Throat swab culture
LAB 1
Throat swab

A throat swab culture is a laboratory test done to isolate and identify organisms that may cause infection in the throat mainly group A beta-hemolytic streptococci.

**Collection of Specimen**

**Who will collect the specimen**
- Physician Or Medical technologist, Microbiologist, experienced nurse.

**Type of specimen**
- Two Swabs from posterior pharynx, tonsils, or other inflamed area.

**Storage**
- Maintain specimen swab at room temperature.
<table>
<thead>
<tr>
<th>Common pathogenic bacteria</th>
<th>Commensals flora</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-haemolytic streptococci group A</strong></td>
<td>The upper respiratory tract includes the epiglottis and surrounding tissues, larynx, nasal cavity, and the pharynx.</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>α haemolytic streptococci</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Neisseria species other than N. gonorrhea</td>
</tr>
<tr>
<td>Klebsiella spp and other Enterobacteriaceae</td>
<td>Coagulase negative staphylococci</td>
</tr>
<tr>
<td>Bacteroides spp. and other anaerobes</td>
<td>Staph. aureus (occasionally)</td>
</tr>
<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>Haemophilus haemolyticus</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Diphtheroides</td>
</tr>
<tr>
<td></td>
<td>Occasionally β-haemolytic streptococcus other than group A</td>
</tr>
</tbody>
</table>
Throat Swabs collection procedure

I. Turn the patients face against the light, ask the patient to open his mouth wide and phonate an “ah” gently depress the patients tongue with a tongue blade so that the throat is well exposed and illuminated.

II. Guide a swab over the tongue into the posterior pharynx.

III. Rub the swab firmly over the back of the throat, both tonsils and any areas of inflammation, exudation or ulceration. Care should be taken to avoid touching the tongue, cheeks or lips with the swab.

IV. Place the swab in the transport medium and push it down to the bottom.
# Throat Swabs Collection Procedure

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection Guidelines</th>
<th>Time and Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1- Depress tongue with tongue depressor.</td>
<td></td>
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<tr>
<td></td>
<td>2- Sample posterior pharynx, tonsils, and inflamed areas with sterile swab.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3- Collect two swabs one for gram stain and the other for culturing.</td>
<td>Swab transport medium $\leq 2h,RT$ $\leq 24h,RT$</td>
</tr>
</tbody>
</table>

### Criteria of specimen rejection
- Inappropriate specimen transport device.
- Mislabeled specimen.
- Unlabeled specimen.
- Dried samples.
- Specimen received after prolonged delay (usually more than 2 hours).
- Specimen received in expired transport media.

*S. pyogenes* is highly resistant to desiccation and remains viable on a dry swab for as long as 48 to 72 hours.
Culture plates should be incubated for at least **48 hours** before reporting as negative for group A streptococci. In addition, the incubation of plates in 5% to 10% CO$_2$. (also aerobic and anaerobic conditions are used but CO2 condition is perfect)
Gram Stain

Direct smear:
- A gram stain from the swab noting the predominant organism.
Throat Swab Culturing

Culture

- Because Streptococcus pyogenes is the primary case of pharyngitis most laboratories routinely screen throat cultures for this organism.

- Classically throat swabs plated on 5% sheep Blood agar plates and Columbia C.N.A, streak the swab across first quadrant of blood agar plate and using a sterile loop streak to produce isolated colonies, make few stabs in the agar plates also, Group A Streptococcus (S. pyogenes) are usually ß- hemolytic the activity of hemolysin enzyme will increased by the stabbing.

