

Respiratory Bench

CLS 417: Clinical Practice in Microbiology

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Types of Specimens

A. Upper Respiratory Tract Specimens include:

- Throat swabs
- Epiglottal swabs
- Nasal/nasopharyngeal swabs / aspirates
- Mouth swabs
- Oral abscess swabs / aspirates
- Sinus or antral aspirates

B. Lower Respiratory Tract Specimens include:

- Sputum
- Bronchial aspirates (washings)
- Bronchial brushings
- Bronchoalveolar lavage (BAL)
- Lung biopsies
- Lung Aspirates
- Open Lung biopsies
- Mucopus aspirated from the nasopharynx for children.

Collection of Sputum

1. Give the patient a clean (need not be sterile), dry, wide-necked, *leak-proof* container, and request him or her to cough deeply to produce a sputum specimen.
2. Use a phenol-containing disinfectant to wipe the outside of the container after collecting the specimen.
3. Label the container, and complete a request form with patient name, no., date, and time of collection.

Important Notes on Sputum Collection

- The specimen *must be sputum, not saliva*.
- Sputum is best collected in the morning soon after the patient wakes and before any mouth-wash is used.
- *When Pneumocystis pneumonia is suspected: Collect broncho-alveolar lavage (BAL) or induced sputum.*

Transport of Sputum

1. When pneumonia or bronchopneumonia is suspected, deliver the sputum to the laboratory with as little delay as possible because organisms such as *S. pneumoniae* and *H. influenzae* require culturing as soon as possible (Do not refrigerate).
2. *When pneumonic plague is suspected: Deliver the sputum to the laboratory as soon as possible. Make sure the specimen is marked HIGH RISK.*

Most Common Pathogens Isolated from Sputum Sample

BACTERIA

Gram positive

Streptococcus pneumoniae

Staphylococcus aureus

Streptococcus pyogenes

Gram negative

Haemophilus influenzae

Klebsiella pneumoniae

Pseudomonas aeruginosa

Proteus species

Yersinia pestis

Moraxella catarrhalis

Also *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

Most Common Pathogens Isolated from Sputum Sample

FUNGI AND ACTINOMYCETES

- *Pneumocystis jiroveci*
- *Blastomyces dermatitidis*
- *Histoplasma capsulatum*
- *Aspergillus species*
- *Candida albicans*
- *Cryptococcus neoformans*
- *Nocardia species.*

PARASITES

- *Paragonimus species*

Commensals

Gram positive

Staphylococcus aureus

Staph. epidermidis (S. albus)

Streptococci Viridans

Streptococcus pneumoniae

Enterococci

Micrococci

Lactobacilli

Diphtheroids

Yeast-like fungi

Gram negative

Neisseria species

Moraxella catarrhalis

Haemophilus influenzae

Fusobacteria

Coliforms

Unsuitable Sputum Specimens

- When the sputum is mostly saliva, report the specimen as 'Unsuitable for microbiological investigation' and request another specimen.
- *Before culturing sputum, many laboratories examine a wet preparation or Field's stained smear microscopically for cells. When large numbers of squamous epithelial cells (often covered with bacteria) are present and only a few or no pus or macrophage cells, this indicates that the specimen is unsuitable for culturing.*

Macroscopic Examination of Sputum

Report whether the sputum is:

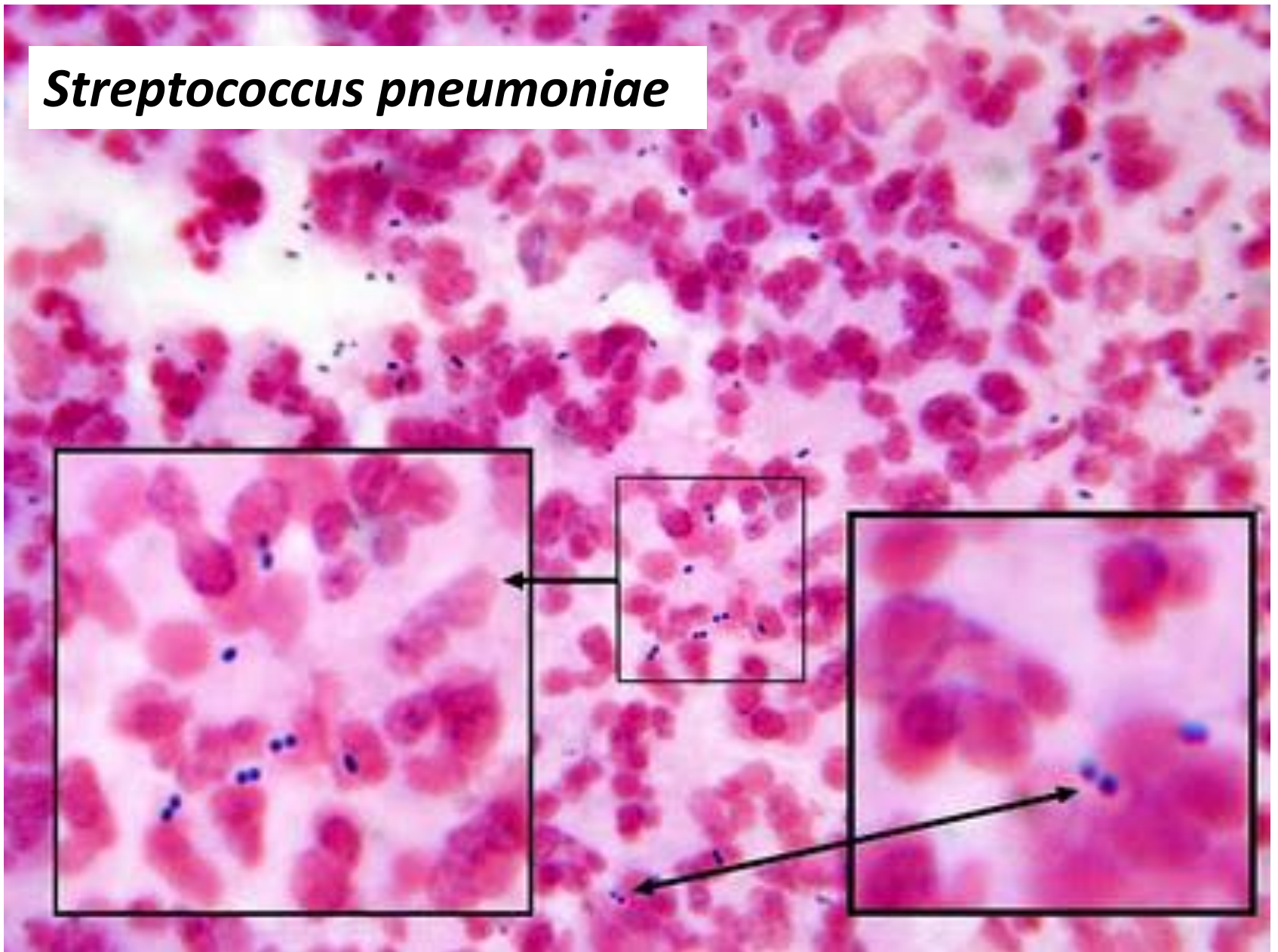
- **Purulent:** *Green-looking, mostly pus*
- **Mucopurulent:** *Green-looking with pus and mucus*
- **Mucoid:** *Mostly mucus*
- **Mucosalivary:** *Mucus with a small amount of saliva*
- When the sputum contains blood, this must also be reported.

