Respiratory Tract Infections (RTI).

A-Lower respiratory tract infections:

Physical examination, X-ray and laboratory investigations focus on :the degree of respiratory compromise and the identity of the causal pathogen.

Since a wide range of candidate pathogens may have to be considered, the number of likely candidates should be reduced as far as possible by searching for clues in the history (a history of tobacco consumption, recent travel, occupation, pets, and contacts with similar symptoms should be sought, examination and preliminary results.

Diagnostic specimens can be optained from the respiratory tract with deceptive ease, but their value is often limited by contamination by the indigenous flora of the oral cavity. **All appropriate specimens should have a direct microscopic examination**.

Doctor may order a sputum Gram's stain to help identify the cause of pneumonia. This is an infection that can affect lower respiratory tract. It's often caused by microorganisms, like bacteria, viruses, or fungi.

The direct examination serves several purposes:

1- The quality of the specimens can be assessed, for example, sputa can be rejected that represent saliva (clear) and not lower respiratory tract secretion by quantitation of white blood cells or sequamous epithelial cells present in the specimens.
2- The microbiologist and clinician can be given an early indication of what may be wrong with the patient (e.g. (++++) G+ve cocci in clusters in an exudate).
3- The workup of the specimen can be guided by comparing what grows in culture to what was seen on original smear.

Note: Direct examinations are usually not performed on throat, nasophryngeal, or stool specimens but are indicated from most other sources due to the presence of abundant normal microbiota.

Sputum.

Expectorated. is a mixture of saliva and mucus that you cough up from your respiratory tract. It's usually colored and thick in consistency,

especially when you there is an infection in the lungs.However, lower respiratory tract secretions will be contaminated with upper respiratory tract secretions, especially saliva, unless they are collected using an invasive technique. For this reason, sputum is among the least clinically relevant specimens received for culture in microbiology laboratories, even though it is one of the most numerous and time-consuming specimens.

Good sputum samples depend on thorough health care worker education and patient understanding throughout all phases of the collection process.

1- If the patient at home,the doctor may ask him to collect the sputum sample himself. They will give him a sterile sample cup to use. Wait until he's ready to collect him sample before opening the lid.
2- The night before patient provides a sample, tries to drink lots of fluids like water or tea. This will help his body make more sputum. Collecting sample first thing in the morning, before eating or drinking anything. There tends to be more bacteria present at this time and can help ensure accurate test results.
3- Food should not have been ingested for 1-2 hours before expectoration and brush teeth and rinse mouth. Don't use antiseptic mouthwash.
4- Patients should be instructed to take a couple of long, deep breaths. Then breathe deeply and cough hard until sputum comes up.
5- Spit out the sputum into sample cup. Keep coughing up sputum until the cup is filled to the marker, which should equal approximately 1 teaspoon.
6- Screw the lid onto the cup and wash and dry the outside of it. Write your name and the date on the label.
7- Take the sample to the clinic or laboratory, following your doctor's instruction. You can refrigerate it for up to 24 hours if needed, but you shouldn't freeze it or store it at room temperature.
8- If you can't cough up enough sputum, try breathing steam in from boiling water, or take a hot steamy shower. The sputum sample must come from deep inside your lungs for the test to be accurate.

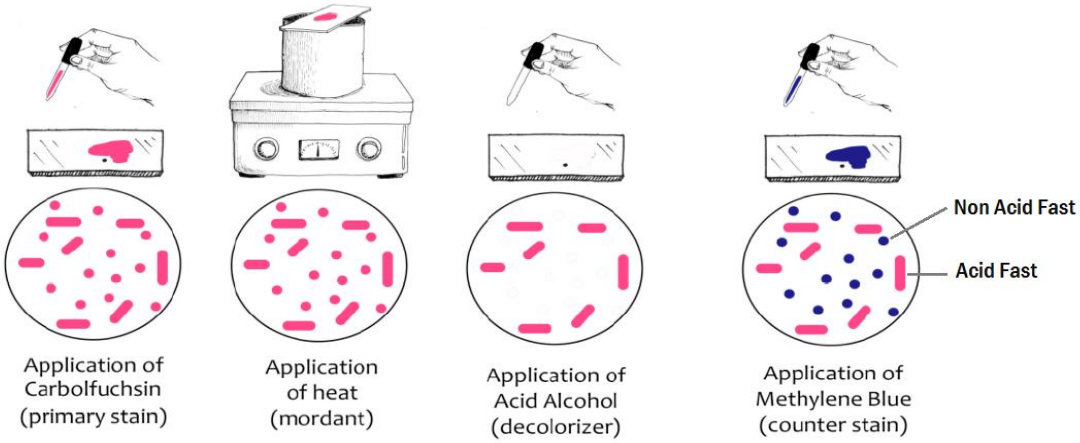
How is sputum sample analyzed?