Note

- Inoculate another Chocolate and MacConkey agar plates also are recommended if organisms other than S. pyogenes is suspected.
Columbia C.N.A. agar with Blood

Ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>Colistin sulphate</td>
</tr>
<tr>
<td>Tryptic digest of beef heart</td>
<td>Nalidixic Acid</td>
</tr>
<tr>
<td>Corn starch</td>
<td>Agar</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Sheep Blood</td>
</tr>
</tbody>
</table>

- **Nalidixic Acid** and **Colistin sulphate** are the antimicrobics suppressing the growth of Enterobacteriaceae and Pseudomonas spp., and allowing yeast, Staphylococci, Streptococci, and Enterococci to grow.
- Certain Gram-negative organisms, such as *Gardnerella vaginalis* and certain Bacteriodes spp., can grow very well on Columbia CNA Agar with blood.
- Colistin disrupts the cell membrane of Gram-negative organisms, particularly effective against Pseudomonas spp.
- Nalidixic Acid blocks DNA replication in susceptible bacteria and acts against many Gram-negative bacteria.
Make few stabs in the agar plates

Note: Make few stabs to increased hemolysin activity of group A Strepto.
Streptococcal Selective agar (SSA)

**Ingredients:**

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<tr>
<td>Peptone</td>
<td>Colistin sulphate</td>
</tr>
<tr>
<td>Peptic Digest of Soybean</td>
<td>crystal violet</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>Agar</td>
<td>Sheep Blood</td>
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- Streptococcal selective agar (SSA) is available commercially.
- A modification of sheep blood agar, this medium contains crystal violet, trimethoprim-sulfamethoxazole, and colistin in concentrations adequate to inhibit most bacteria except for *Streptococcus pyogenes* and *S.agalatiae*.
- Beta hemolysis is readily observed.
- The medium is effective for primary plating of throat swabs for detection of group A streptococci.
Direct antigen detection

Identification of group A Streptococcal antigen in throat specimens are available now by using different methods including latex agglutination, enzyme immunoassay and gene probe technology, that allow detection of Streptococcal group A antigen within at little as 10 minutes.

Antistreptolysin O titre (ASOT).
1. **Lateral flow test**
The sample is applied to a strip of nitrocellulose film and, if GAS antigens are present, these will migrate along the film to form a visible line of antigen bound to labeled antibodies.

2. **Immunoassay**
The newest and more expensive test. It involves mixing the sample with labeled antibodies and then with a special substrate on a film which changes colour to signal the presence or absence of GAS antigen.
Note  small, gray white, transparent to translucent colonies, beta hemolysis (complete lysis of the red blood cells around the colonies; see arrows), and sensitive to the antibiotic bacitracin.
Outline of differentiation between Gram-Positive cocci

Gram Positive Cocci Flow Chart

- Gram Positive Cocci → Catalase Test
  - Positive
    - Staphylococcus species → Coagulase Test
      - Positive → Staphylococcus aureus
      - Negative → A Coagulase-Negative Staphylococcus species
  - Negative
    - Streptococcus species
      - Growth on Sheep's Blood Agar
        - Beta Hemolytic
          - No Sensitive
            - Streptococcus pyogenes (GAS)
          - Yes Resistant
            - Another β-hemolytic streptococcus
        - Alpha Hemolytic
          - No Sensitive
            - Streptococcus pneumoniae
          - Yes Resistant
            - α-hemolytic streptococci
Differentiation between β-hemolytic streptococci

- The following tests can be used to differentiate between β-hemolytic streptococci
  - Lanciefield Classification
  - Bacitracin susceptibility Test
    - Specific for *S. pyogenes* (Group A)
  - CAMP test
    - Specific for *S. agalactiae* (Group B)
Bacitracin sensitivity

- **Principle:**
  - Bacitracin test is used for presumptive identification of group A
  - To distinguish between *S. pyogenes* (susceptible to B) & non group A such as *S. agalactiae* (Resistant to B)
  - Bacitracin will inhibit the growth of gp A *Strep. pyogenes* giving zone of inhibition around the disk

- **Procedure:**
  - Inoculate BAP with heavy suspension of tested organism
  - Bacitracin disk (0.04 U) is applied to inoculated BAP
  - After incubation, any zone of inhibition around the disk is considered as susceptible
CAMP test