Microscopic Examination of Sputum

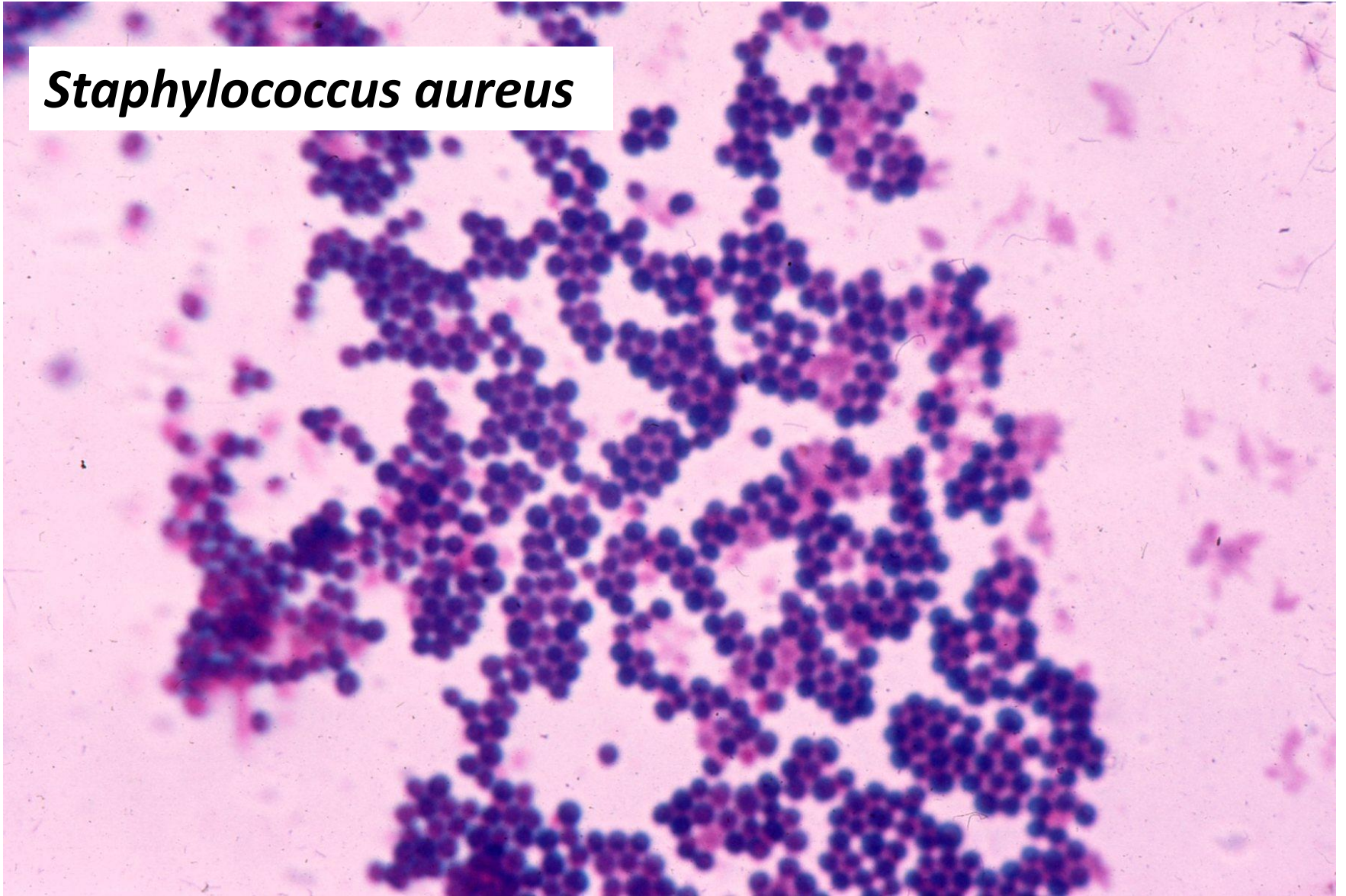
Gram smear

1. Using a piece of stick, transfer a *purulent part of the* sputum to a glass slide, and make a thin smear.
2. Allow the smear to air-dry in a safe place. Fix as and stain by the Gram technique.
3. Examine the smear for pus cells and bacteria:
 - ✓ Gram positive diplococci (capsulated) that could be *S. pneumoniae*.
 - ✓ Gram positive cocci in groups that could be *S. aureus* but not often seen.
 - ✓ Gram negative rods and cocco-bacilli that could be *H. influenza*.
 - ✓ Gram negative capsulated rods that could be *K. pneumoniae*, but not often seen.
 - ✓ Gram negative diplococci in and between pus cells that could be *M. catarrhalis*.

