Lower Respiratory Tract Infection

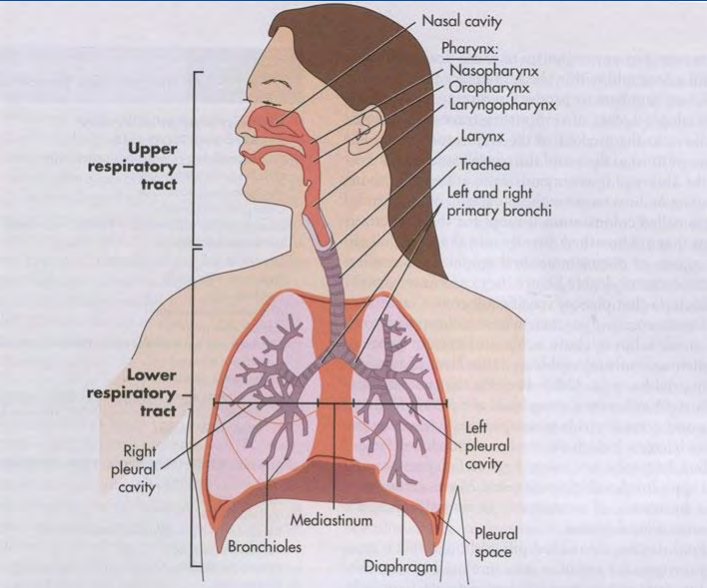


FIGURE 56-1 Anatomy of the respiratory tract, including upper and lower respiratory tract regions.

- Clinical Diseases and etiologic agents

-Sample Collection and transport

-Direct Smear

(interpretation and Report)

-Culture methods

(interpretation and Report)

“ The culture of lower respiratory specimens may result in more unnecessary microbiologic effort than any other type of specimen.”
Raymond C Bartlett

Lower Respiratory Tract Infections Epidemiology

- Pneumonia is the sixth leading cause of death in US
- Increasing numbers of patients at risk
  - Aging population
  - Increase in patients with immunocompromising conditions

Lower respiratory infections

- Overtreatment has lead to resistance
  - Multidrug resistant *Streptococcus pneumoniae*
  - Resistance among hospital acquired pathogens such as *Acinetobacter, Pseudomonas aeruginosa E.coli K.pneumonia (ESBLs) MRSA* and others

Categories of Lower Respiratory Tract Infections

- Acute bronchitis
- Community acquired pneumonia
- Hospital acquired pneumonia
- Pneumonia in the immunocompromised host

Acute Bronchitis

- Part of or preceded by an URTI
- Sputum is often clear at the onset but may become purulant
- Etiologic agents:

- adults viral
- Infant and preschool children: bordetella pertusis

Chronic bronchitis

Common condition affecting about 10-30% adults

Coughing up sputum on most days during at least 3 consecutive months for more than 2 successive years

Viruses are frequent causes

Non encapsulated H.influ/S.pneumoniae/M.catharalis

Bronchiolitis

Human metapneumovirus

RSV 40-80% cases

Pneumonia

*Children 2 month to 5 years 80% viral (RSV/Parainflu/Adeno/Metapneumovirus/C.pneumoniae/M.pneumoniae)

*Children 5-14 years 70 % viral and up to 80% of cases mixed infection

*Young adults<30 years M.pneumoniae/Influ/C.pneumoniae

TABLE 1. Most common pathogens implicated in lower respiratory tract syndromes and their relative contributions[a]

Disease and pathogen	% of cases
Acute bronchitis	
Respiratory viruses[b]	90
Bordetella pertussis-Bordetella parapertussis	5–10[c]
Mycoplasma pneumoniae	5–10[c]
Chlamydia pneumoniae	5–10[c]
Community-acquired pneumonia	
Streptococcus pneumoniae	66
Haemophilus influenzae	1–12
Legionella species	2–15
Mycoplasma pneumoniae	2–14
Klebsiella species	3–14
Enteric gram-negative bacilli	6–9
Staphylococcus aureus	3–14
Chlamydia species	5–15
Influenza virus	5–12
Hantaviruses	<1–2
Other viruses	<1–12
Mycobacterium tuberculosis	<1–10
Moraxella catarrhalis	<1–2
Unknown	23–49

Hospital Acquired Pneumonia

*5-10 % hospital in – patients develop infection during administration to ICU.

*80% of hospital associated pnemonia is linked with mechanical ventilation(VAP)

*10-30% of critical care patients develop VAP

*VAP is the most common and fatal infection of ICU

*VAP may account for up to 60% of all HAI

Hospital-acquired pneumonia	
Gram-negative bacilli	
Pseudomonas aeruginosa	16
Enterobacter species	11
Klebsiella pneumoniae	7
Other enteric gram-negative bacilli	9
Acinetobacter	3
Legionella species	0–2
Haemophilus influenzae	0–2
Other	0–10
Gram-positive cocci	
Staphylococcus aureus	17
Streptococcus pneumoniae	2–20
Other	2–5
Anaerobes	10–20
Fungi	0–10
Mixed	13–54

Organism isolated from tracheal tube aspirates in Milad Hospital

organism	No(%)
K. pneumoniae	67(20)
P. aeruginosa	62(18.5)
A. baumannii	60(18)
S. aureus	51(15.5)
S. marsescense	32(9.5)
E. coli	22(6.6)
Enterobacter spp	10(3)
Candida spp	5(1.5)
Others	26(7.7)

Rhbar and Hajia accepted for publication in ICHE

Aspiration pneumonia

Anaerob

S.aureus

Enterobacteriacae

Pseudomona

H.influ

Legionella

Acinetobacter

Moraxella

Chronic LRTI

*M.tuberculosis

*M.avium intracellular

*Actinomyces/Nocardia

*Fungi

Cystic Fibrosis

*Mucoid Pseudomona

*S.aureus

*H.influ

*Burkhholderia cepacia

RSV/Flu/Aspergylus

Immunocoprommised patients

*Neoplasm

*Transplant Recipents

*HIV

Community Acquired Pneumonia Diagnosis

Available Test Methodologies

- Sputum Gram stain and culture
- Blood cultures
- Serologic studies
- Antigen detection tests
- Nucleic acid amplification tests

Specimen collection

Sputum Collection

- Proper patient instruction
  - Food should not have been ingested for 1-2 h prior to expectoration
  - The mouth should be rinsed with saline or water
  - Avoid using mouth wash solution
  - Patient should breathe and cough deeply
  - Patient should expectorate into a sterile container
- Transport container immediately to lab
- Perform Gram stain and plant specimen as soon as possible

Sputum collection

- Sputum of less than 2ml should not be processed unless obviously purulent
- Only 1 sputum per 24hr .submitted
- Transportation in <2 hr is recommended with refrigeration if delays anticipated.
- Handle all samples using universal precautions.
- Some scoring system should be used to reject specimen that re oral contamination.

Induced sputum

*Patients who are unable to produce sputum may be assisted by respiratory therapy technician. Aerosol induced specimen are collected by allowing the patient to breath aerosolized droplets of a solution of 15% sodium chloride and 10% glycerin for approximately 10 minute . Obtaining such specimen may avoid the need for a more invasive procedures ,such as bronchoscopy or needle aspiration, in many cases.

*Specimen resemble saliva

*Should be accepted in the laboratory without prescreening

*High diagnostic value in pneumocystis,mycobacterium and fungi

Gastric aspiration

- The gastric aspiration is used exclusively for isolation of acid-fast bacilli and may be collected from patients who are unable to produce sputum, particularly young children. The relative resistance of mycobacteria allows them to remain viable for a short period. Gastric lavage must be delivered to the lab immediately so that the acidity can be neutralized. Specimen can be first neutralized and then transported if immediate delivery is not possible.

IDSA Practice Guidelines Diagnostic Tests for CAP

- Outpatients
  - Empiric therapy with a macrolide, doxycycline, or a fluoroquinolone
- Hospitalized patients with CAP
  - Gram stain and culture of sputum
  - 2 pretreatment blood cultures
  - Studies for Mtb, Legionella in select patients

Bartlett JG. 2003. *Clin Infect Dis* 37:1405-33

ATS Guidelines
Diagnostic Tests for CAP

- Empiric therapy for outpatients
  - Macrolide or tetracycline
- Hospitalized patients with CAP
  - 2 sets of pre-treatment blood cultures
  - Pleural fluid Gram stain/culture when appropriate
  - Studies for Legionella, Mtb, fungi in select patients
  - Sputum Gram stain/culture only if resistant or unusual pathogen is suspected
  - Avoid extensive testing