• Principle:
  – Group B streptococci produce extracellular protein (CAMP factor)
  – CAMP act synergistically with staph. \( \beta \)-lysin to cause lysis of RBCs

• Procedure:
  – Single streak of *Streptococcus* to be tested and a *Staph. aureus* are made perpendicular to each other
  – 3-5 mm distance was left between two streaks
  – After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis
  – *S. agalactiae* is CAMP test positive while non gp B streptococci are negative
CAMP Factor Test

Group B Streptococcus
(CAMP Factor)

S. aureus
(Spingomyelinase C)

Group A Streptococcus

Enhanced Zone of Hemolysis

CAMP is an acronym for the authors of this test (Christie, Atkinson, Munch, Peterson). The CAMP test takes advantage of the capacity of GBS-group B strep to produce this CAMP factor; most other hemolytic streptococci do not produce CAMP factor. Enhances the ability of S. aureus to produce Beta hemolysis.
Differentiation between $\alpha$-hemolytic streptococci

- The following definitive tests used to differentiate between *S. pneumoniae* & viridans streptococci
  - Optochin Test
  - Bile Solubility Test
Optochin Susceptibility Test

• **Principle:**
  – Optochin (OP) test is presumptive test that is used to identify *S. pneumoniae*
  – *S. pneumoniae* is inhibited by Optochin reagent (<5 μg/ml) giving a inhibition zone ≥14 mm in diameter.

• **Procedure:**
  – BAP inoculated with organism to be tested
  – OP disk is placed on the center of inoculated BAP
  – After incubation at 37°C for 18 hrs, accurately measure the diameter of the inhibition zone by the ruler
  – ≥14 mm zone of inhibition around the disk is considered as positive and ≤13 mm is considered negative

• *S. pneumoniae* is positive (S) while *S. viridans* is negative (R)
Optochin Susceptibility Test

Optochin resistant
S. viridans

Optochin susceptible
S. pneumoniae
Bile Solubility test

• Principle:
  – *S. pneumoniae* produce a self-lysing enzyme (autolysin) to inhibit the growth
  – The presence of bile salt accelerate this process

• Procedure:
  – Add ten parts (10 ml) of the broth culture of the organism to be tested to one part (1 ml) of 2% Na deoxycholate (bile) into the test tube
  – Negative control is made by adding saline instead of bile to the culture
  – Incubate at 37°C for 15 min
  – Record the result after 15 min
Bile Solubility test

• Results:
  – Positive test appears as clearing in the presence of bile while negative test appears as turbid
  – *S. pneumoniae* soluble in bile whereas *S. viridans* insoluble
Differentiation between β-hemolytic streptococci

<table>
<thead>
<tr>
<th></th>
<th>Hemolysis</th>
<th>Bacitracin sensitivity</th>
<th>CAMP test</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pyogenes</td>
<td>β</td>
<td>Susceptible</td>
<td>Negative</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>β</td>
<td>Resistant</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Differentiation between α-hemolytic streptococci

<table>
<thead>
<tr>
<th></th>
<th>Hemolysis</th>
<th>Optochin sensitivity</th>
<th>Bile solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>α</td>
<td>Sensitive (≥ 14 mm)</td>
<td>Soluble</td>
</tr>
<tr>
<td>Viridans strep</td>
<td>α</td>
<td>Resistant (≤ 13 mm)</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>
Outline of differentiation between Gram-Negatives

Gram negative

Coccobacilli
- H. influenzae - X & V factors required
- B. pertussis - growth on Bordet-Gengou medium, oxidase +
- Brucella spp. - aerobic
- F. tularensis - requires cystein for growth
- P. multocida - oxidase +, catalase +
- L. pneumophila - growth on charcoal yeast agar with iron and cysteine

Cocci = spp. Neisseria
- N. meningitidis glucose & maltose +
- N. gonorrhoeae glucose +

Lactose +
- Fast fermenter
  - Klebsiella urease +
  - E. coli indole +
  - Enterobacter
- Slow fermenter
  - Citrobacter
  - Serratia
  - Others