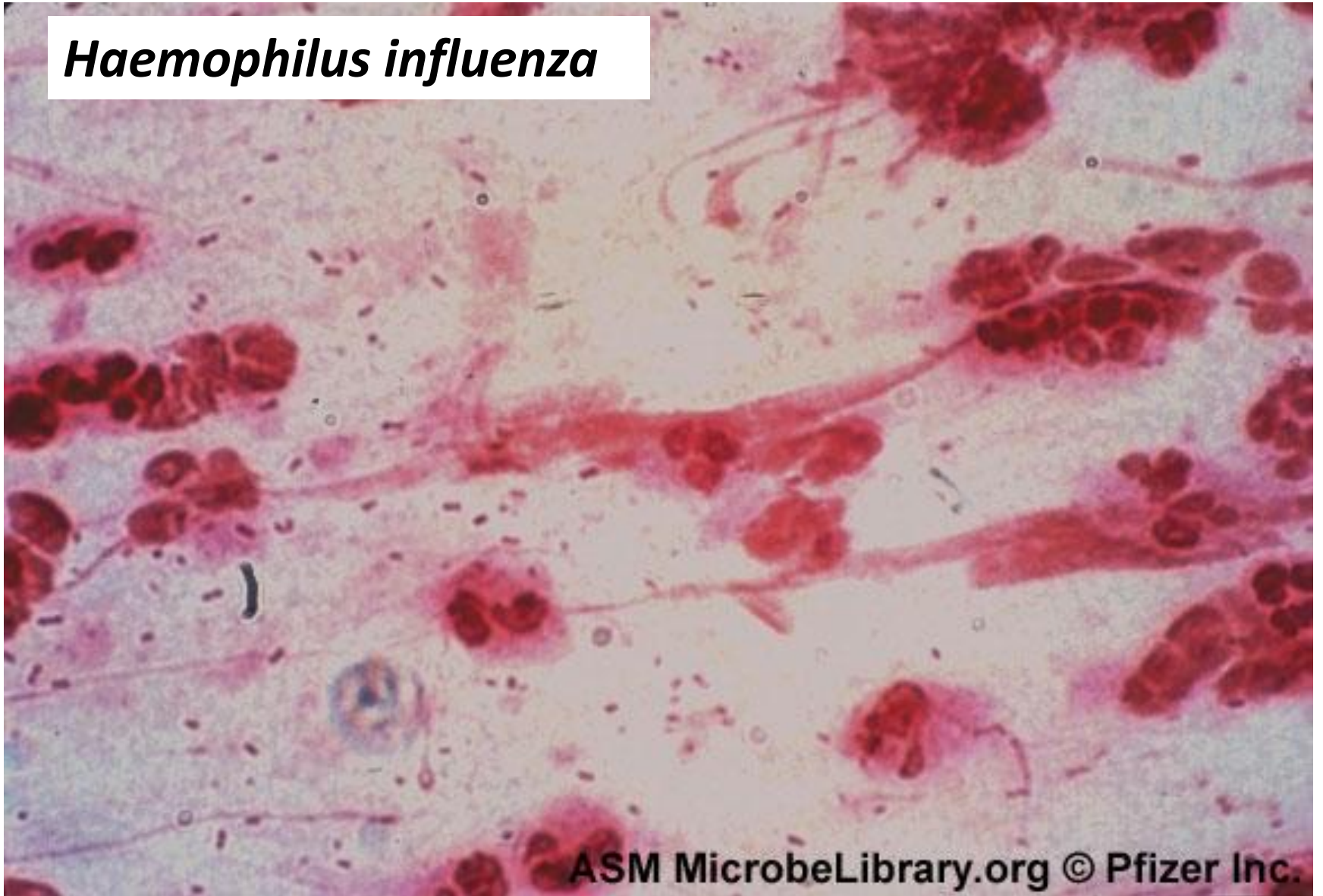
Streptococcus pneumoniae



Staphylococcus aureus

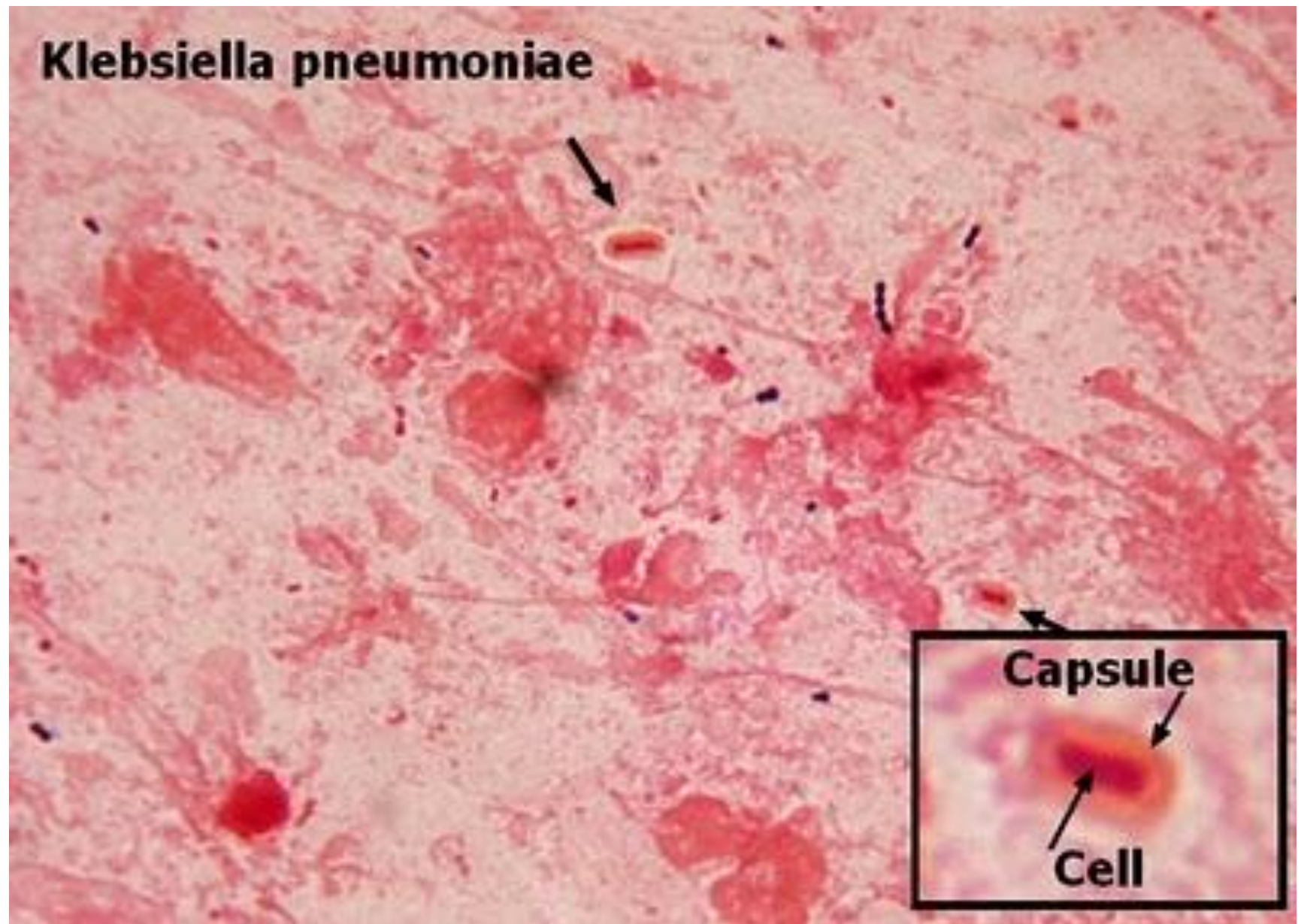


Haemophilus influenza