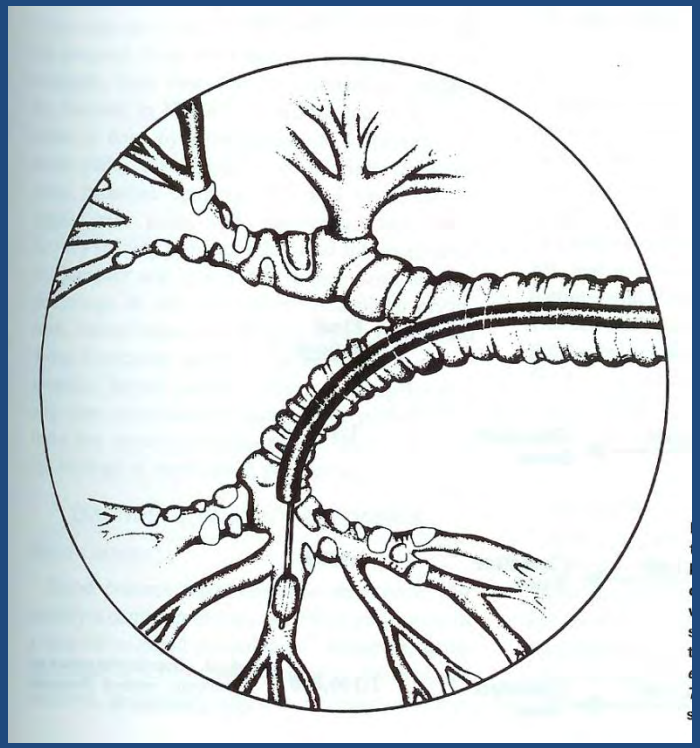
ATS. 2007. *Clin Infect Dis* 44:S27-72

Hospital Acquired Pneumonia Diagnosis

- American College of Chest Physicians: Clinical findings are not sufficient for definitive diagnosis
- Qualitative culture or endotracheal sputum has poor predictive value
- Endotracheal aspiration or suction samples should be treated as sputum by laboratory(GNB clonization)
- Bronchoscopy is recommended by many pulmonologists
  - Bronchial washes (contamination with URT flora)
  - Protected specimen brushing(Aspiration pneumonia)
  - Bronchoalveolar lavage specimens (BAL)
  - Transbronchial biopsy

Respiratory Specimens

- Protected Brush Specimen
  - To procure uncontaminated lower airway secretions
  - Brush within 2 catheters



Respiratory Specimens

- Bronchoalveolar Lavage (BAL)
  - Samples large area of the lung
  - Performed using a bronchoscope
  - 100 to 250 ml of saline injected
  - Injected saline along with secretions is collected by aspiration
- Transthoracic Aspiration
  - Involves percutaneous introduction of a needle directly into the infiltrate

Direct Gram Smear

Prescreening for adequacy

Specimen Acceptability Criteria Gram Stain Smear

*Expectorated sputum
*Endotracheal aspiration or suction

Sputum Gram Stain

Good quality specimens

- Quantify number and types of inflammatory cells
- Note presence of bronchial epithelial cells
- Concentrate on areas with WBCs when looking for organisms

Table 2. Screening criteria for microscopic examination of sputum samples in the diagnosis of lower respiratory tract infections.

Author	Method	Criteria for acceptance of sample (score)
Bartlett	SEC, PMN	Positive numerical score
Murray	SEC/LPF	<10
Geckler	SEC/LPF	<25
Van Scoy	PMN/LPF	>25
Barry	SEC, PMN, mucus	Positive numerical score
Heineman	PMN:SEC	>10

NOTE. LPF = low-power field; PMN = polymorphonuclear leukocytes; SEC = squamous epithelial cells.

TABLE 1. Summary of six published criteria for judging acceptability of sputum specimens

Author (reference)	Method[a]	Criteria for acceptability
Bartlett (3)	Assign + and − values; +2 if >25 WBC; +1 if 10–25 WBC; +1 if mucus seen; −2 if >25 EPI; −1 if 10–25 EPI	Any positive score (sum of + and − values assigned)
Murray and Washington (7)	Avg no. of EPI/LPF	<10 EPI/LPF
Geckler et al. (4)	Avg no. of EPI/LPF	<25 EPI/LPF
Van Scoy (8)	Avg no. of WBC/LPF	>25 WBC/LPF
Barry (1)	Assign + and − values: +3 if >150 WBC; +2 if 76–150 WBC; +1 if 1–75 WBC; −3 if >25 EPI; −2 if 16–25 EPI; −1 if 5–15 EPI	Any positive score (sum of + and − values assigned)
Heineman and Radano (6)	Avg ratio of WBC to EPI	>10 WBC/EPI

[a] Stained smears were examined under 100× magnification, and the number of squamous epithelial cells (EPI) or white blood cells (WBC) per LPF was determined.

WBC

Number of WBC may not relevant because

*many patients are severely neutropenic from disease or treatment

*Defect in effective inflamatory response such as Immunocompromosed patients (bone marrow implant)