Lactose -
- Oxidase +
  - V. cholerae glucose +
  - P. aeruginosa
- Oxidase -
  - Strict anaerobe
    - B. fragilis

Urease +
- P. mirabilis
- H. pylori
- Y. pestis, bipolar staining
- Y. enteroltyca, motile at 25°C, non-motile at 37°C
- grows on campy agar

Urease -
- C. jejuni, grows on campy agar
- S. dysenteriae, non-motile
- Salmonella spp. motile & produces H₂S
Corynebacterium Diphtheria
Klebs-Löffler bacillus

- Potassium tellurite medium (Hoyle's Agar)—black colonies within 48hrs
- Loefflers serum slope—creamy white colonies in 6-8 hrs
Bordetella pertussis

Bordet-Gengou agar

- blood, potato extract, and glycerol,
- with an antibiotic such as cephalaxin or penicillin and sometimes nicotinamide

Regan-low charcoal agar

- charcoal, blood, and antibiotic (cephalexin)
- Medium of choice
Haemophilus influenzae b

M. catarrhalis

Chocolate agar

Chocolate or blood agar
**Legionella**

buffered charcoal yeast extract (BCYE) agar

Cysteine, Iron

incubation for up to 10 days

**N. gonorrhoeae**

Thayer martin agar

Chocolate sheep blood plus antibiotics (vancomycin, colistin, nystatin, and TMP-SMX)
Post specimen processing

Interfering factors:
- Patient on antibiotic therapy.
- Improper sample collection.

Result reporting:
- Report Gram stain finding as an initial report.
- Report the isolated and its sensitivity pattern as a final report.

Turn around time:
- Gram stain result should be available half hour after specimen receipt.
- Isolation of a possible pathogen can be expected after 2-4 days.
- Negative culture will be reported out 1-2 days after the receipt of the specimen.
LAB 2

Sputum sample collection
Sputum Definition

1. It is a secretion that is produced in the lungs and the bronchi (tubes that carry the air to the lung), also known as phlegm
2. This mucus-like secretion may become infected, blood stained, or contain abnormal cells that may lead to a diagnosis
3. Tracheobronchial sections are an inconstant mixture of plasma, water, electrolytes and mucin
4. As these mixture pass through the lower and upper respiratory tract, they become contaminated with cellular exfoliations, nasal and salivary gland secretions and normal bacterial flora of the oral cavity
Sputum specimens

Ordered to identify organisms growing in sputum

➢ C&S
➢ AFB
  ➢ 3 consecutive, early am
➢ Cytology
  ➢ Abnormal lung cancer by cell type
  ➢ 3 early am
Sputum collection

Sputum (Expectorate):
1. Collect early morning specimen under the direct supervision of a nurse or a physician.
2. Have patient rinse or gargle with water to remove superficial flora.
3. Instruct patient to cough deeply to produce a lower respiratory specimen.
4. Exam specimen to make sure it contains thick mucus. Do not submit saliva.

Sputum (Induced):
1. Have patient rinse mouth with water after brushing gums and tongue.
2. With the aid of a nebulizer, have patients inhale about 25 mL of 3 to 10% sterile saline.
3. Collect the induced sputum in a sterile container.
Sputum Collection

1. Drinking a lot of water and other fluids the night before the test may help to get the sample
2. To be asked to cough deeply and spit any sputum in a sterile cup
3. The sputum is then taken to the laboratory
4. There, it is placed in a special substance (medium) under conditions that allow the organisms to grow
Physical Properties of Sputum

1. Appearance
   • It may be described as liquid (serous), mucoid, purulent, bloody or combination of these.

2. Color
   • Normal sputum is either white or colorless.
   • Yellow to green sputum can be an indication of pus, infection such as pneumonia.
   • Blood in sputum is called hemoptysis which could be due to e.g.; lung cancer, tuberculosis, lung abscess, hemorrhage.
   • Rust color is due to decomposed Hemoglobin and it is typical for S. pneumonia.
   • Very thick (viscose) sputum is a characteristic of cystic fibrosis.
   • Parasites in sputum can occur as in Ascaris.
3. **Odor**

