed by age 4.
- First appeared in orphanages during WWII
- Widespread during HIV epidemic in the 1980s
- Increasing among non-HIV patients
  - Hematologic malignancies
  - Solid tumors
  - Long-term high dose steroids use
  - Stem cell transplants
  - Patient on immunosuppressive drugs
- High mortality
  - 80-93% in HIV patients
  - 40-71% in non-HIV patients

Bateman M, Oladele R, Kolls JK. Diagnosing Pneumocystis jirovecii pneumonia: A review of current methods and novel approaches. Med Mycol. 2020 Nov 10;58(8):1015-1028. doi: 10.1093/mmy/myaa024.
CDC. Laboratory Identification of Parasites of Public Concern, Pheumocystis. CDC - DPDx - Pneumocystis

Pneumocystis jirovecii

- Diagnosis – Samples
  - Lower Respiratory Tract
    - Sputum (induced)
    - Bronchoalveolar lavage (BAL)
    - Oral washings
    - Nasopharyngeal aspirate
    - Blood/serum
    - Urine

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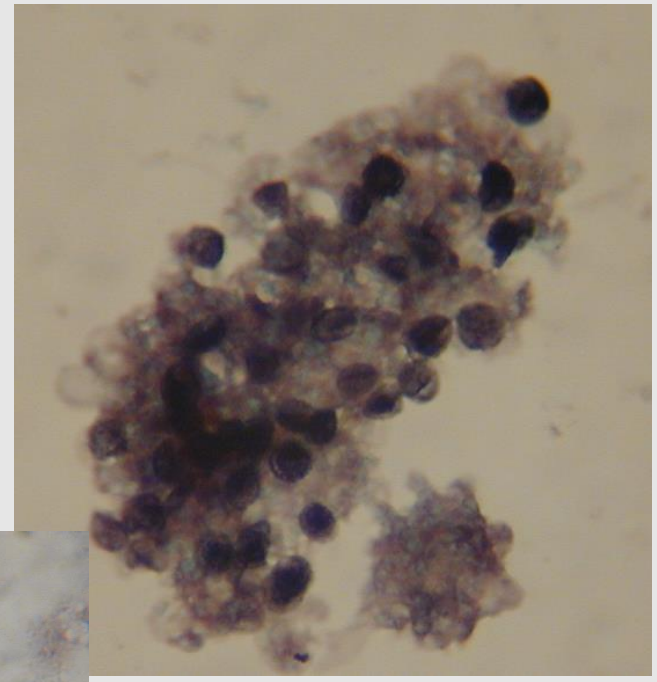
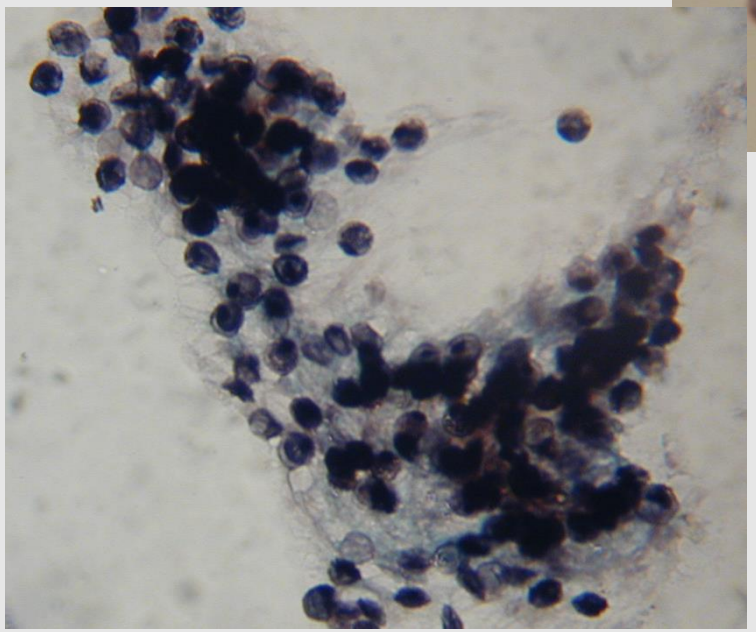
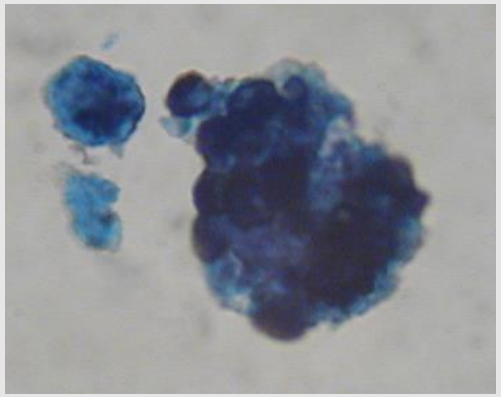
Pneumocystis jirovecii