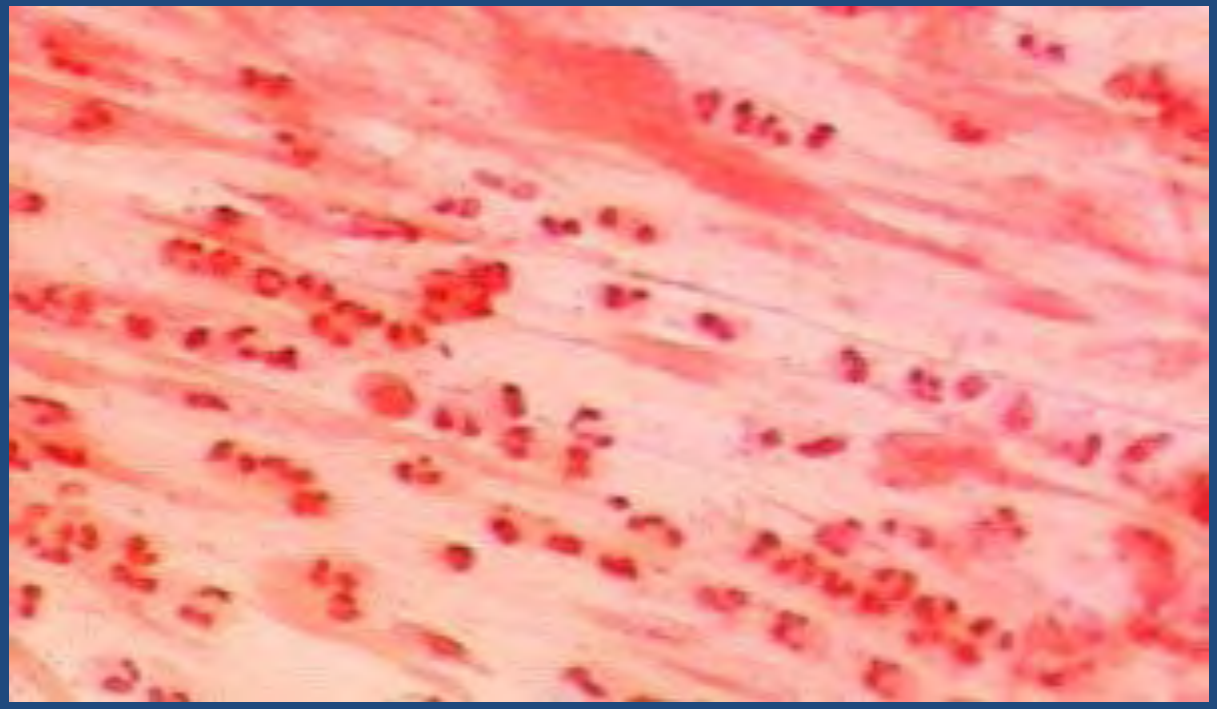
*Legionella pneumonia sputum

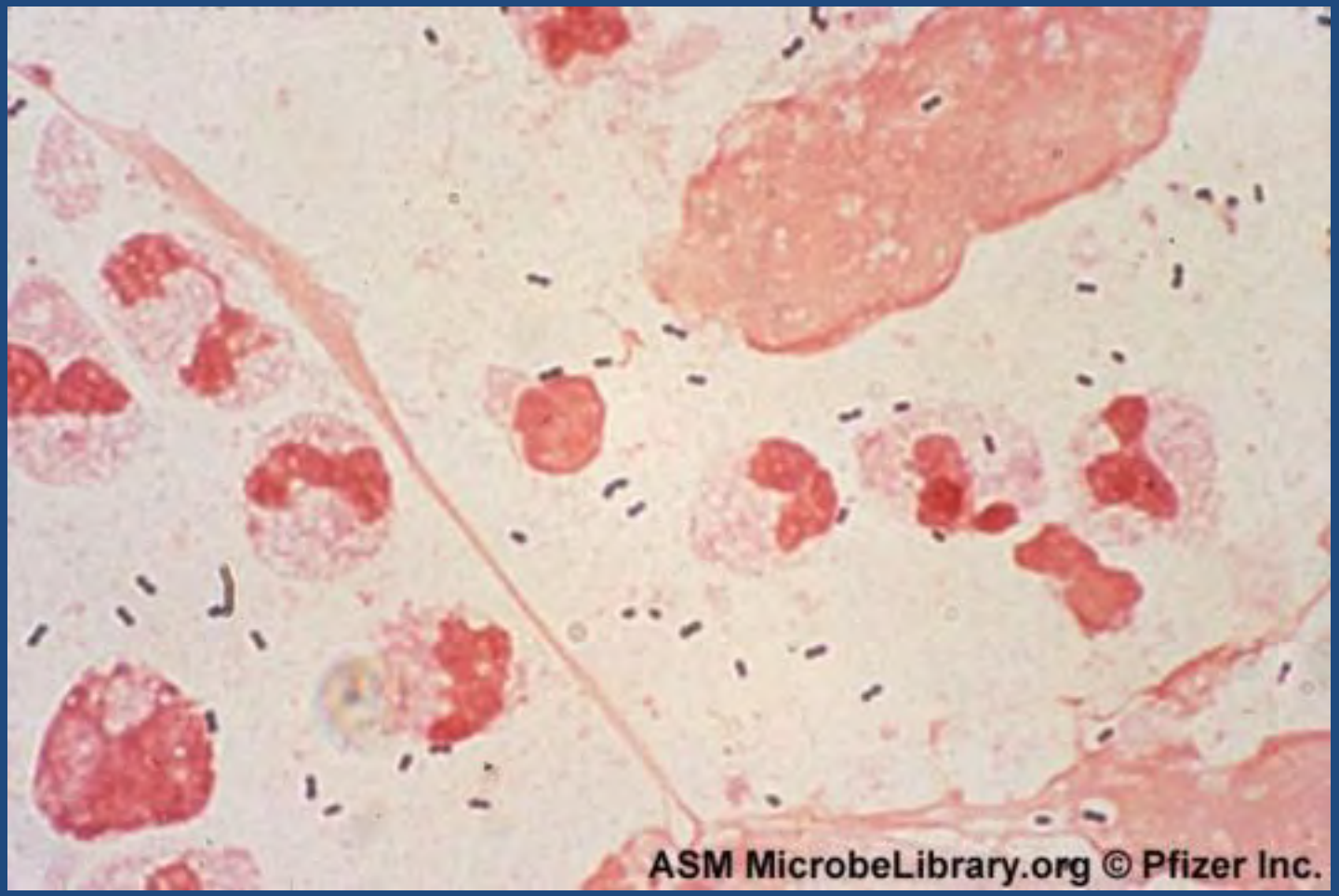
*Forign body (endotracheal catheter)

Sputum Gram Stain
Poor Quality

ASM MicrobeLibrary.org © Pfizer Inc.