At the laboratory, sputum sample will be analyzed using a sputum Gram's stain. By placing a thin layer of sputum on a slide and allow it to dry. Then staining the smear with Gram's stain.

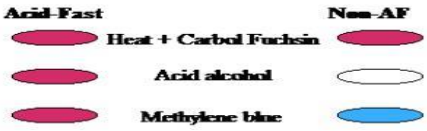
What do the results of mean?

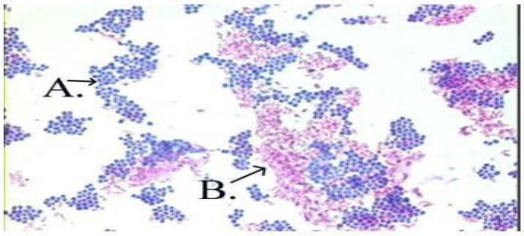
A-If the test results from sputum Gram's stain are abnormal, it means that bacteria and white blood cells have been detected. The bacteria found will be Gram-positive [Common Gram-positive bacteria detected by the test include: *Staphylococcus, Streptococcus, Bacillus, Listeria, Enterococcus, Clostridium*.], or Gram-negative. [Common Gram-negative bacteria detected by the test include: *E. coli, Klebsiella species, Proteus species Pseudomonas aeruginosa*.].

B- A normal test result means that few white blood cells and no bacteria have been found in your sputum sample, and your symptoms may be due to other causes.



Acid Fast Results





A = Non acid fast

B = Acid Fast Bacteria

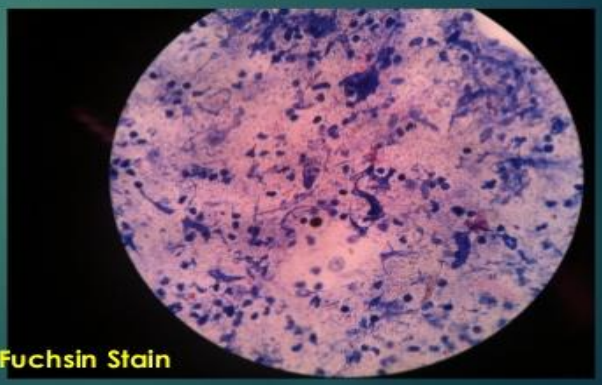
ACID FAST BACTERIA VERSUS NON ACID FAST BACTERIA

Acid fast bacteria	Non acid fast bacteria
Acid fast bacteria are a type of bacteria that resist decolorizing by acid after staining	Non acid fast bacteria are a type of bacteria that are readily decolorized by acid after staining
Final color is pink or red	Final color is blue
Stained with the primary stain	Stained with the counter stain
Consist of mycolic acid in their cell wall	Do not consist of mycolic acid in their cell wall
Mycobacterium is an example	*Escherichia coli* is an example

Visit www.pediaa.com

ACID FAST BACILLI:

- *Mycobateria are virtually the only bacteria that are acid-fast because of the presence of* **mycolic acid and their lipid-rich cell walls**, *which are relatively impermeable to various basic dyes unless the dyes are combined with phenol.*
- *Once stained, the cells resist decolourization with acidified organic solvents and are therefore called "acid-fast".*



Carbol Fuchsin Stain

Routine Culture.