- Diagnosis – Testing
- Stains
  - Giemsa, Wright, Silver (GMS), calcofluor white
  - Positive results confirms diagnosis
  - Negative result does not rule out presence of PCP
  - Reliant upon sample quality
  - Reader competency







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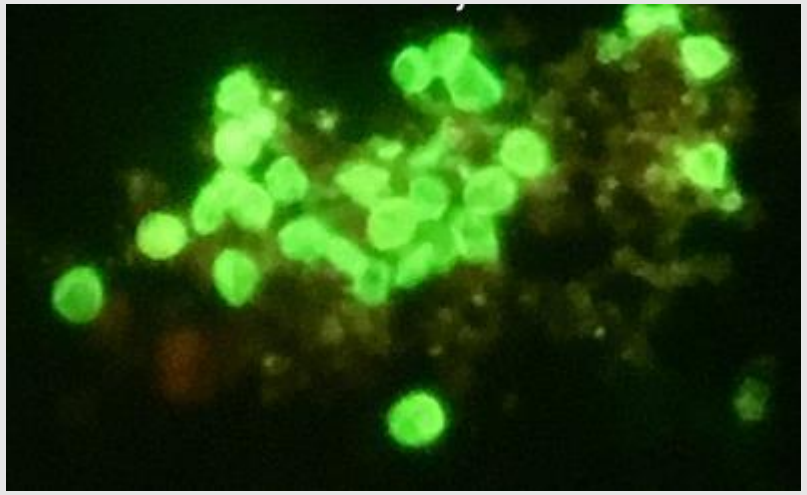
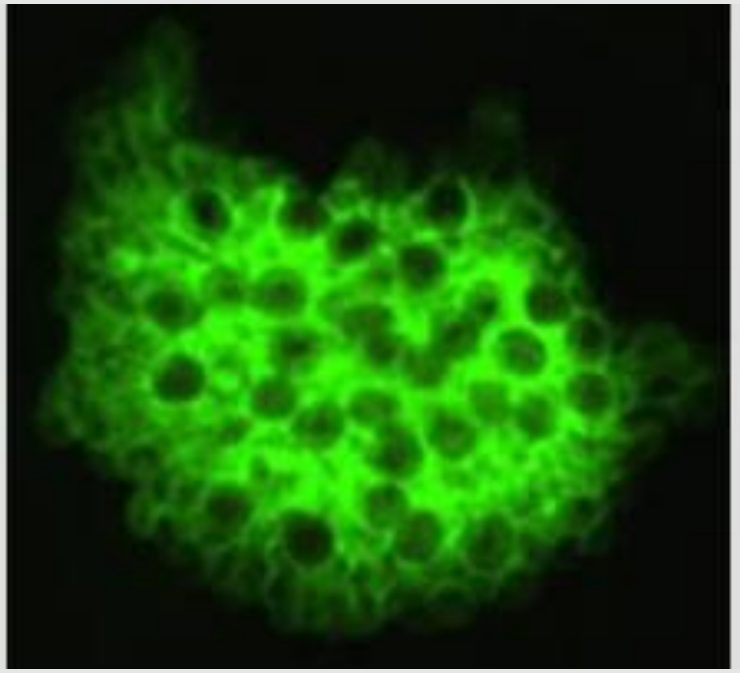
Pneumocystis jirovecii

- Diagnosis – Testing
- Immunofluorescent Stain
  - Higher sensitivity and specificity than traditional stains
  - Easier to perform, easier to interpret





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Pneumocystis jirovecii

- Diagnosis – Testing
- Molecular methods
  - PCR
    - High sensitivity and high specificity
    - Both HIV and non-HIV patients
  - Loop-mediated isothermal amplification (LAMP)
    - Better than stains
    - Detection rates similar to PCR
  - Flow cytometry
  - Antibody assays
    - Different immune response
    - Past exposure or infection
  - Blood antigens

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Trivia Time

- What was the name of the stadium the Milwaukee Brewers played in before moving to Miller Park in 2003?