Sputum Gram Stain
Good Quality

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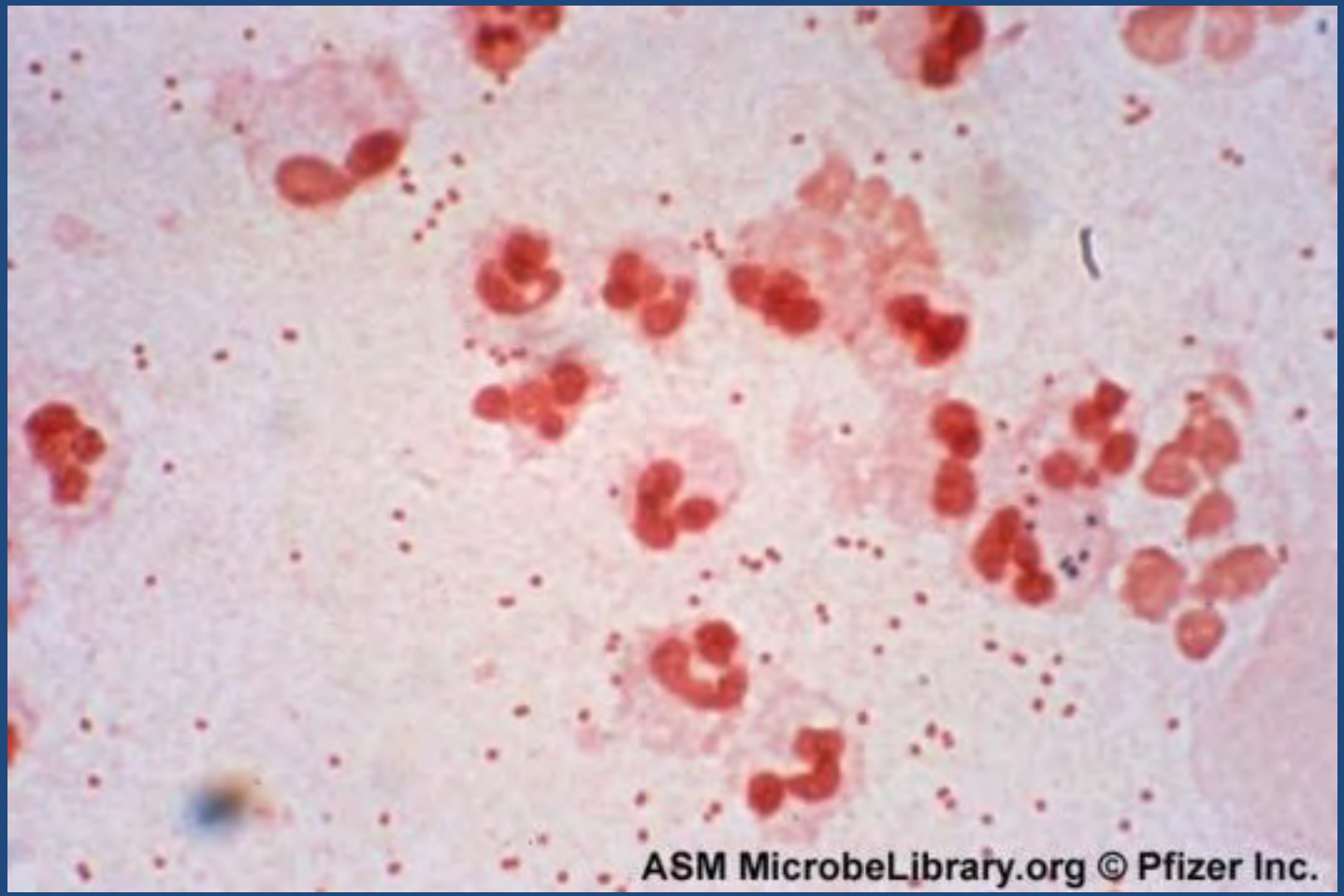
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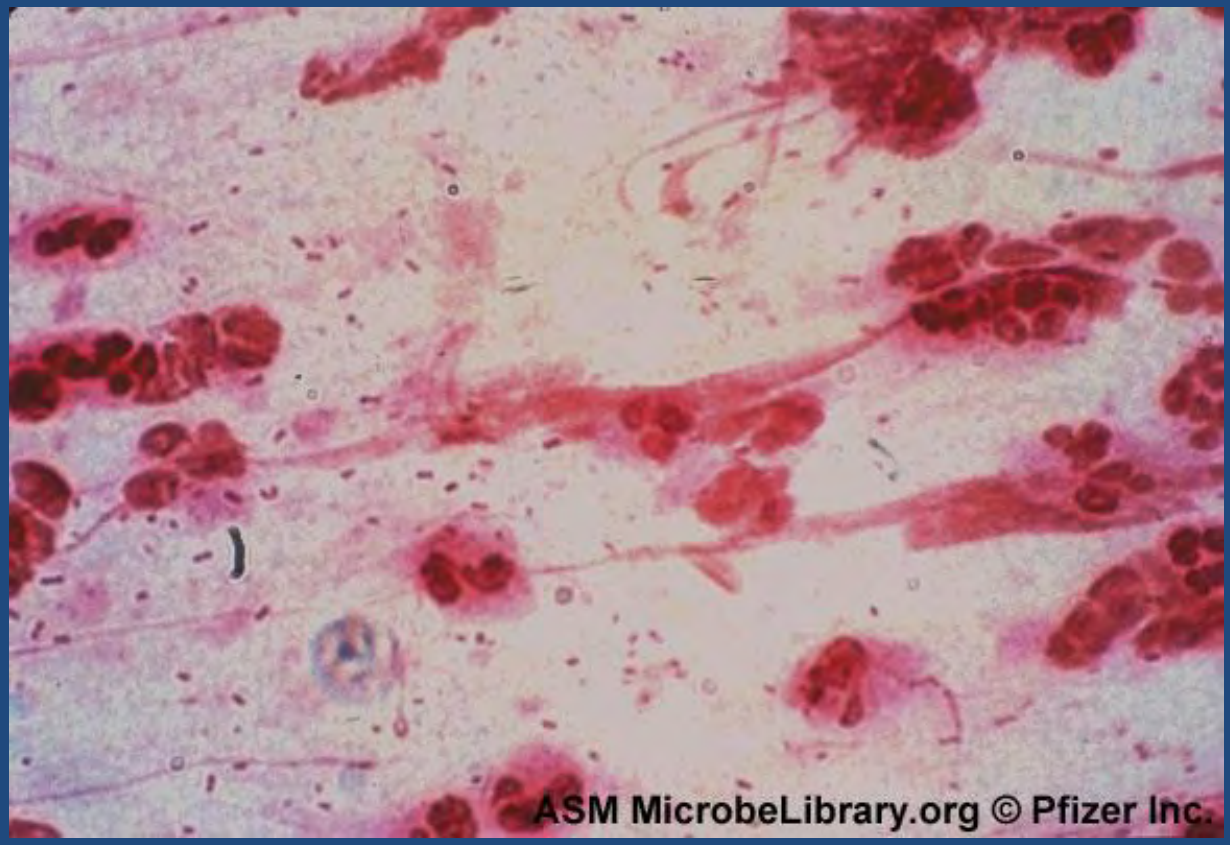
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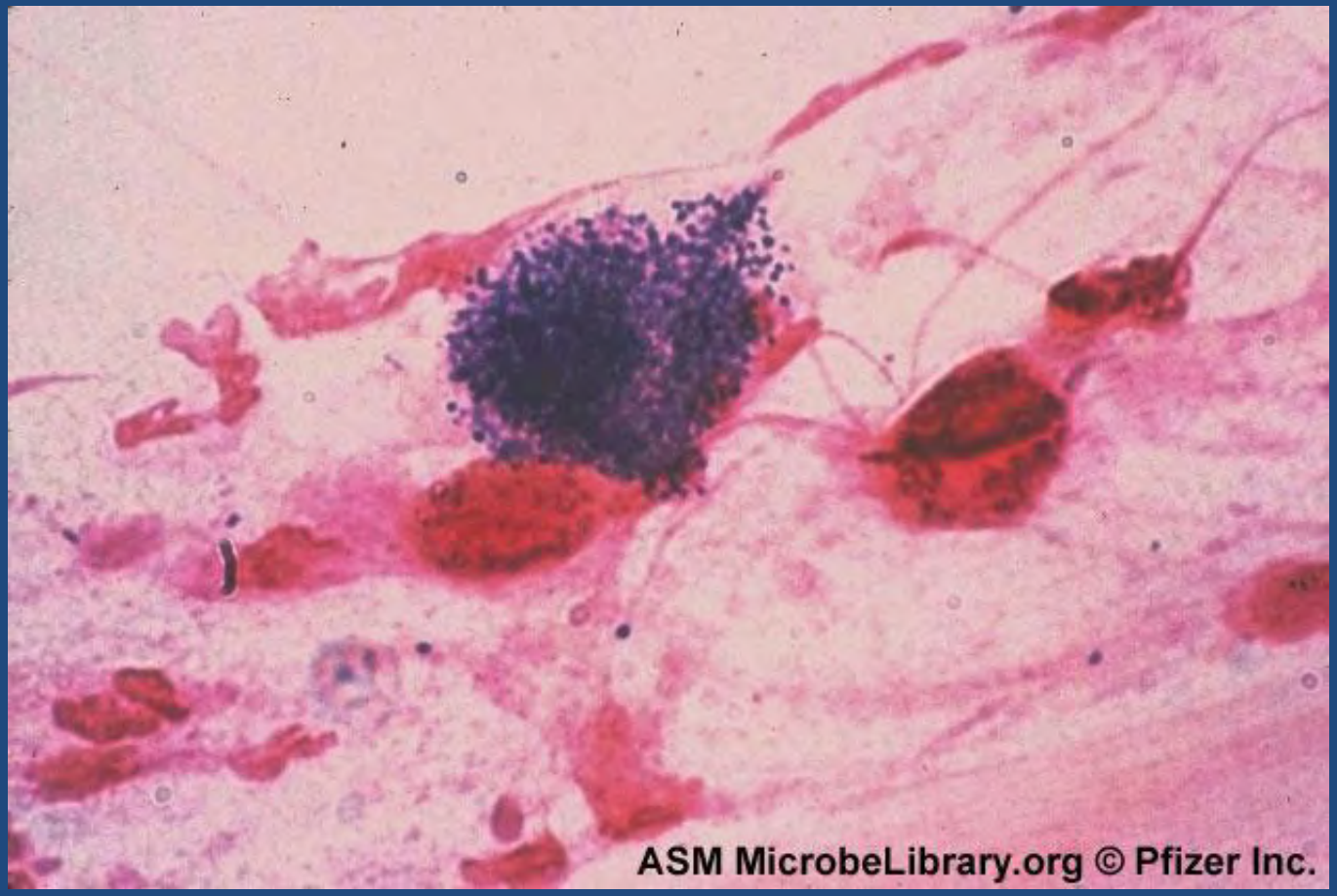
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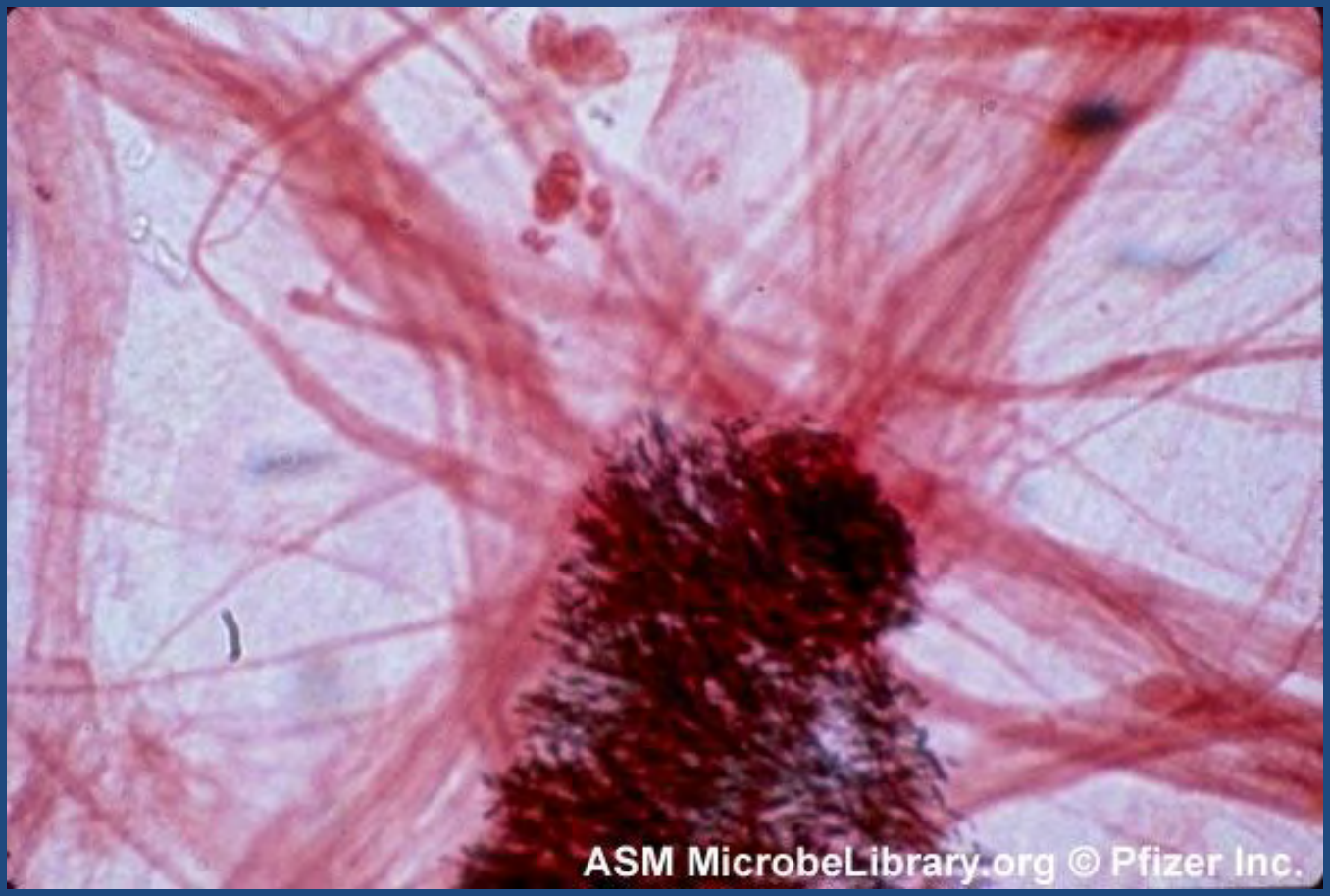
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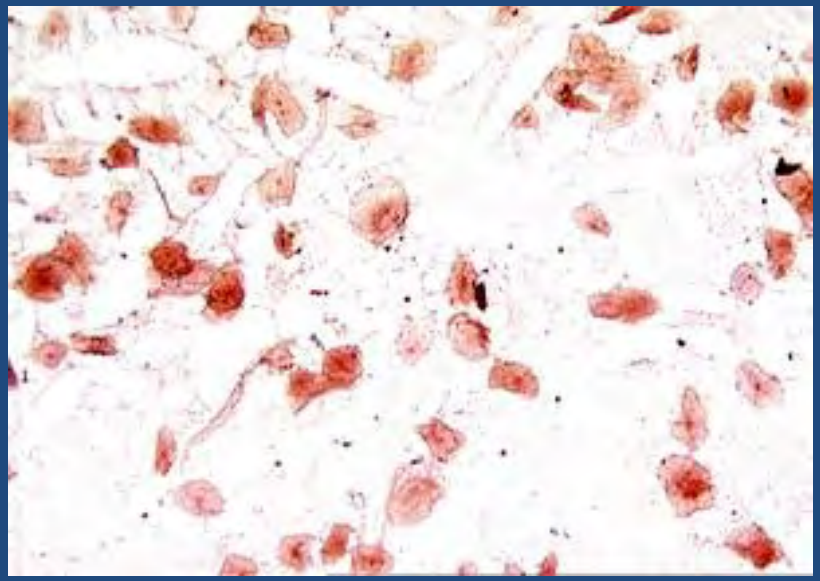
Bronchoalveolar Lavage (BAL) Specimen Acceptability