Most of the commonly sought etologic agents of lower respiratory tract infection are isolated on routine media:

- 5% sheep blood agar, MacConkey agar for the isolation and differentiation of gram negative bacilli.
- Chocolate agar for *Haemophilus* and *Neisseria spp*.
- Because of contaminating oral flora, sputum specimens obtained by bronchial washing and lavage, tracheal aspirates, and tracheostomy or endotracheal tube aspirates are not inoculated to enrichment broth or incubated anaerobically

- After 24-48 hours of incubation, the numbers and types of colonies are recorded.
- Sputum specimens from patients known to have cyctic fibrosis (CF) should be inoculated to selective agar, such as specific chromogenic agar, for recovery of *S.aureus* and selective horse blood-bacitracin, inoculated anaerobically and aerobically, for recovery of *Haemophilus influenza* that may be obscured by the mucoid *Pseudomonas* on routine media.
- The use of selective medium for *Burkholderia cepacia*, such as *Burkholderia cepacia* agar (BC) or (Oxidation Fermentation Polymyxin Bacitracin Lactose Agar(OFPBL) agar, is also necessary.
- To ensure optimum culture reporting, conditions must be well defined in terms of an objective grading system for streaked plates.
- Finally, the clinical significance of culture findings depende not only on standardized and appropriate laboratory methods but also on how specimens are collected and transported, other laboratory data, and the patient's clinical presentations.
- Numerous bacterial agents that cause lower respiratory tract infections are not detected by routine bacteriologic culture.
- Mycobacteria, *Chlamydia*, *Nocardia*, *Bordetella pertussis*, *Legionella*, and *Mycoplasma pneumoniae* require special procedures for detection, this also applies to viruses and fungi.

- Optimal recovery for *Mycobacterium tuberculosis* requires multiple specimens for acid-fast staining, culture and at least one sample for molecular testing.

B- Upper Respiratory Tract Infections and Other Infections of the Oral Cavity and Neck.

It is important to keep in mind that upper respiratory tract infections may spread and become more serious because the mucosa (mucous membrane) of the upper tract is continuous with the mucosl lining of the sinuses, eustachin tube, middle ear and lower respiratory tract.

Pathogenesis.

Pathogenic mechanisms differ and depend on the organism causing the pharyngitis. For example, some organisms directly invade the pharyngeal mucosa (e.g. *Arcanobacterium haemolyticum*), others elaborate toxins and other virulence factors at the site (e.g. *Corynebacterium diphtheria*), and others invade the pharyngeal mucosa and elaborate toxins and other virulence factors (e.g. group A streptococci *Streptococcus pyogenes*).

Note: The type of swab used for collection is very important.

- For example, cotton swabs should never be used for culture because because fibers contain fatty acids on the surface, which are capable of killing *Bordetella*. Calcium alginate or Darcon swabs are acceptable for

obtaining nasopharyngeal swab specimens, with calcium alginate being optimal for culture.

- However, if polymerase chain reaction (PCR) is to be performed, Darcon or rayon swabs on plastic shafts are preferred.
- Specimens for *B.pertussis* ideally should be inoculated directly to fresh culture media at the patient's bedside. If this is not possible, transport for less than 2 hours in 1% Casamino acid medium at room temperature is acceptable. If specimens are plated on for a day after collection, Amies transport medium with charcoal is acceptable.
- If specimens are plated more than 24 hours after collection, Regan-Lowe or Jones-Kendrick transport medium is optimal, both contain charcoal, starch and nutrients as well as cephalexin. If lengthy delays in transport are expected, transport of specimens in Regan-Lowe medium at 4C° is recommended.

Direct visual examination or detection.

- A Gram stain of material obtained from upper respiratory secretions or lesions may not improve diagnosis.
- Yeast-like cells can be identified, which are helpful in identifying thrush, and the characteristic pattern of fusiform and spirochetes of Vincent's angina may be visualized.
- Gram's crystal violet (allowed to remain on the slide for (1 min) before rinsing with tap water) and the Gram stain can be used to identify the spirilla and fusiform bacilli of Vincent's angina caused by *Borrelia vincentii*.(G-ve)
- If crystal violet is used, the smear should be very thin because everything will be intensely Gram positive, making a thick smear difficult to read.
- Spirilla and bacilli may be stained using a dilute solution of carbol fuchsin.

Note: For causes of pharyngitis, Gram stains are unreliable. Direct smears of exudate from membrane-like lesions used to differentiate diphtheria from other causes are also not reliable or recommended.

Pathogenes and Cultures.

Streptococcus pyogenes (Beta-Hemolytic Group A Streptococci).