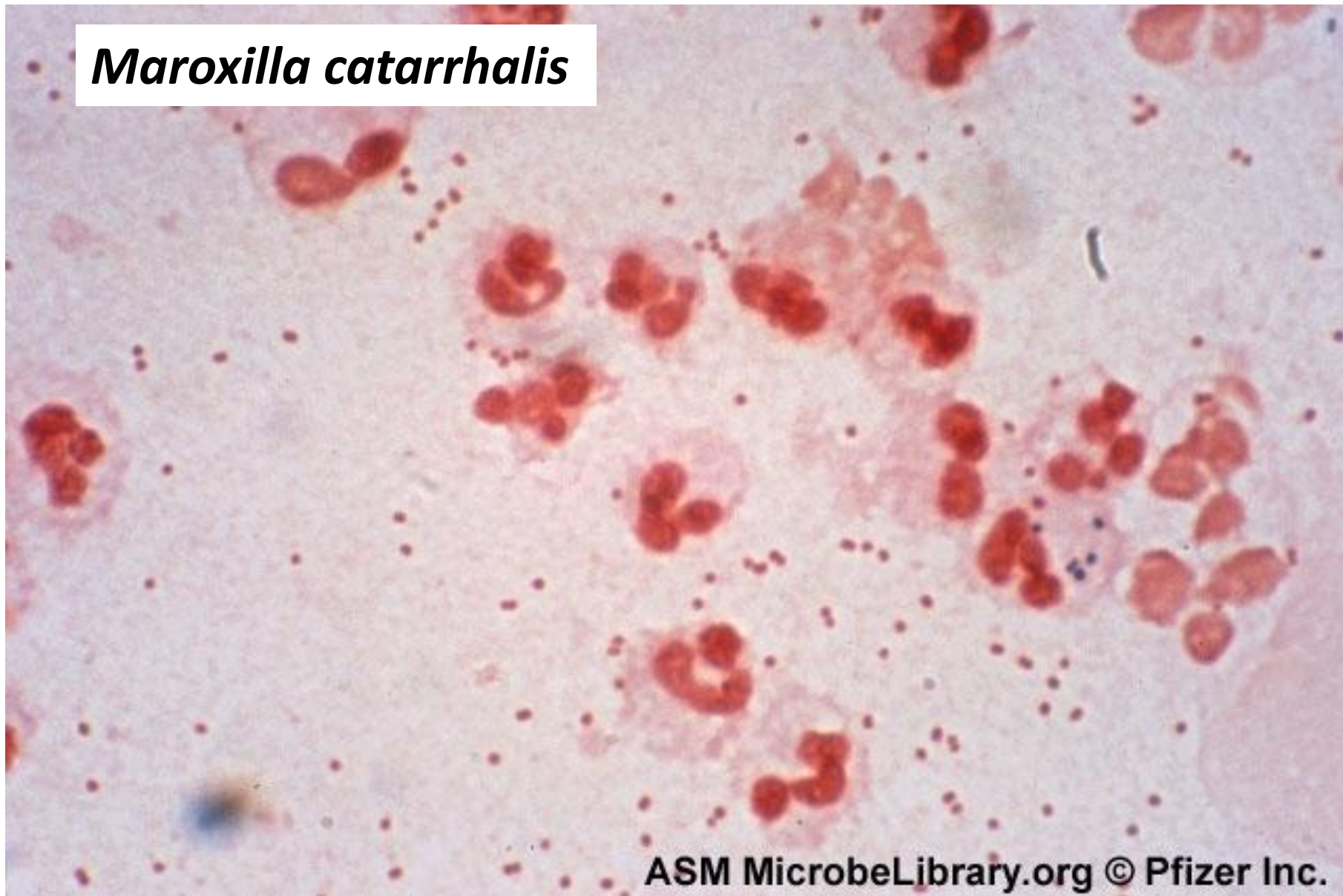


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Klebsiella pneumoniae



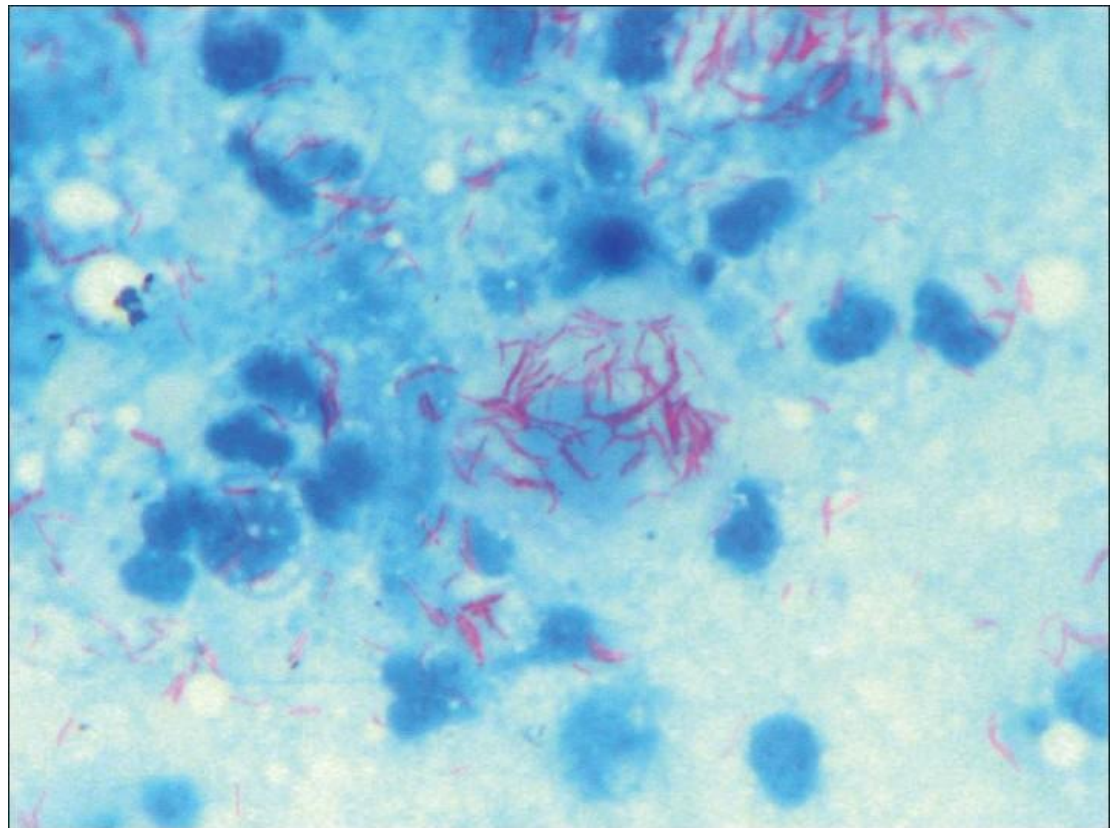
Maroxilla catarrhalis



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Additional Staining Techniques

- ***Ziehl-Neelsen Smear when *Mycobacterium tuberculosis* is suspected. Or send directly to Tb lab.***