A. City Stadium
B. County Stadium
C. Athletic Field
D. Valley Park

Those Less Frequent…

Core Pathogens	Opportunistic Pathogens	Common Indigenous Flora
Bacillus anthracis	*Acinetobacter spp.*	Capnocytophaga
Bordetella spp.	*Actinomyces spp.*	Coagulase negative staph.
Cryptococcus neoformans	*Burkholderia cepacia*	*Corynebacterium spp.*
Fracisella tularensis	β-hemolytic strep C or G	*Enterococcus spp.*
Haemophilus influenzae	Enterobacterales/GNR	*Eikenella*
Legionella spp.	*Moraxella catarrhalis*	*Haemophilus spp not influenzae*
Molds	*Neisseria meningitidis*	*Micrococcus*
Mycobacterium	*Pasteurella spp.*	*Neisseria spp. not listed*
Neisseria gonorrhoeae	*Pseudomonas aeruginosa*	*Rothia spp.*
Nocardia	*S. aureus*	Strep. anginosus group
Streptococcus agalactiae	*Streptococcus agalactiae*	Streptococcus viridans group
S. pneumonia	*Stenotrophomonas maltophilia*	Yeast not Cryptococcus
Streptococcus pyogenes		

Francisella tularensis

- Humans infected via zoonotic exposure
  - Tick and deer fly bites
    - Ulceroglandular
    - Most common form of tularemia
  - Skin contacted with infected animals
    - Rabbits
    - Prairie dogs/rodents
    - Muskrats
    - Domestic cats
- Dust/aerosol exposure during farming/landscaping
    - Causes Pneumonic tularemia, most serious form

Francisella tularensis

Pathogen	Identification Guide	Sensitivity	Reporting Guidelines
Francisella tularensis	Scant Growth on BAP No Growth on MAC Slow growing Grey/White Opaque, entire/smooth Shiny surface Tiny faint GNCB Oxidase Negative BSC II	No Sensitivity	Follow LRN reporting guidelines

Nocardia spp.

- More common amongst weak immune system
  - Diabetes
  - Cancer
  - HIV/AIDS
  - Alcoholism
  - Transplant recipient
  - Male (3:1)
- Generally an environmental transmission
  - Standing water, decaying plants & soil
- Exposure through inhalation, cut/scrape, contaminated medical equipment or post-surgical
- Most often a Lung infection
  - If left untreated, can spread to other parts of the body including brain
  - 44% of brain/spinal infections are lethal

Nocardia spp. (cont.)

- Don’t include specific media; if found report
- If seen in Gram stain, attempt to isolate
- If suspected, providers should notify the lab
- Symptoms consistent with:
  - Tuberculosis
  - Community acquired pneumonia
  - Fungal pneumonia
  - Lung cancer

Nocardia spp. (cont.)

Pathogen	Identification Guide	Sensitivity	Reporting Guidelines
Nocardia spp.	Branching variable GPB Dry adherent colony, chalky Various colonial colors Modified acid-fast	Send out for Sensitivity if requested	Report any amount, hold plates for 7 days