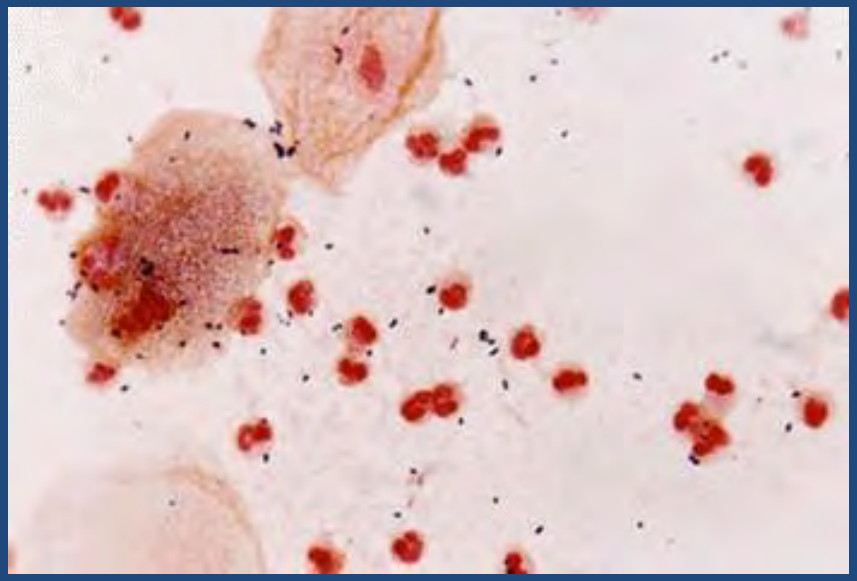
- Microscopic examination of Gram-stained smear
  - Acceptable
    - <1% of cells present are squamous epithelial cells
  - Unacceptable
    - >1% of cells present are squamous epithelial cells
    - **Presence of ciliated columnar bronchial epithelial cells/goblet cells or pulmonary macrophage in specimen obtained by brochoscop or BAL indicates a specimen from LRT**

Gram Stain Smear

Interpretation and Report

-No squamous epithelial cell seen
-Few squamous epithelial cell seen
-Abundant (many) squamous epithelial cell seen indicating oropharyngeal contamination
-Contamination with saliva please submit another specimen
-No WBC Seen





Smears with predominant EP and without WBC

No need to culture and report of organisms

Smears with EP and WBC

-Predominant organism

Gram negative bacilli predominant organism seen on Gram smear

-Presence of other organisms

Mixed bacterial morphotypes also present on Gram smear

-Without predominant organism

Mixed bacterial morphotypes seen on Gram smear

-Without organism

No organisms seen

-One morphotype

Report morphotype as few/moderate and many

Gram Stain Reports

- Be as descriptive as possible
  - Moderate neutrophils
  - Moderate Gram positive diplococci suggestive of *Streptococcus pneumoniae*
  - Few bacteria suggestive of oral flora
- Keep report short—avoid line listing of all morphotypes present

Culture

Routine culture

- *Blood Agar*
- *Chocolate Agar*
- *Mac Conkey*
- *Thioglycolate(for aspirated specimen biopsy)*

Routine culture

- Because of contaminating oral flora ,sputum specimens ,specimens obtained by bronchial washing, and lavage trachestomy, or endotracheal tube aspirates are not inoculated to enriched broth or incubated anaerobically.

- Only specimens obtained by percutaneous aspiration (including transtracheal aspiration)and by protected bronchial brush are suitable for anaerobic culture

Culture

- Transtracheal and percutaneous lung aspiration material may be inoculated to enriched thioglycollate ,as well as to solid media. For suspected cases of legionella disease buffered charcoal yeast extract (BCYE) agar and selective BCYE are inoculated.

Culture

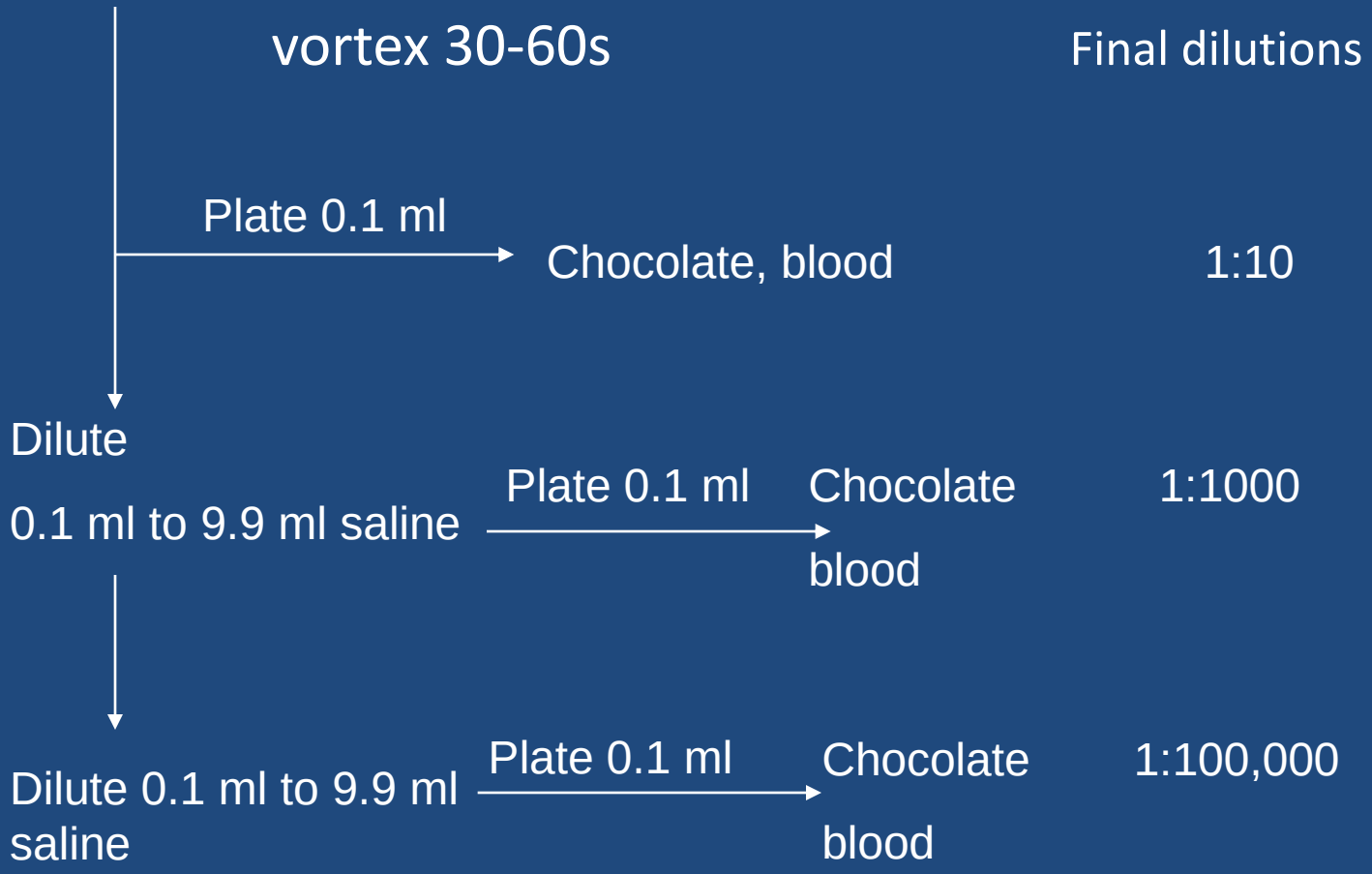
- Sputum specimens from patients known to have cystic fibrosis should be inoculated to selective agar ,such as manitol salt agar for recovery of *S .aureus* and selective horse blood-bacitracin ,incubated anaerobically and aerobically ,for recovery of *H.influenzae* that may be obscured by the mucoid *P.aeroginosa* on routine media.