Streptococcus pyogenes is the primary cause of bacterial pharyngitis in North America, so most laboratories routinely screen throat cultures for this organism. Group A streptococcus are usually β-hemolytic, with less than 1% being nonhemolytic. Three variables must be taken into consideration regarding successful culture of group A streptococcus from pharyngeal specimens: medium, atmosphere, and duration of incubation. There are four combinations of media and atmosphere of incubation for throat specimens have been recommended as listed in the table(1).

Regardless of the medium and atmosphere of incubation, culture plates should be incubated for at least 48 hours before reporting as negative for group A streptococci.

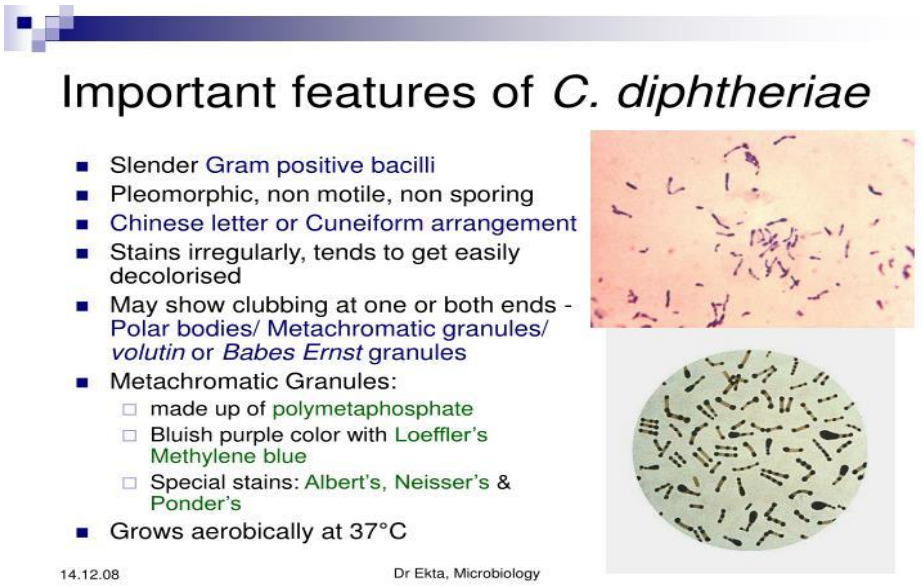
In addition, the incubation of sheep blood agar in (5-10)% CO_2 was strongly discouraged. Drawbacks to culture include an extended incubation time of 24-48 hours for visible colony formation with additional manipulations of the β-hemolytic organisms for definitive identification. If sufficient numbers of pure colonies are not available for identification, subculture requiring additional incubation is necessary.

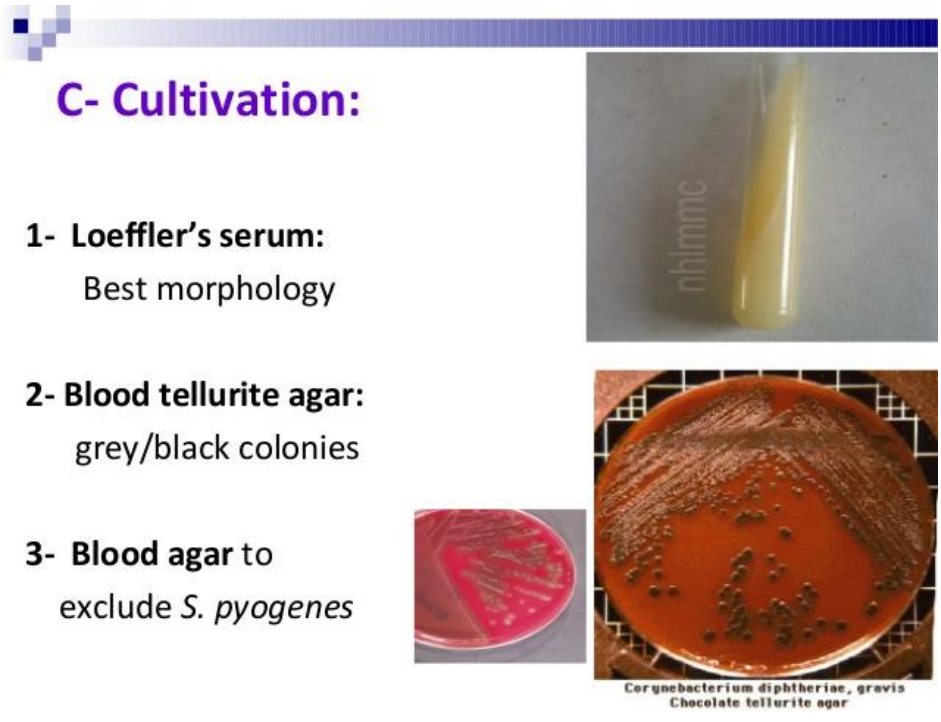
Table(1):Medium and atmosphere for incubation of culture to recover group A streptococci from pharyngeal specimens.