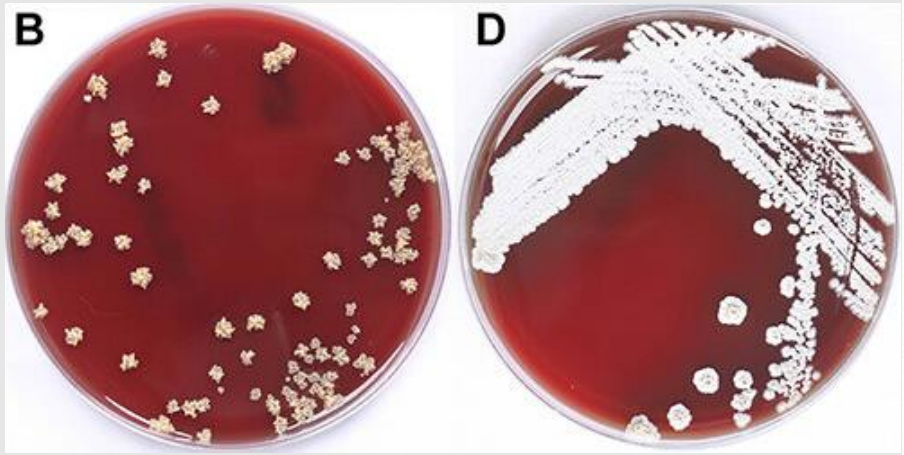


Figure 1: *Nocardia spp.*

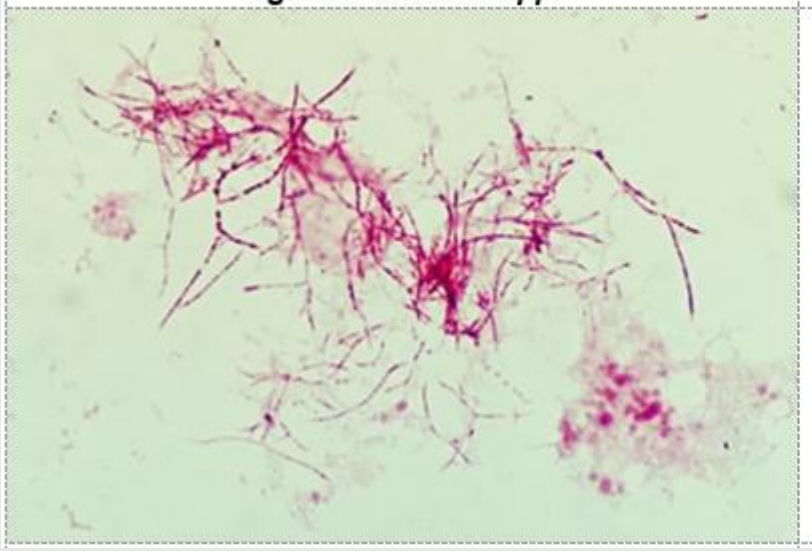
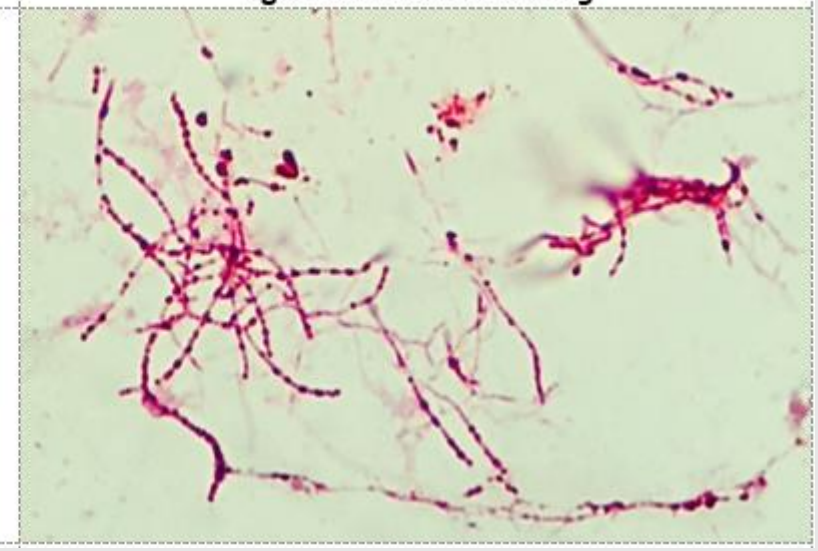


Figure 2: *Beaded staining*



Yeasts and Molds

- May represent normal oral flora:
  - *Candida spp.*
- May represent common environmental saprophytes or serious infection:
  - *Cryptococcus neoformans*
  - *Aspergillus spp.*
  - Zygomycetes
- Generally associated with infection:
  - Dimorphic fungi (e.g. *C. immitis*)