Bronchoscopy Specimens Processing

- Bronchoscopy brush protected
  - Aerobic bacterial culture and Gram stain
  - Anaerobic bacterial culture
  - Limited volume
- Bronchoscopy brush, unprotected
  - No anaerobic culture
  - Limited volume
- Bronchial washings
  - Useful only for pneumonia caused by strict pathogens
  - Reasonable requests: Mtb, Fungi, *Legionella*, *Pneumocystis*
- Bronchoalveolar lavage
  - No anaerobe culture

Bronchoscopy Samples Quantitative Methods

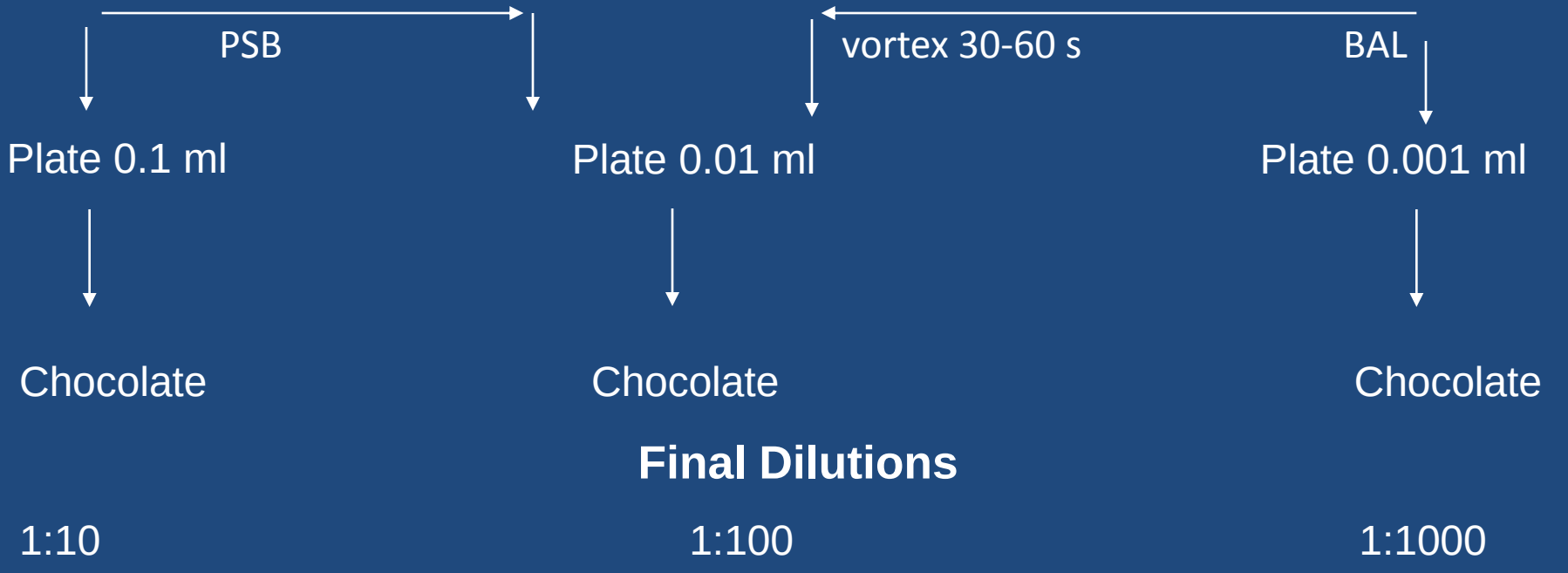
PSB or BAL **Baselski and Wunderink. 1994. *Clin Micro Rev* 7:546.**



Bronchoscopy Samples Quantitative Methods

Calibrated loop method

Baselski and Wunderink. 1994. *Clin Micro Rev* 7:547



Interpretation of Quantitative PSB/BAL

- Dilution Method
  - Quantify each morphotype present and express as CFU/ml
- Calibrated Loop Method
  - Quantify each morphotype present and express as $\log_{10}$ colony count ranges
- Thresholds for significance
  - PSB > 10^3 CFU/ml
  - BAL > 10^4 CFU/ml

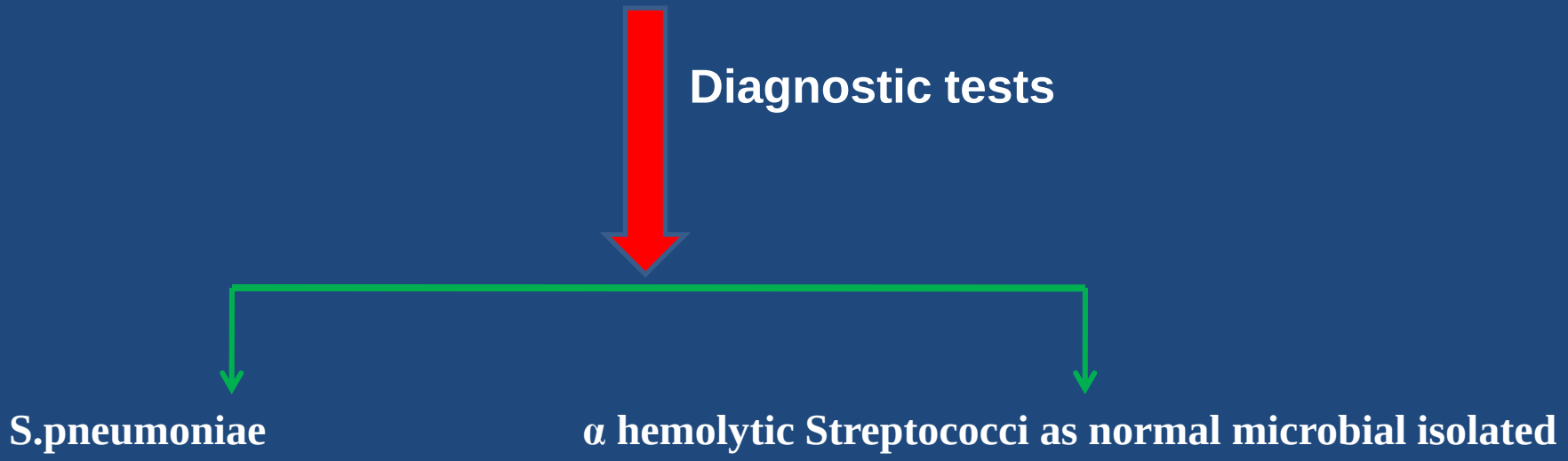
Baselski and Wunderink. 1994. *Clin Micro Rev* 7:547