- Usually no odor is present in normal and pathological sputum, but if bacterial decomposition has been taken place within the body or after expectoration, a variety of odor will be present.
# Sputum Chemical Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>% of Total Weight</th>
<th>% of Total Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>95 %</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>5 %</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Enzymes, α-antitrypsin, LDH, lysozyme, lactoferrin</td>
<td>Variable</td>
<td></td>
</tr>
</tbody>
</table>
Sputum Analysis: Pneumonia

- *Moraxella catarrhalis*, a large number of Gram negative (red) cocci (di-) are seen and many appear to be attaching to or residing within the PMNs
- Some physicians confuse these organisms with the Gram negative coccobacillary
Sputum Analysis: Pneumonia

• *Hemophilus* influenzae pneumonia demonstrating the typical Gram negative coccobacillary forms

• Because of the red background produced by the Gram stain method, these organisms can be difficult to see (oil immersion, 1000x)
Sputum Analysis: Pneumonia

- *Klebsiella pneumoniae* demonstrating Gram negative bacillary organisms. (oil immersion, 1000x)
Klebsiella pneumoniae

MacConkey agar

- It contains bile salts (inhibit Gram-positive bacteria), crystal violet dye (inhibits Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), lactose and peptone.
- Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium
Sputum Analysis: Pneumonia

• Gram stain of the sputum from a patient with *Staphylococcus* aureus pneumonia demonstrating clusters of Gram positive cocci some of which are associated with the PMNs (oil immersion, 1000x)
Sputum Analysis: Pneumonia

• Gram-positive, spherical bacteria, *Streptococcus pneumoniae*, is the cause of many human diseases, including pneumonia
Sputum Analysis: Pneumonia Histoplasma capsulatum

- At 25°C forming macroconidia
- At 37°C yeast form

Grows on Blood agar, Chocolate agar and Sabouraud’s agar (dextrose and peptone; PH 5.6). Takes few weeks to grow.
Coccidioides immitis

Mold form in environment and in cultures regardless of incubation temp

*C. Immitis* arthroconidia

C. *Immitis* spherule form in tissue

Culture of *Coccidioides immitis* on a Medium with Cycloheximide

*C. Immitis* spherule
Sputum Analysis: Pneumonia
Aspergillus fumigatus

• Conidial head

Aspergillus require 1-3 weeks for growth. The colony begins as a dense white mycelium which later assumes a variety of colors, according to species.
Ziehl Nielsen's Stain

1. Smear the sputum
2. Fix by Heating
3. Pour carbol fuchsin and heat it from below, Keep for 5 min.
4. Wash with water, Decolorize with 20%H₂SO₄
5. Wash with Löffler's methylene blue for 1 min.
6. Wash & dry
7. Mount under oil immersion
1. Cover smear with carbolfuchsin. Steam over boiling water for 8 minutes. Add additional stain if stain boils off.

2. After slide has cooled decolorize with acid-alcohol for 15 to 20 seconds.

3. Stop decolorization action of acid-rinsing briefly with water.

4. Counterstain with methylene blue for 30 seconds.

5. Rinse briefly with water to remove excess methylene blue.

6. Blot dry with bibulous paper. Examine directly under oil immersion.

Ziehl-Neelsen acid-fast staining procedure
Mycobacterium tuberculosis Ziehl-Neelsen stain
Löwenstein–Jensen medium

- The usual composition as applicable to *Mycobacterium tuberculosis* is:
  - Malachite green
  - Glycerol
  - Asparagine
  - Potato starch
  - Coagulated eggs
  - Mineral salt solution
  - Penicillin and nalidixic acid
Alternative culture media

- Egg-based – Petragnini medium and Dorset medium
- Middlebrook 7H10 Agar
- Middlebrook 7H9 broth