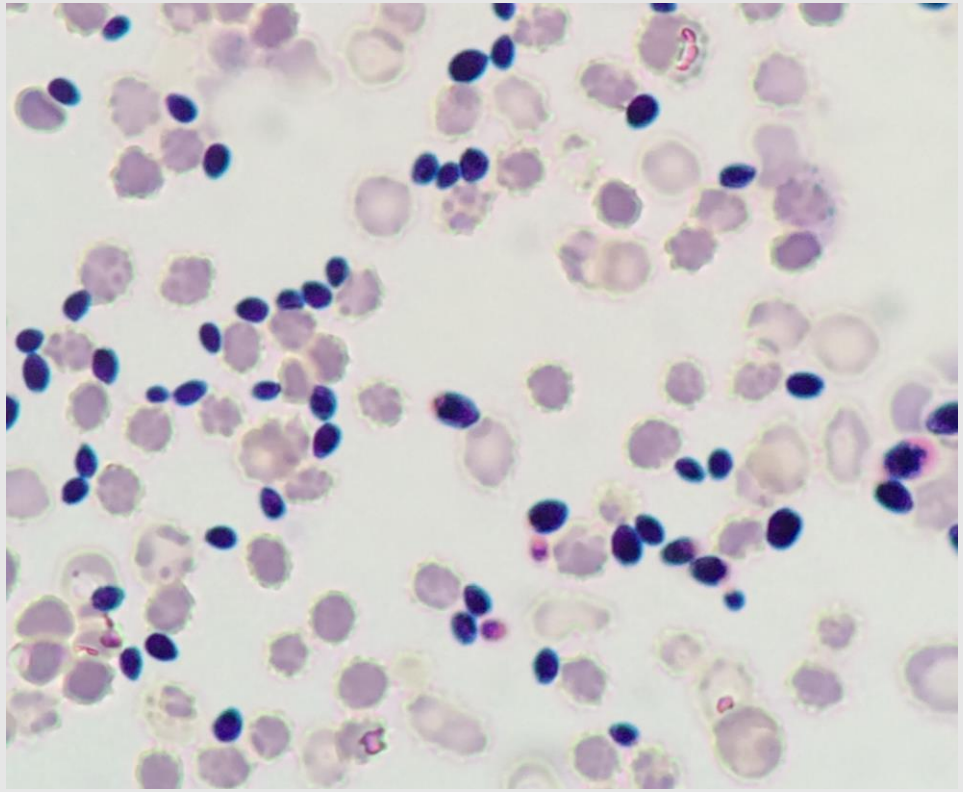
If a yeast is the predominant organism in a sputum specimen and no other obvious pathogens were present would you…

A. Ignore it as part of normal flora
B. Rule out *Cryptococcus* and then ignore it
C. Rule out *Cryptococcus* and report generically (e.g. Yeast not *Cryptococcus*)
D. Identify it and report to at least genus
E. Identify it to species and perform AST

Yeasts

Common respiratory yeasts you may encounter:

- *Candida spp.*
- *Cryptococcus neoformans*
- Dimorphic fungi (e.g. *B. dermatitidis*)



Candida spp.

Common Microbiota	Identification Guide	Sensitivity	Reporting Guidelines
Other Yeast	Medium, Creamy No or Poor growth on MAC Catalase + Calcofluor white Urease θ Instrument ID – If Indicated	None	Do not report unless directed by ID. Candida are not a cause of pneumonia except possibly in oncology or lung transplant patients.

- Part of normal oral flora
- Some circumstances may be necessary to rule out *Candida auris*

Cryptococcus neoformans

Core Pathogen	Identification Guide	Sensitivity	Reporting Guidelines
Cryptococcus neoformans	Yeast colonies Calcofluor white Urease + Instrument ID	No Sensitivity	Report any amount