Special notes

Specimen without EP or few EP

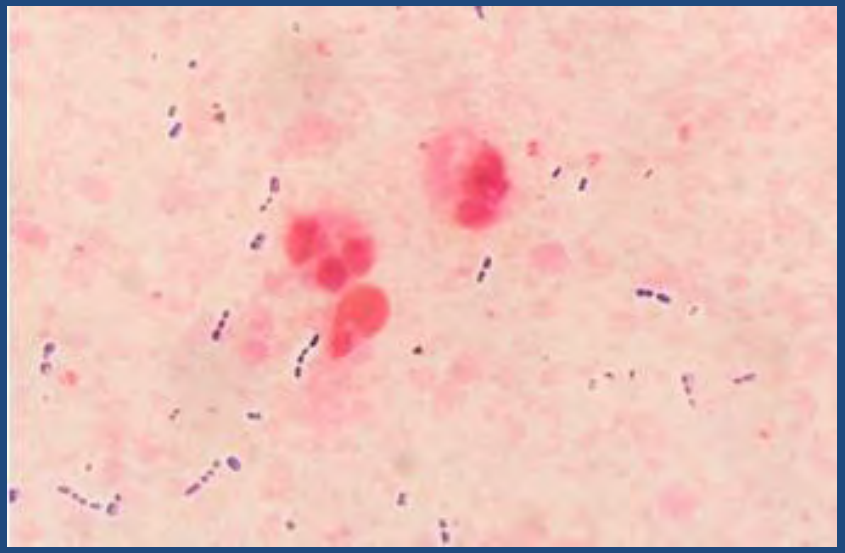
α hemolytic Streptococci

Heavy or moderate (predominant) growth or colony resemble S.pneumoniae

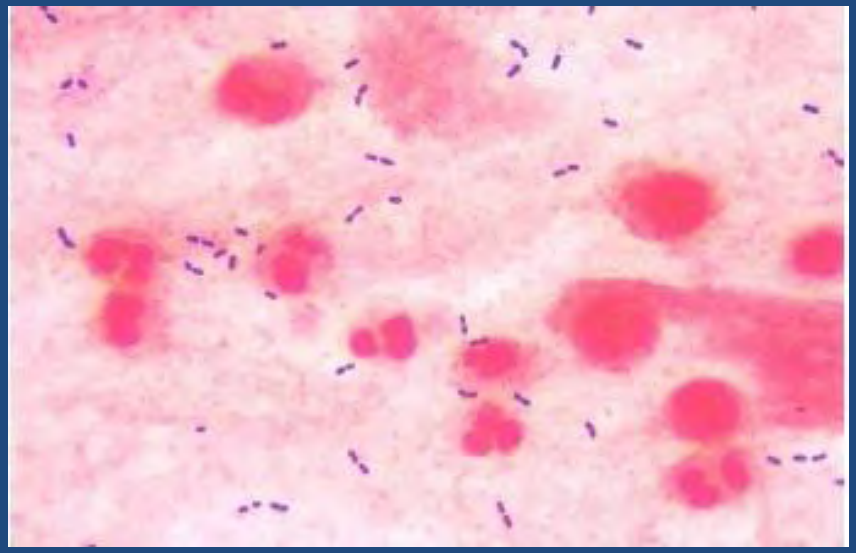


S.pneumoniae

S.pneumoniae mucoid colony

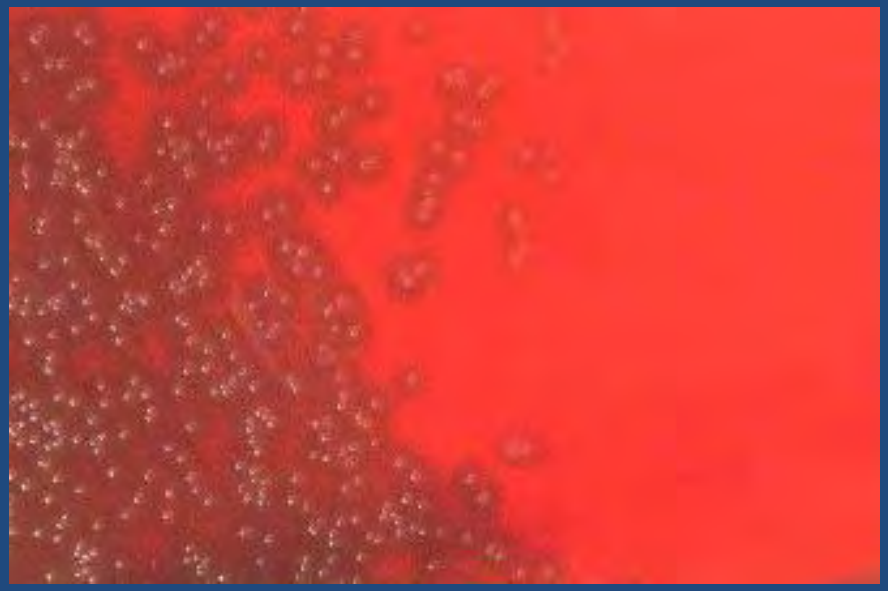


S.pseudopneumoniae

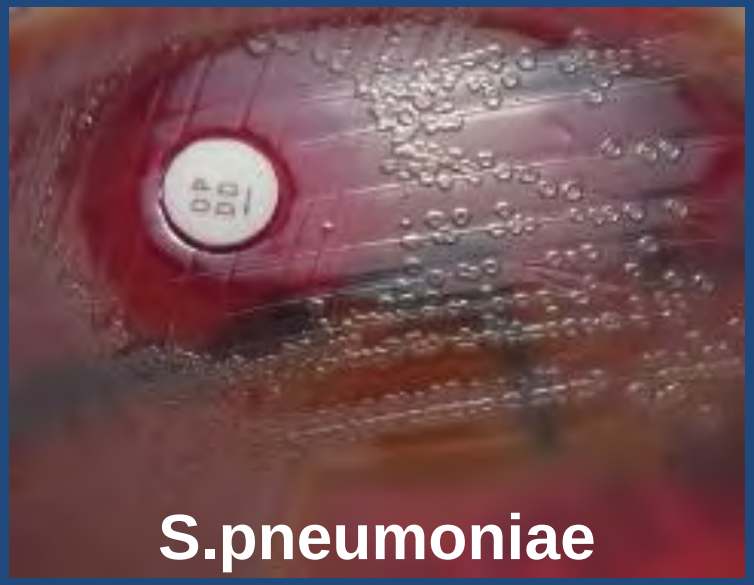


S.pneumoniae

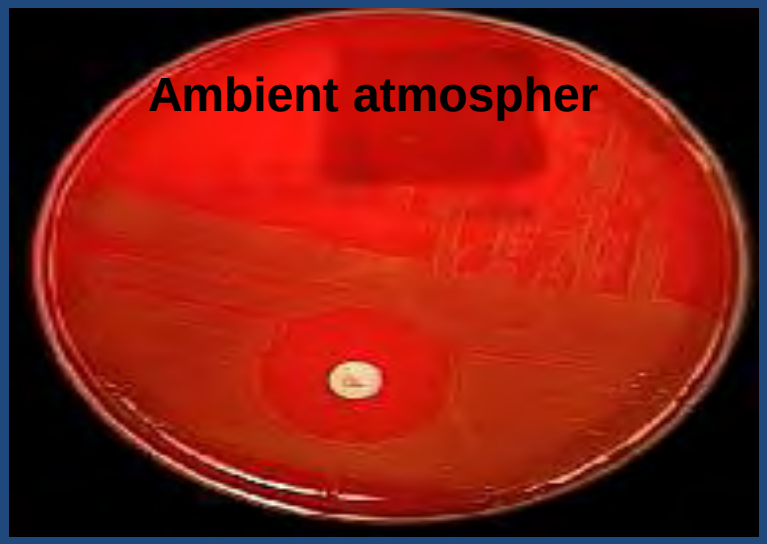
S.pneumoniae

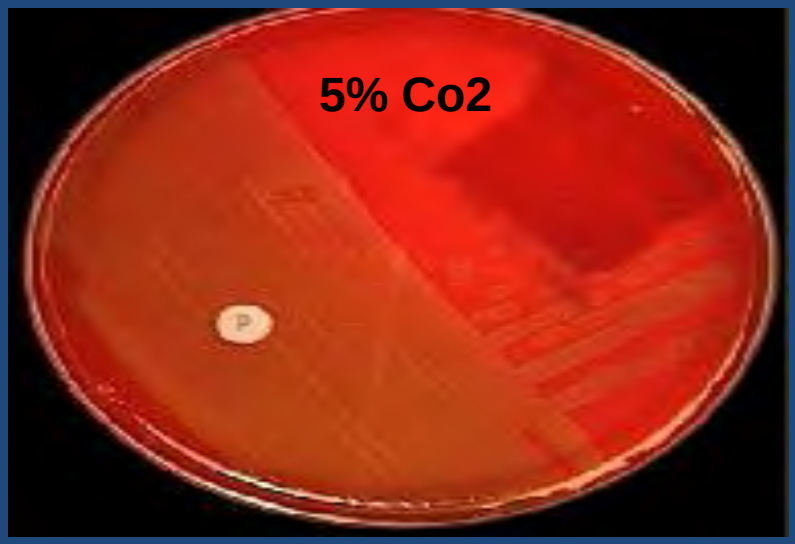


S.pseudopneumoniae



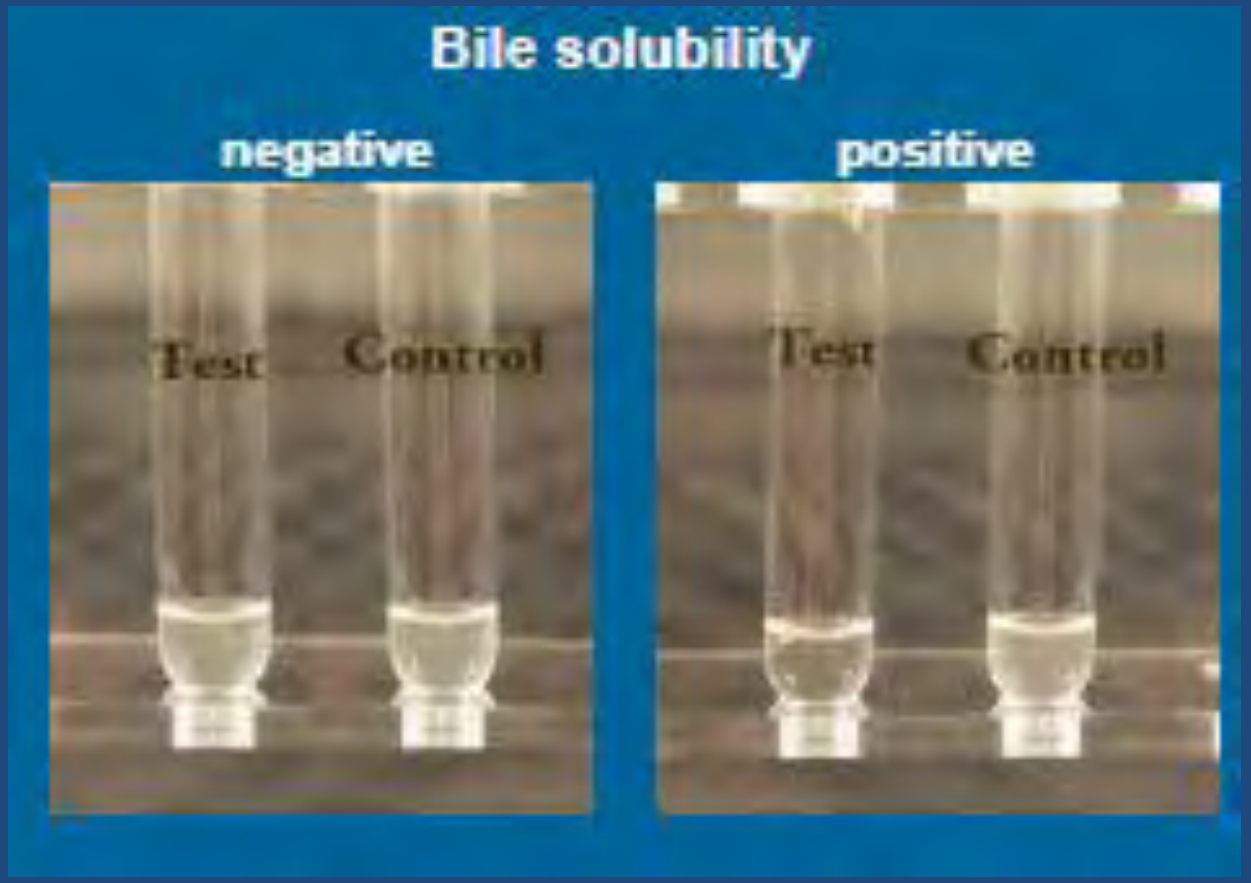
S.pneumoniae





S.pseudopneumoniae

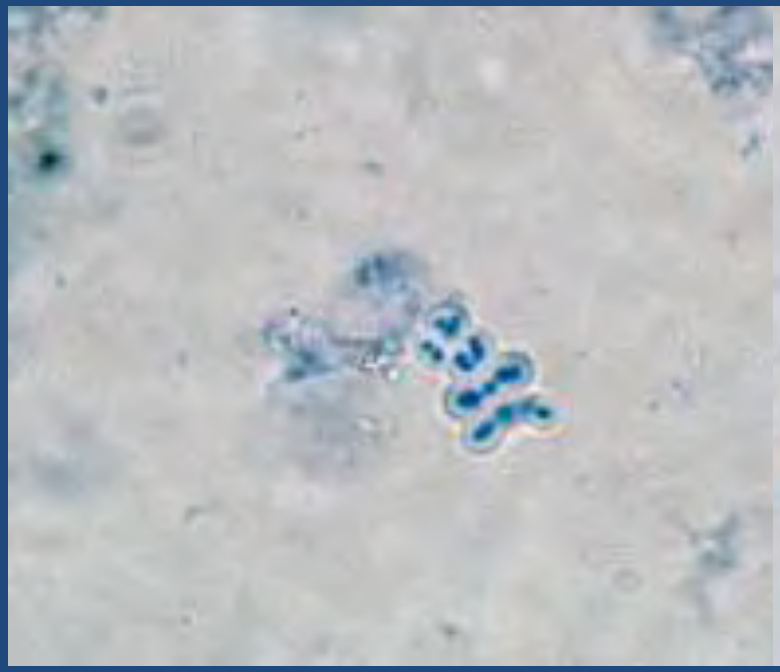
It is not soluble in bile



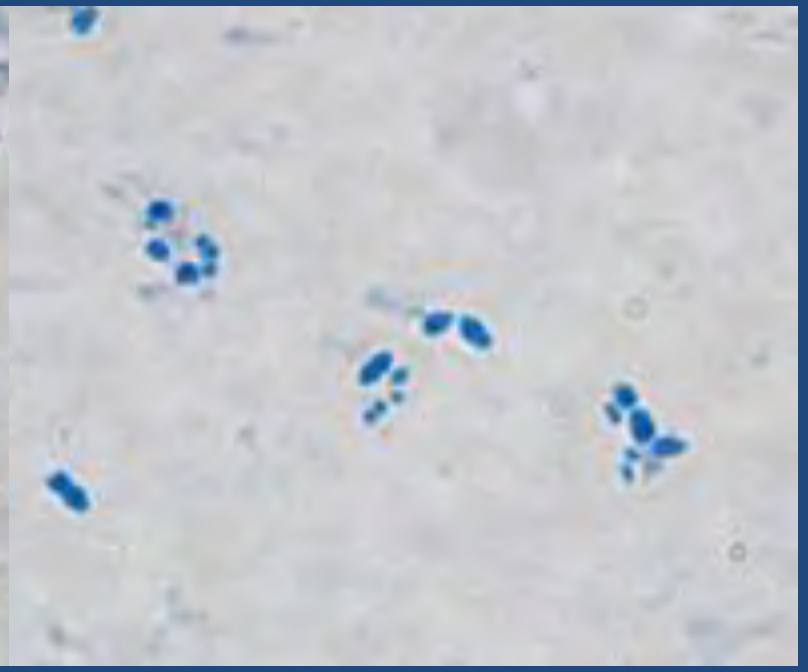
S.pseudopneumoniae

S.pneumoniae

There is no pneumococcal capsule (and is therefore not typable)



S.pneumoniae



S.pseudopneumoniae

β hemolytic Streptococci

Mixed with other colony or few colony

Good growth

Isolation and subculture(stabing)

Diagnosis tests

Group A Streptococci Isolated

Serotyping for other

Hemophilus

Predominant culture GNB resemble Hemophilus

Isolation on choc

Diagnosis test

Nieserria and Moraxella

Predominant culture GNDB or GNDB in Gram smear

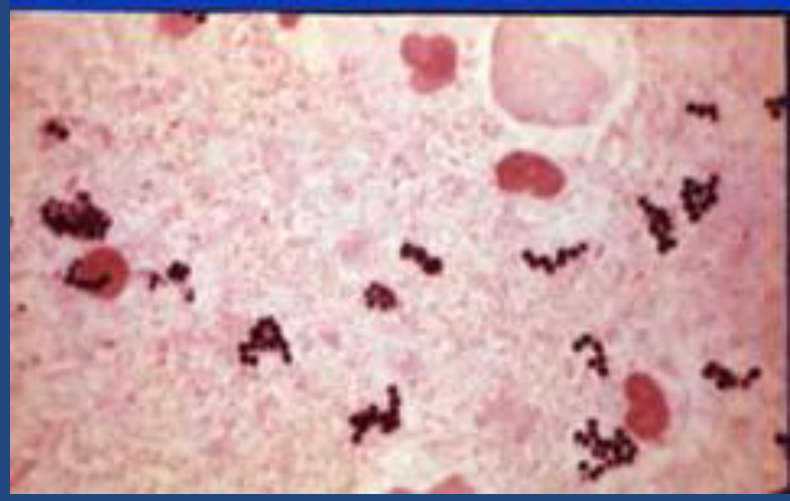
Diagnosis test

Other Gram Negative

No. of: Colony types	Colonies	Normal microbiota	Action*
1-2	Rare to few	Absent to few, or if gram-negative bacilli predominant on Gram stain	ID and AST
		Moderate to abundant	ID and AST only if requested
1-2	Moderate to abundant	Present or absent	ID and AST
> 2	Few to abundant	Present or absent	ID and AST only if requested

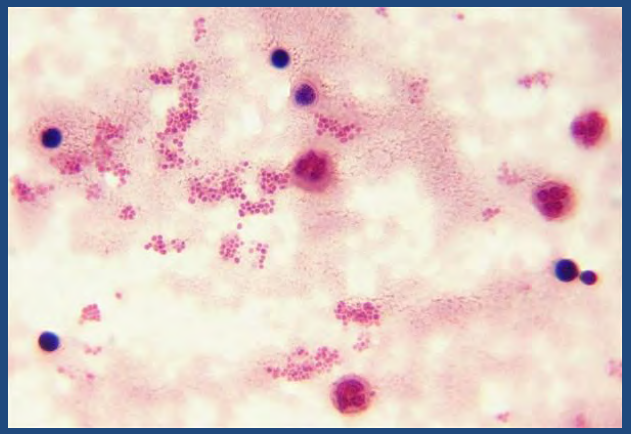
* ID, identify; AST, perform antimicrobial susceptibility testing

Staphylococci



