Examination of throat and mouth specimens

COLLECTION AND TRANSPORT OF THROAT AND MOUTH SWABS

1- In a good light and using the handle of a spoon to depress the tongue, examine the inside of the mouth. Look for inflammation, and the presence of any membrane, exudate, or pus.

– With diphtheria, a greyish-yellow membrane (later becoming greyish green-black and smelly) can often be seen extending forwards over the soft palate and backwards onto the pharyngeal wall.

– With a streptococcal sore throat, the tonsils are inflamed and often covered in yellow spots.

2- Swab the affected area using a sterile cotton wool swab. Taking care not to contaminate the swab with saliva, return it to its sterile container.

***Important**:* For 8 hours before swabbing, the patient must not be treated with antibiotics or antiseptic mouth-washes (gargles).

3- Within two hours of collection, deliver the swab with a completed request form to the laboratory.

4- Transport of swabs in tubes containing silica gel It has been shown that *S. pyogenes* will remain viable for at least 3 days (at ambient temperatures) on swabs stored in tubes containing 3–5 g of *desiccated* silica gel.

LABORATORY EXAMINATION OF THROAT and MOUTH SPECIMENS

Possible pathogens

BACTERIA

Gram positive

Streptococcus pyogenes

Corynebacterium

diphtheriae

Corynebacterium ulcerans

Note: Pathogens in the upper respiratory tract such as *Bordetella pertussis*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*, are usually more successfully isolated from naso-pharyngeal secretions collected by aspiration.

Commensals

Gram positive	***Gram negative***
Viridans streptococci	*Moraxella catarrhalis*
Non-haemolytic streptococci	*Neisseria pharyngitidis*
	Fusobacteria
Streptococcus pneumoniae	Coliforms
Staphylococcus epidermidis	*Bacteroides* species
Micrococci	*Haemophilus influenza*
Lactobacilli	(mostly non-capsulate strains)
Diphtheroids	

Day 1

1- Culture the specimen

Blood agar

– Inoculate the swab on a plate of blood agar. Use the loop to make also a few stabs in the agar (well area). Colonies of *S. pyogenes* growing below the surface will show more distinct zones of hemolysis because of the anaerobic conditions provided.

– When a swab is received in silica gel, moisten it first with sterile nutrient broth and then inoculate the plate.

– Add a 0.05-unit bacitracin disc to the plate. This will help in the identification of *S. pyogenes*. Some workers also add a co-trimoxazole disc (as used for susceptibility testing) which prevents the growth of other bacteria, making it easier to see *beta* hemolytic *S. pyogenes* colonies.

– Incubate the plate preferably anaerobically or, when this is not possible, in a carbon dioxide enriched atmosphere overnight at 35–37 C. Candle jar incubation will detect most *beta* hemolytic streptococci.

Note: *Beta*-hemolytic streptococci produce larger zones of hemolysis when incubated anaerobically.

Culture of specimen when diphtheria is suspected

When diphtheria is suspected and culture is *specifically requested*, inoculate the swab on Tinsdale medium or tellurite blood agar. Incubate the plate aerobically at 35–37 C for up to 48 hours, examining for growth after overnight incubation**.**

Examine the specimen microscopically *Gram smear*

Make an evenly spread smear of the specimen on a slide. Allow the smear to air-dry in a safe place. Fix and stain by the Gram technique. Use dilute carbolfuchsin (1 in 10 dilution) as the counterstain in preference to safranin or neutral red.

Other bacteria: No attempt should be made to report *routinely* other bacteria in a Gram stained smear from a throat swab because the throat contains a wide variety of commensals that cannot be distinguished morphologically from pathogens.

Albert stained smear when diphtheria is suspected

Prepare the smear as described previously under Gram smear. Fix with alcohol and stain by the Albert staining technique. Examine the smear for bacteria that could be *C. diphtheriae*. Look for pleomorphic rods containing dark-staining volutin granules. The pleomorphic rods tend to join together at angles giving the appearance of Chinese letters.

Note: In Gram stained smears, *C. diphtheriae* stains variably and weakly Gram positive, whereas commensal diphtheroids appear strongly Gram positive.

Day 2 and Onwards

1- **Examine and report the cultures *Blood agar culture***

Look for *beta*-hemolytic colonies that could be *Streptococcus pyogenes* (Lancefield Group A *Streptococcus*). Most strains are sensitive to bacitracin. However, bacitracin sensitivity cannot be completely relied on to identify *S. pyogenes*. The organism should be tested serologically to confirm that it belongs to Lancefield Group A or tested biochemically using the PYR test. PYR test for the presumptive identification of *S. pyogenes*

Isolation and identification of C. diphtheriae

The cultural features of *C. diphtheriae* on Tinsdale medium and tellurite blood agar (TBA). Identify as follows: –

Examine a Gram stained smear for variable staining pleomorphic rods, Sub inoculate two slopes of Dorset egg medium or Loeffler serum agar (The medium enhances the development of metachromatic granules as seen in methylene blue stains. Formation of the granules demonstrates the characteristic cellular morphology of *C. diphtheriae*.). Incubate at 35–37 _C for 6 hours or until sufficient growth is obtained.

Corynebacterium diphtheriae cultivated on Tinsdale agar (TIN). Tinsdale agar is used for the primary isolation and identification of *Corynebacterium diphtheriae*. It contains **L-cysteine** and **sodium thiosulfate** that are H_2S indicators. **Potassium tellurite** is the selective agent (inhibits most of the upper respiratory tract normal flora) that turns the media brown-black as a result from the reduction of potassium tellurite to metallic tellurite.

Tellurite Blood Agar is a selective medium used for isolation and cultivation of Corynebacterium species.Potassium tellurite acts as a selective agent and has inhibitory activity against most gram-positive and gram-negative bacteria except Corynebacterium species.
C.diphtheriae reduces potassium tellurite to tellurium and thereby produce gray-black coloured colonies.

1- **Antimicrobial susceptibility testing**

WHO in its publication *Basic Laboratory Procedures in Clinical Bacteriology* advises that routine susceptibility tests on throat or pharyngeal isolates are most often not required, and may even be misleading. The major pathogens involved in bacterial pharyngitis are *S. pyogenes* and *C. diphtheriae*. Benzylpenicillin and erythromycin are considered as the antibiotics of choice to treat both types of infection. In cases of diphtheria, treatment with antitoxin is also indicated.

Summary of Microbiological Examination of Throat and Mouth Swabs

Day 1

			ADDITIONAL INVESTIGATIONS
1	**Culture Specimen**	■ *Blood agar* – Add a bacitracin disc – Incubate, preferably anaerobically (or in CO_2)	■ *MTM or TBA:* When diphtheria suspected
2	**Examine Microscopically**	■ *Gram smear* Look for: – Pus cells and Gram negative Vincent's organisms – Gram positive pleomorphic rods when diphtheria suspected – Gram positive yeast cells when thrush suspected	■ *Giemsa or Wayson's smear:* When diphtheria suspected

Day 2 and Onwards

3	**Examine and Report Cultures**	■ *Blood agar culture* Look for *beta*-haemolytic streptococci, sensitive to bacitracin. *Identify as S. pyogenes* Lancefield group PYR test	■ *MTM or TBA cultures* Examine for growth of *C. diphtheriae*

Key: MTM = Modified Tinsdale medium, TBA = Tellurite blood agar