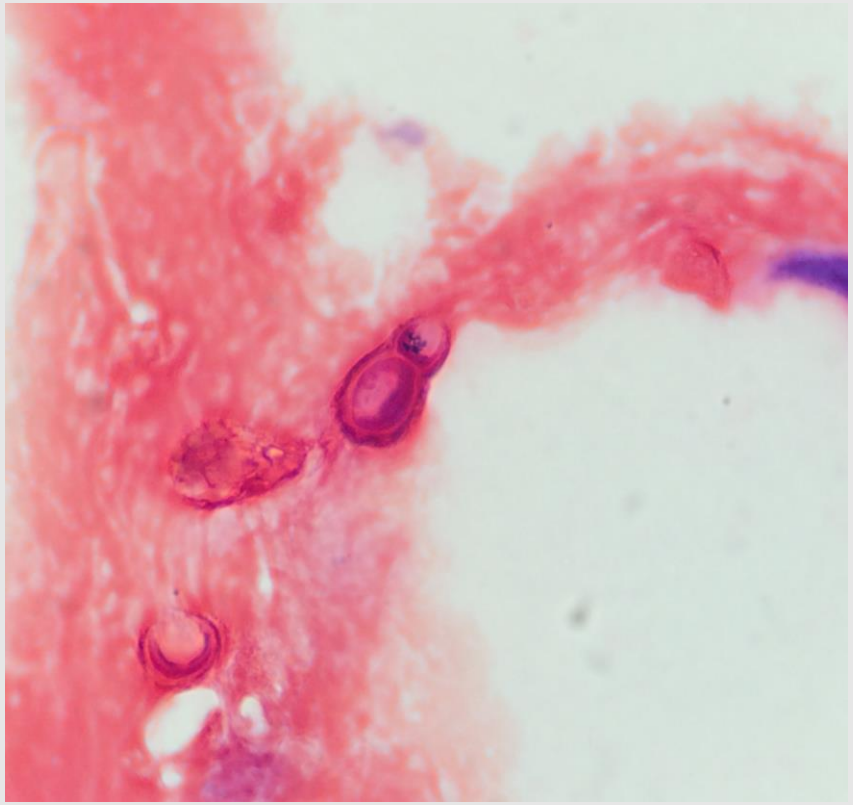
- Commonly associate with pigeon droppings
- Associated with:
  - Pneumonia
  - Meningitis
  - Disseminated disease
- Most important in immunocompromised

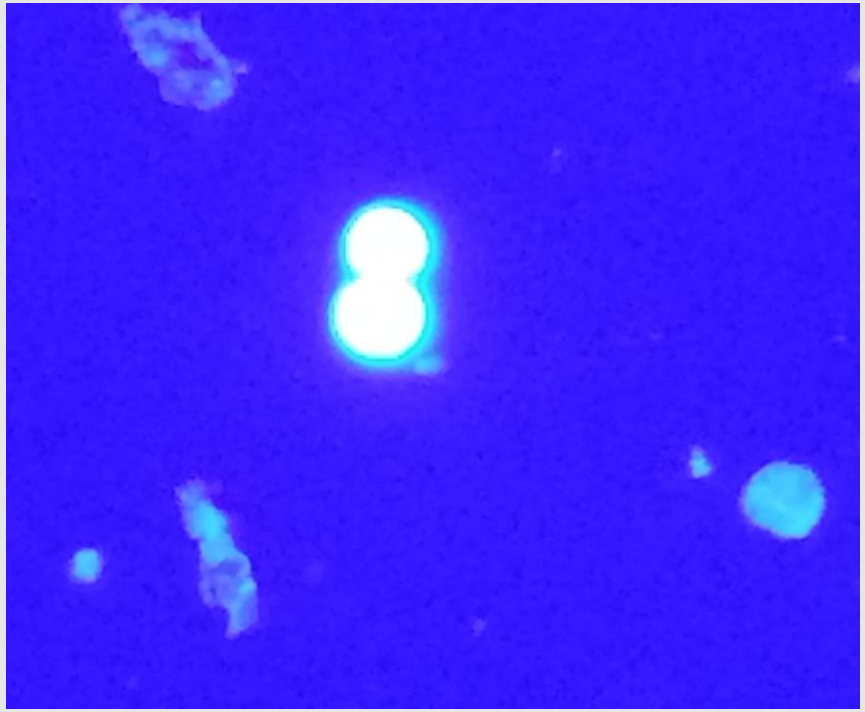
Molds

- **Common molds you may encounter include:**
  - Environmental saprophytes (*Aspergillus spp.*)
  - Dimorphic fungi (e.g. *Blastomyces dermatitidis*)
  - Zygomycetes (e.g. *Rhizopus*)
- With the exception of dimorphs; primarily an issue in immunocompromised
- Can be contaminants or life threatening