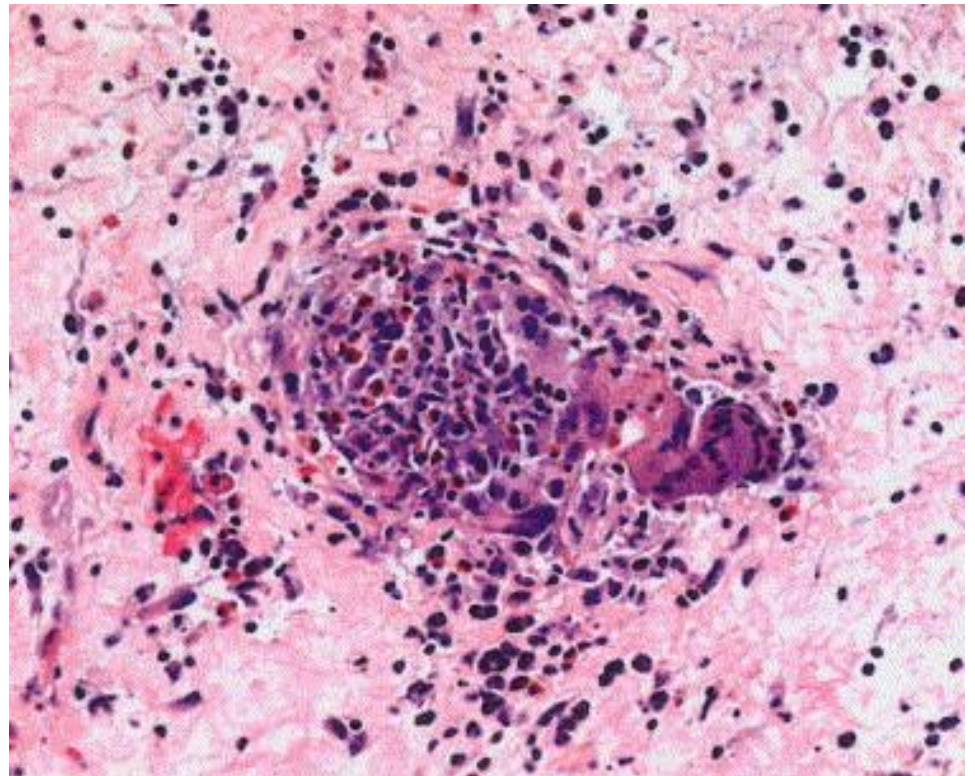
Additional Staining Techniques

- *Saline preparation when paragonimus eggs is suspected.*



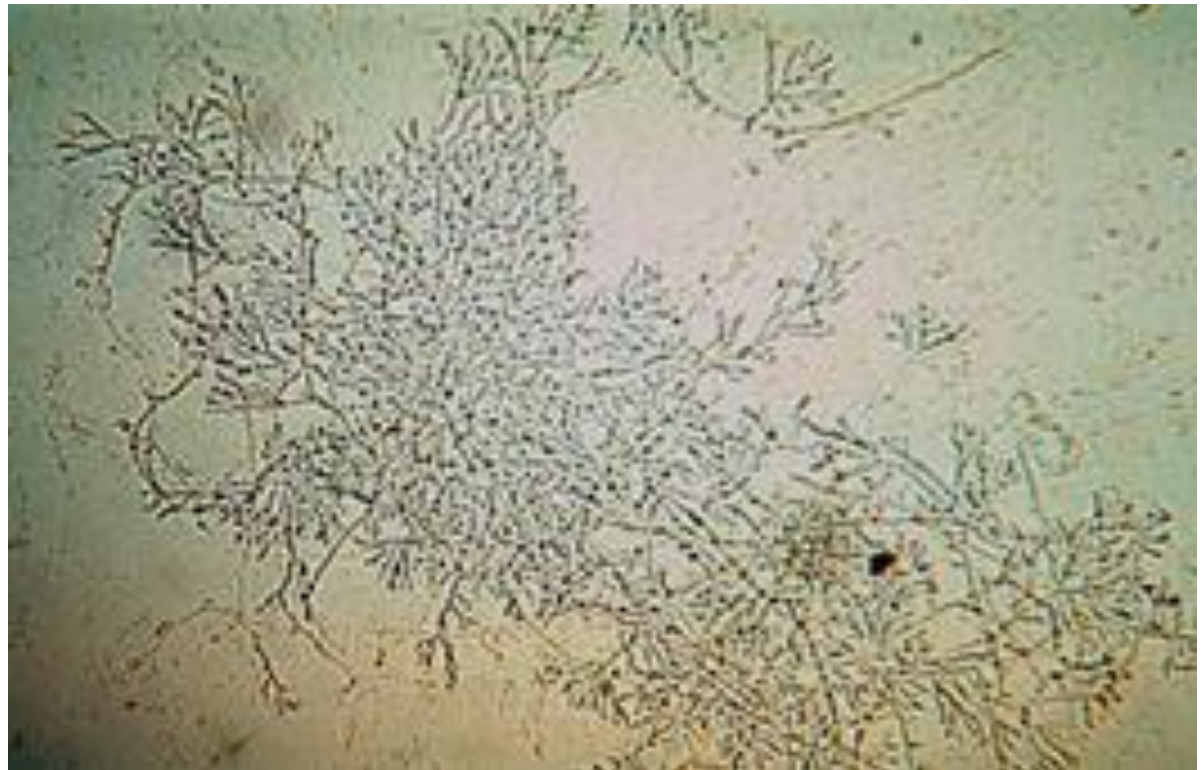
Additional Staining Techniques

- *Eosin preparation when an allergic condition requires investigation.*



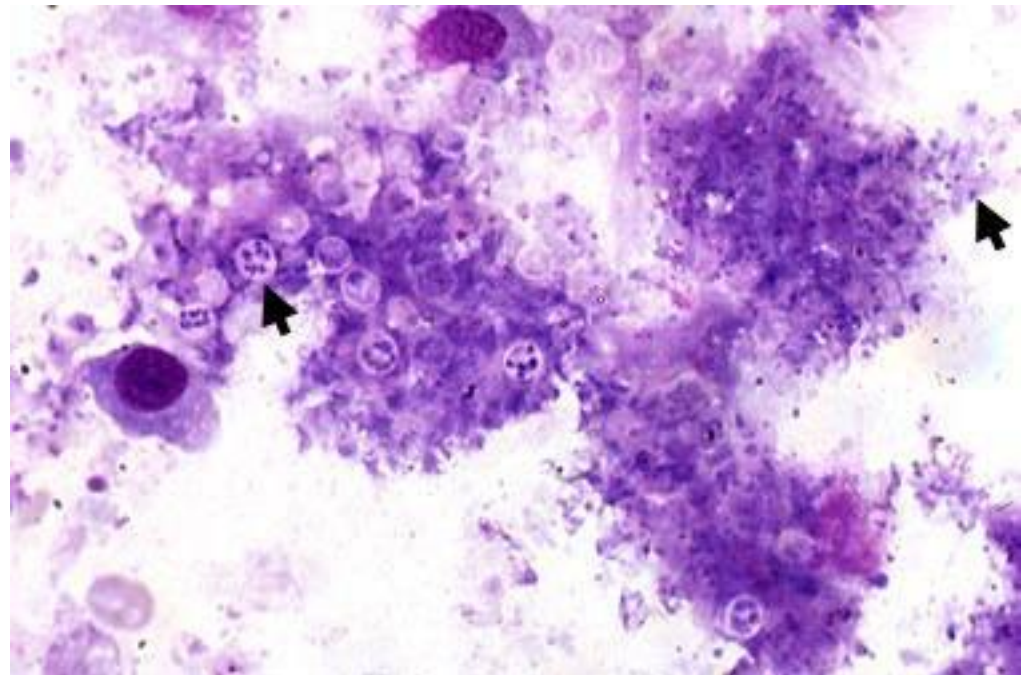
Additional Staining Techniques

- *Potassium hydroxide (KOH) preparation when Aspergillus infection is suspected.*