Media	Atmosphere of Incubation
Sheep blood agar	Anaerobic.
Sheep blood agar with coverslip over the primary area of inoculation	Aerobic.
Sheep blood agar with trimethoprime-sulfamethoxazole	(5-10)% CO_2 or anaerobic.

Corynebacterium diphtheria.

It is G+ve small rods. If diphtheria is suspected, the physicians must communicate this information to the clinical laboratory. Because streptococcal pharyngitis is included in the differential diagnosis of diphtheria and because dual infections occur, cultures for *Corynebacterium diphtheria* should be plated onto sheep blood agar or selective agar, as well as onto special media for recovery of this agent. These special media include Loeffler's agar slant and cysteine-tellurite agar plate.





Bordetella pertussis.

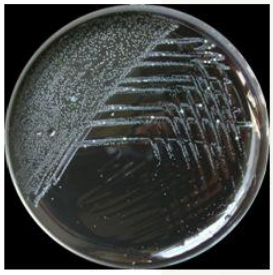
Freshly prepared Bordet-Gengou agar was the first medium developed for isolation of *Bordetella pertussis*. However, because it was inconvenient to use, other media were subsequently developed. Today, Regan-Lowe or charcoal horse blood agar is recommended for use in diagnostic laboratories. Specimens should be plated directly onto media if possible, because the organisms are extremely delicate.

Bordetella pertussis

- Whooping cough
- One most highly communicable diseases of childhood
- DPT vaccine

Laboratory

- Tiny GNR or GNCB
- Slow growing 3-5 days
- **Regan-Lowe** Charcoal media
  - Young colonies - mercury droplets, as age turn whitish gray
- **Bordet-Gengou Potato** Infusion agar
  - Small, white, domed "pearls"
  - *B. pertussis* & *B. parapertussis* – hemolytic
- **Nucleic acid detection by PCR**
  - Primary rapid diagnostic tool
  - Nasopharyngeal swabs