Molds

- If seen in Gram Stain, add fungal media
  - Not to be confused with Yeast with pseudohyphae!
  - Perform Calcoflour white/KOH stain to assist discrimination



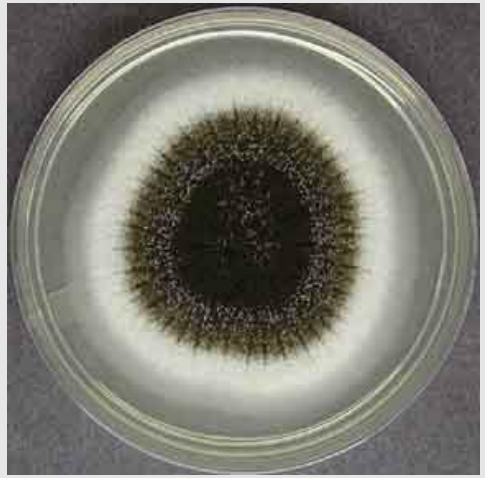


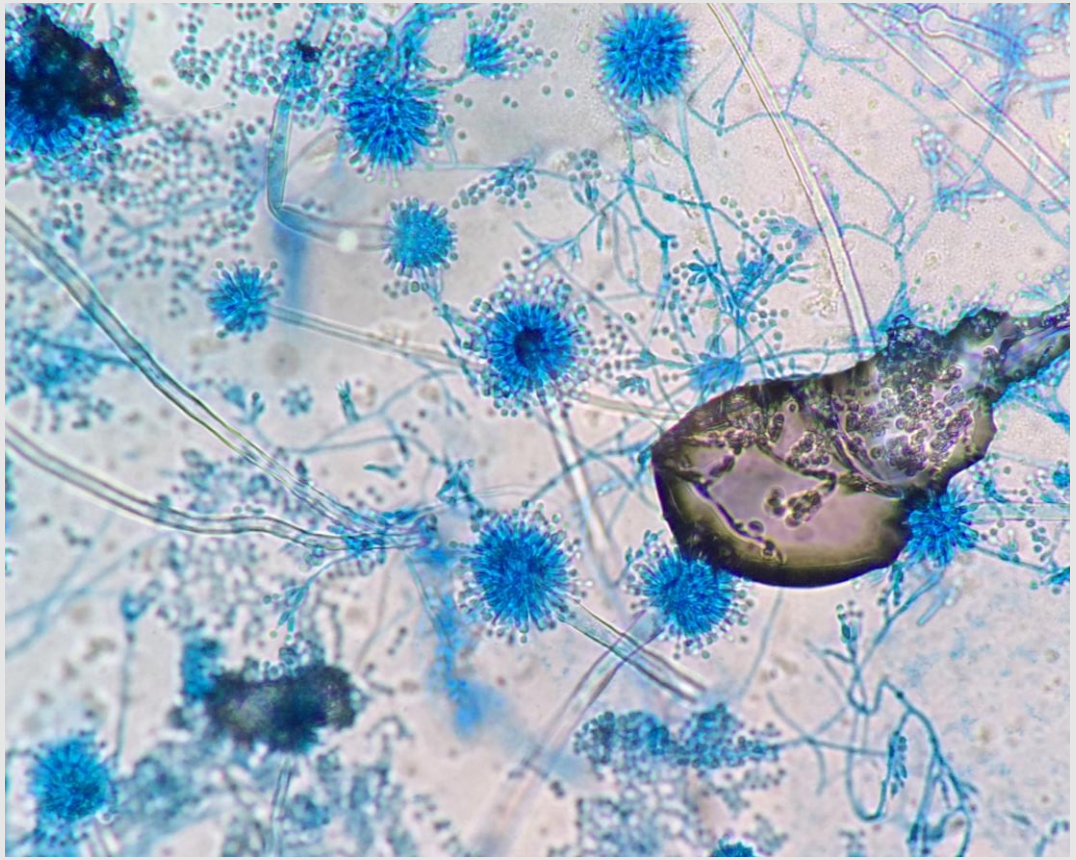
Molds

Pathogen	Identification Guide	Sensitivity	Reporting Guidelines
Molds	Dry or fluffy colonies Gram Stain BSC II Calcofluor White/KOH prep Tape plates & Hold 1 Week	No Sensitivity	“Fungus isolated, request further ID if warranted”

Aspergillus spp.