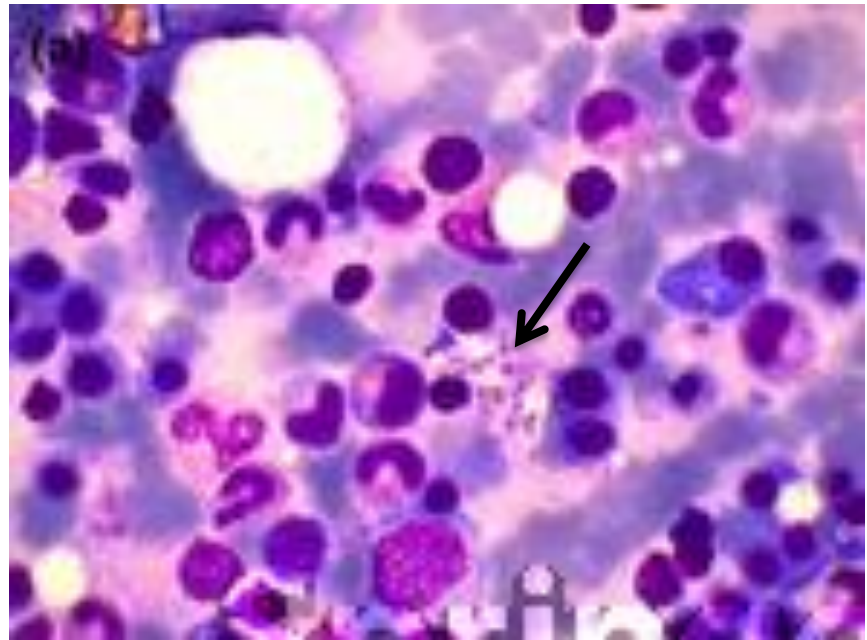
Additional Staining Techniques

- ***Giemsa and toluidine blue O preparations when *Pneumocystic* cyst is suspected (AIDS patients):*** on broncho-alveolar lavage (BAL) or induced sputum.



Additional Staining Techniques

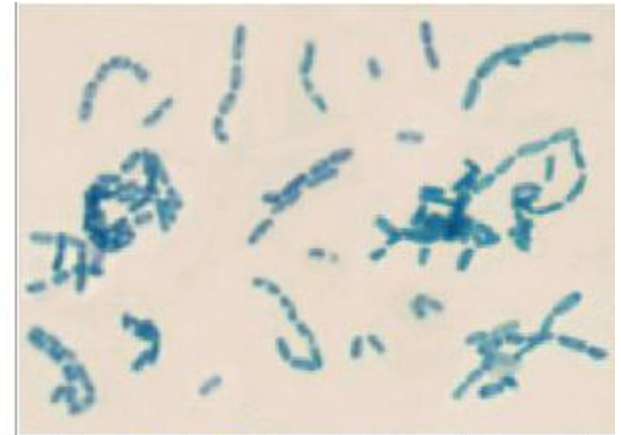
- *Giemsa stained preparation when histoplasmosis is suspected.*



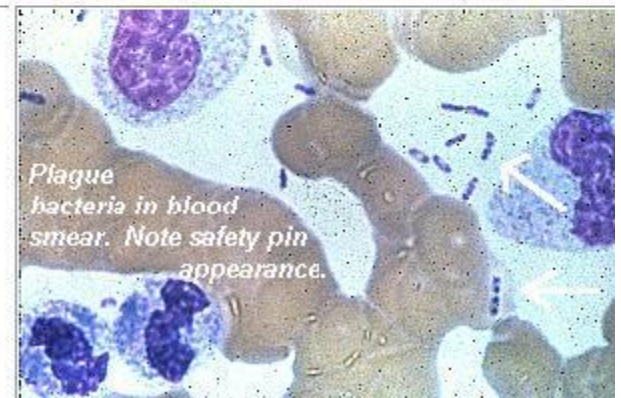
Additional Staining Techniques

- *Giemsa or Wayson stained preparation when pneumonic plague is suspected (Y. pestis: Hazard group 3).*