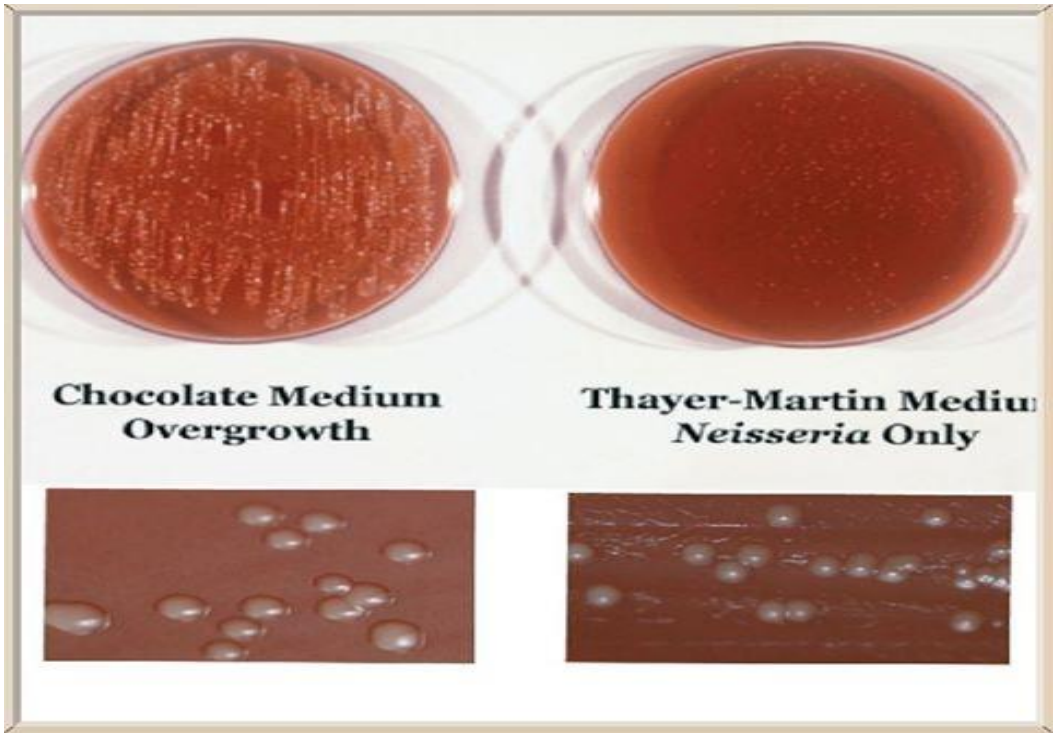


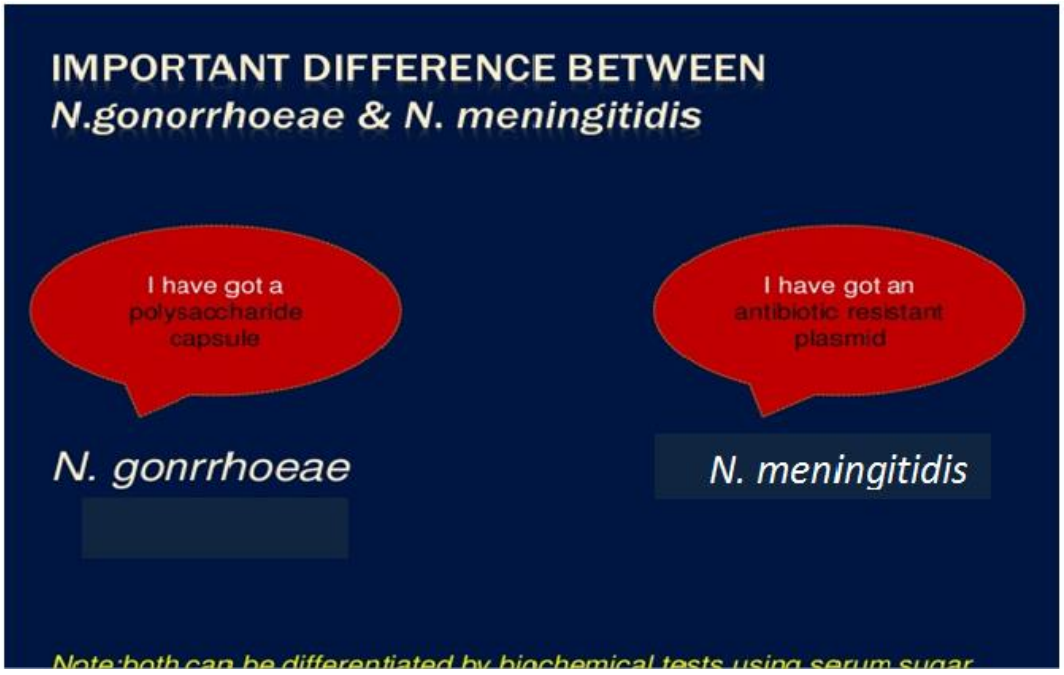
Cultivation

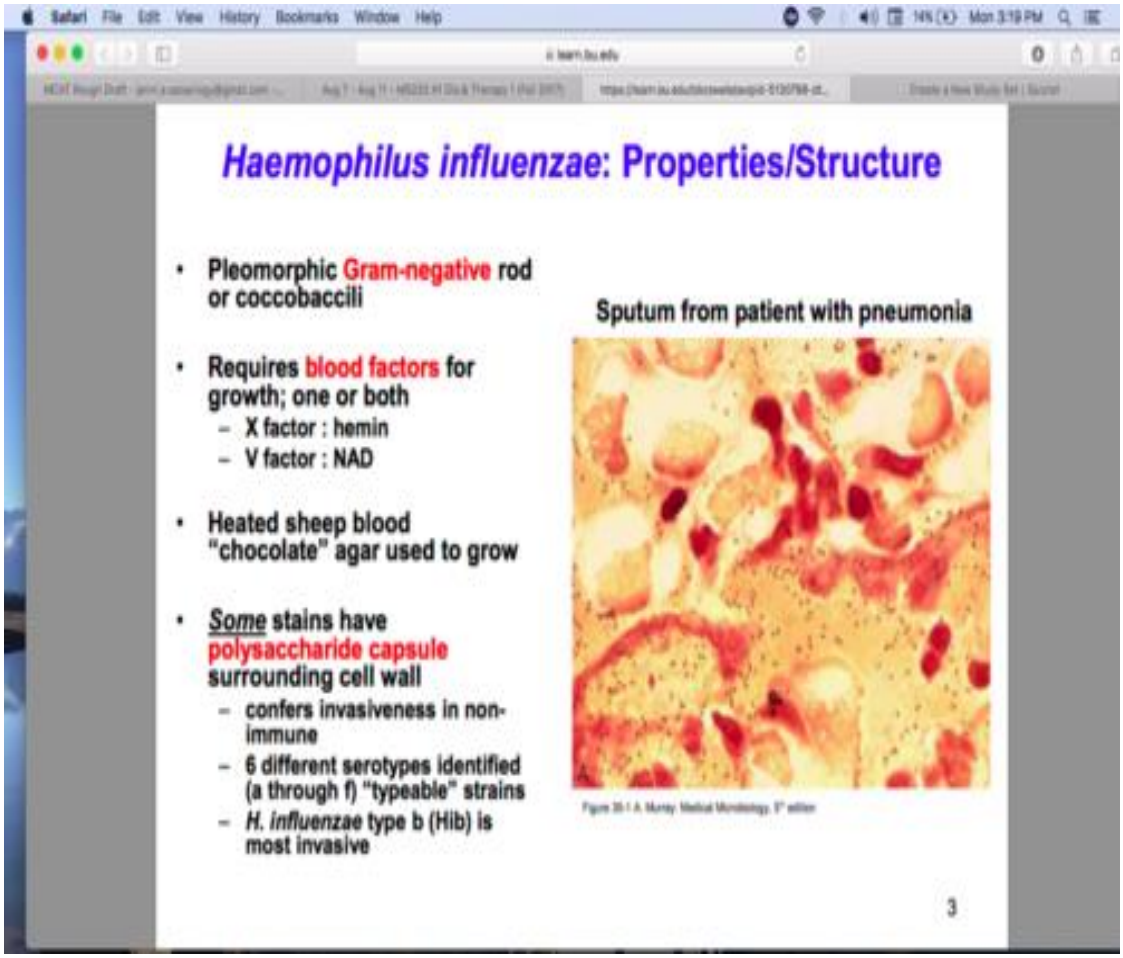
- *B. pertussis* is fastidious (it doesn't grow on typical blood agar)
- Growth after 3-5 days at 35°C in a humidified atmosphere without elevated carbon dioxide on Regan-Lowe medium
- Regan-Lower is a charcoal agar with 10% horse blood and cephalexin antibiotic
- It appears as small, smooth shiny colonies with a pearl-like luster resembling mercury droplets surrounded by a zone of hemolysis

Neisseria.

It is G-ve diplococci. Specimens received in the laboratory for isolation of *Neisseria meningitides* (for detection of carriers) or *N.gonorrhoeae* should be plated onto selective medium either modified Thayer-Martin or Martin-Lewis agar. After 24-48 hours of incubation in (5-10)% CO_2, typical colonies of *Neisseria* spp. May be visible.






Haemophilus influenzae: Properties/Structure
• Pleomorphic Gram-negative rod or coccobaccili
• Requires blood factors for growth; one or both
– X factor : hemin
– V factor : NAD
• Heated sheep blood "chocolate" agar used to grow
• Some stains have polysaccharide capsule surrounding cell wall
– confers invasiveness in non-immune
– 6 different serotypes identified (a through f) "typeable" strains
– H. influenzae type b (Hib) is most invasive
Sputum from patient with pneumonia
3