No. of colonies	Colony morphotypes	Normal microbiota	Action	Report
Any no.	CNS	Moderate to abundant	None	Normal microbiota
Moderate, abundant, or predominant	CNS	Absent to few	ID	CNS as normal microbiota
Rare to few	*S. aureus*	Moderate to abundant	None	Normal microbiota
Rare to few	*S. aureus*	Absent to few or gram-positive cocci predominant on Gram stain	ID, AST	*S. aureus*
Moderate to abundant, or predominant	*S. aureus*	Present or absent	ID, AST	*S. aureus*

Yeast



Rare to moderate of yeast
Moderate to abundant growth of normal flora

↓

Normal microbiota

Rare to moderate of yeast
No growth of normal flora or predominant yeast cell in Gram smear

↓

ID

Predominant culture

↓

ID

Special notes

Specimen with many EP and WBC

predominant organisms in gram stain not seen

No need to additional diagnosis and complementary culture

One organism seen in gram stain

-Growth on culture ID / AST

-Normal flora no need to ID

-Predominant GNB in gram smear and isolation of one type colony or predominant colony

ID / AST

-Two or more colony type of GNB without predominant type

Multiple species of gram negative bacilli, no predominant organism isolated

Culture without predominant organisms

Mixed bacterial morphotypes

College of American Pathologist Recommendation

***Examine all sputum specimen microscopically**
***Report microscopic finding quantitavely**
***Use microscopic criteria for accetability**
***compare Gram stain and culture results**
***Oganisms seen in the smear don't grow in culture**
***Organism that grow in moderate quantity or less are not seen in the Gram smear**
***List both the Gram smear and culture results together in the same report**

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