Wayson stain from culture

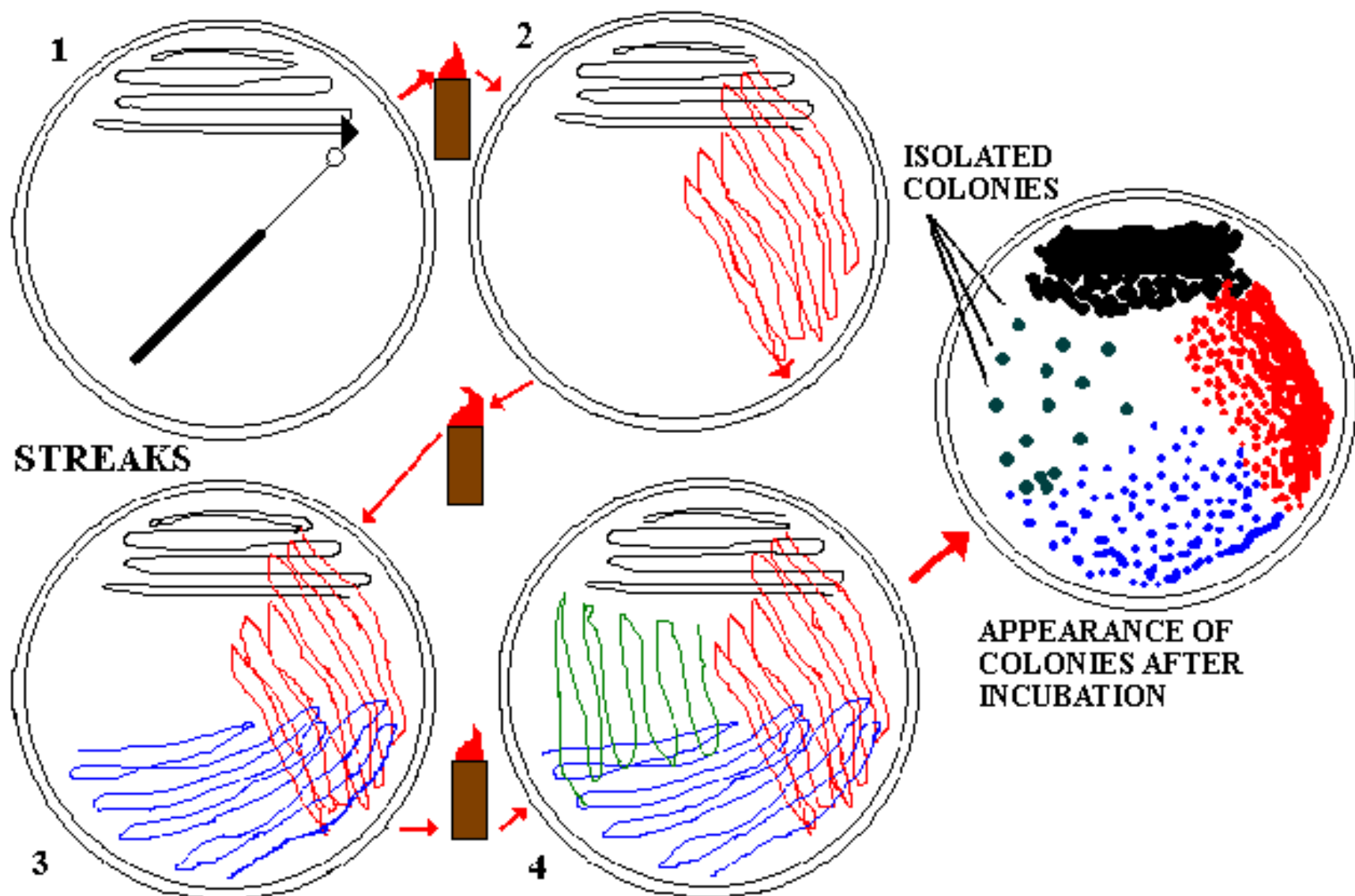


Giemsa stain from blood smear



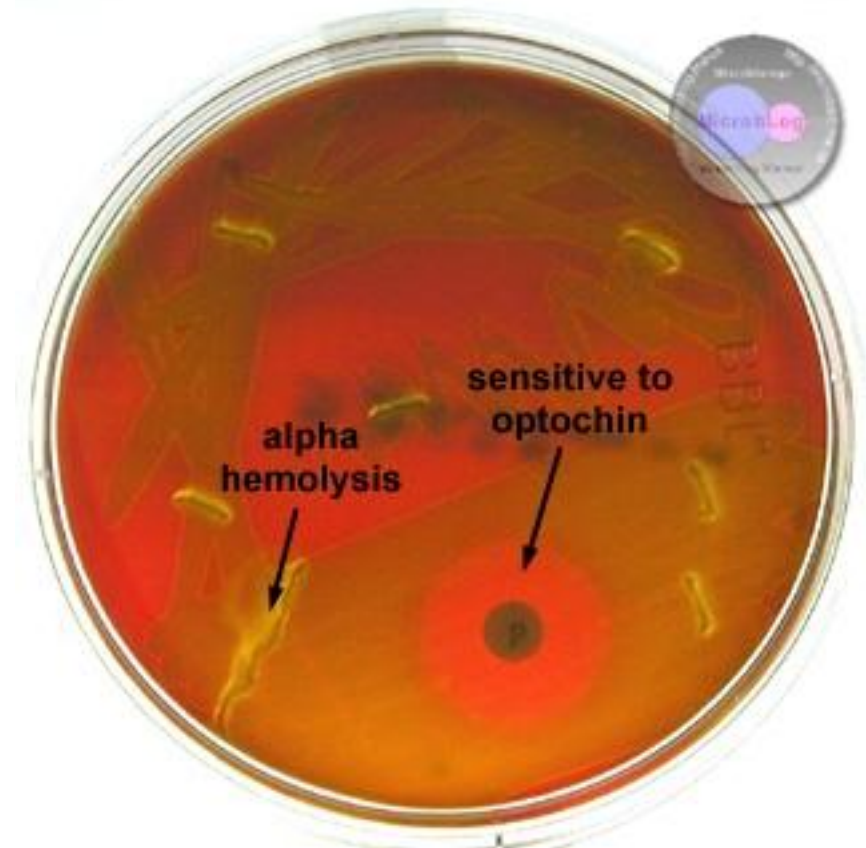
Culturing the Sputum

- To obtain as pure a culture as possible of a respiratory pathogen it is necessary to reduce the number of commensals inoculated. Ways of reducing commensal numbers include washing the sputum free from saliva or liquefying and diluting it.
1. Wash a purulent part of the sputum in about 5 ml of *sterile physiological saline*.
 2. **Inoculate the washed sputum on plates of:**
 - Blood agar aerobically.
 - Chocolate agar in CO₂.
 - MacConkey agar.



To Isolate *S. pneumoniae*

- Add an optochin disc to the blood agar plate within the area of 2nd spread. This will help to identify *S. pneumoniae*



Examine and Report the Cultures

	Mac	BA	Choc.
<i>S. pneumoniae</i>	No growth	α -haemolytic ring shaped colonies	Grow well in CO ₂
<i>S. aureus</i>	LF small colonies	Most are β -haemolytic colonies	Yellow to cream colonies
<i>H. influenzae</i>	No or Poor growth	No or Poor growth	Mucoid colonies
<i>K. pneumoniae</i>	LF mucoid pink colonies	Klebsiellae produce large grey-white usually mucoid colonies	Same as BA
<i>M. catarrhalis</i>	No growth	Grey-white Waxy appearance colonies	Same as BA

Antimicrobial Susceptibility Testing

- Susceptibility tests should be performed only when the amount of cultural growth of a pathogen is significant.
- Strains of *S. pneumoniae* should be tested on blood agar for susceptibility to penicillin, tetracycline, and erythromycin. Penicillin susceptibility is best determined using an oxacillin 1 g disc. A zone size less than 20 mm indicates reduced susceptibility.
- *H. influenzae* strains should be tested for **β-lactamase** production and susceptibility to ampicillin, tetracycline, and co-trimoxazole.