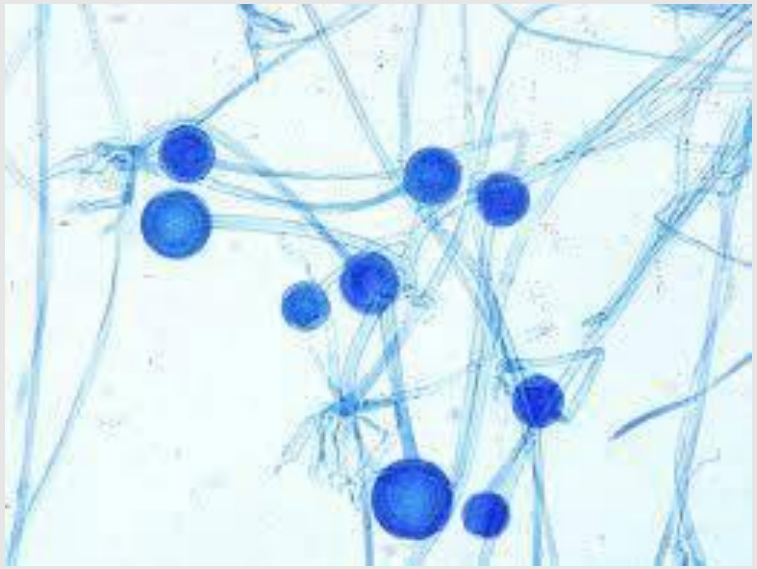
- Common environmental mold, but can cause
  - Aspergilloma (fungus ball)
  - Allergic bronchopulmonary aspergillosis
    - Asthmatic patients
    - Cystic Fibrosis patients
  - Invasive pulmonary aspergillosis
    - Weakened immune systems
    - Healed cavitary lesions

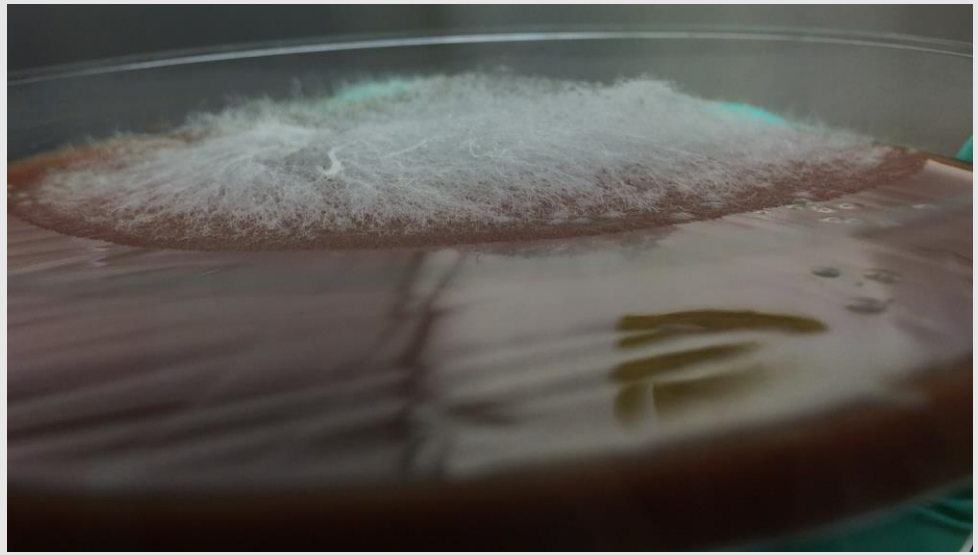




Zygomycetes

- Common environmental mold
- Primarily infects immunocompromised
- Very difficult to treat; surgical intervention is often required
- Report in any level





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