

### AIMS

This practical class will illustrate some of the characteristics of pyogenic (pus forming) cocci. These include *Staphylococcus aureus* and *Neisseria* species.

#### 20.1 STAPHYLOCOCCAL COAGULASE ACTIVITY

*Staphylococcus aureus* is a common cause of food poisoning (through the production of an exotoxin) and is also the most frequent cause of boils. It is frequently found in nosocomial infections. Pathogenic *Staphylococci* characteristically produce coagulase enzymes that coagulate plasma. The production of coagulase is a characteristic indicator that a strain of *Staphylococcus* is *S. aureus*. In Public Health Laboratories the assay for coagulase activity is now usually performed using latex bead agglutination, however the test below is the classical assay for coagulase activity and a positive result produces a clearly visible veil of polymerized fibrin.

##### **Materials: Tube method (for free coagulase):**

1. Two glass plugged test tubes.
2. Lyophilised Rabbit plasma, diluted 1:5
3. Broth cultures of two different strains of *Staphylococci* (labelled A and B).
4. Automatic pipettor.

##### **Procedure:**

1. Work in pairs. Label the tubes A and B. Pipette 0.5 ml of plasma into each tube.
2. Add 0.5 ml of broth culture A to tube A.
3. Add 0.5 ml of broth culture B to tube B.
4. Mix the contents of the tubes well and incubate in the water bath @ 37°C for 1½ hour.
5. Carefully remove the tubes after incubation, hold them to the light, tilt, rotate, and gently shake them.
6. A positive reaction results in the formation of fibrin that polymerizes and becomes evident as a delicate veil of protein.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_SP_16.jpg	<a href="#">Coagulase test</a>		<a href="#">Coagulase test</a>

#### 20.2 CATALASE TEST: AN EXAMPLE OF A BIOCHEMICAL ASSAY

You may find colonies of *Staphylococcus epidermidis* difficult to distinguish from non-haemolytic *Enterococci*. However, Gram-stained *Staphylococci* may be seen as grape-like clusters of round cocci whereas non-haemolytic *Enterococci* usually form pairs of smaller, oval cocci. A further distinguishing feature is provided by the catalase test in which oxygen is visibly liberated from H<sub>2</sub>O<sub>2</sub> by *Staphylococci*, but not by *Enterococci*.

##### **Materials:**

1. Plate culture (20.2) bearing colonies of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*.
2. Hydrogen peroxide (Note: H<sub>2</sub>O<sub>2</sub> will produce an unpleasant burning sensation if you get it on your skin. Wash well with water if required).
3. Capillary tubes.

##### **Procedure:**

1. Touch the colony with one end of the capillary tube so as to remove a tiny sample on to the inside of the tube without blocking it.

2. Dip the opposite end of the capillary briefly into the peroxide provided.
3. Tilt the tube so that the peroxide runs down onto the bacterial sample.
4. A positive reaction is indicated by the evolution of oxygen within the capillary.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_MX_24.jpg	<a href="#">Catalase test</a>		<a href="#">Catalase test</a>

### 20.3 LANCEFIELD GROUPING OF STREPTOCOCCI

Rebecca Lancefield (1895-1981) showed that most species of *Streptococci* have characteristic carbohydrate antigens in their cell walls. Using the presence of these antigens as a guide, Streptococci can be divided into groups, for example *Streptococcus pyogenes* is in Group A. This procedure speeds the recognition of human and veterinary pathogenic *Streptococci*. To carry out a grouping test, the antigens are extracted in soluble form and identified with antisera.

In the photograph provided, an enzyme extract of the bacterial cell wall material containing fragments on which the group-specific antigens were exposed, was mixed with latex particles that had been pre-coated with group-specific antibodies. The photograph shows a positive result in which the latex particles have been agglutinated by the presence of homologous antigen, and a negative result in which no such agglutination has occurred.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_ST_26.jpg	<a href="#">Lancefield grouping</a>		<a href="#">Lancefield grouping</a>

### 20.4 IDENTIFICATION OF STREPTOCOCCUS PNEUMONIAE

*Streptococcus pneumoniae* is a common cause of bacterial pneumonia, ear infections and meningitis but it may also be a component of the normal flora of the nasopharynx. *Streptococcus pneumoniae*, the pneumococcus, is a capsulated diplococcus that forms  $\alpha$ -haemolytic colonies on blood agar. Upon ageing the colonies tend to lyse spontaneously causing collapse of the central portion.

#### Materials:

1. A pure culture of *Streptococcus pneumoniae* on a blood agar plate.
2. Photograph of *Streptococcus pneumoniae* treated to reveal the capsule.

#### Procedures:

1. Examine the pneumococcal colonies on the blood agar plate. Note the characteristic colony morphology.
2. Examine the photograph and note the presence of the capsule.

Catalogue No	Small Image	Image Map	Large Image
M_BI_ST_27.jpg	<a href="#">St. pneumoniae – top and back lit</a>		<a href="#">St. pneumoniae – top and back lit</a>
M_BI_ST_16.jpg	<a href="#">St. pneumoniae – Gram's stain</a>		<a href="#">St. pneumoniae – Gram's stain</a>
M_BI_ST_28.jpg	<a href="#">St. pneumoniae. – capsule stain</a>		<a href="#">St. pneumoniae – capsule stain</a>

### 20.5 NEISSERIA

Bacteria belonging to the genus *Neisseria* are Gram-negative cocci. The most important pathogenic species are *N. gonorrhoeae*, the causative agent in gonorrhoea and *N. meningitidis*, a prominent cause of meningitis. The related species, *Neisseria pharyngis*, is a non-pathogenic commensal in the upper respiratory tract. *N. gonorrhoeae* is a fastidious bacterium and for growth in culture it requires a rich medium and increased CO<sub>2</sub>.

## Morphology of colonies and of individual organisms:

### Materials:

1. A blood agar plate culture of *Neisseria gonorrhoeae* grown in 10% CO<sub>2</sub>.
2. A blood agar plate culture of *Neisseria pharyngis*.
3. Slides and a bacteriological loop.
4. Gram's stain.

### Procedure:

1. Prepare a smear of any **one** of the organisms.
2. Stain by Gram's method.
3. Examine under oil immersion.

### A useful biochemical test for *Neisseria* : the Oxidase Reaction:

This is used to locate scanty *Neisseria* colonies in a background of mixed colonies e.g. to detect vaginal carriers of *N. gonorrhoeae*. In the plate provided, *N. pharyngis*, is used to demonstrate the reaction.

### Materials:

1. A blood agar plate sown with *Neisseria* sp.
2. The oxidase reagent : tetramethyl-paraphenylene diamine.
3. A capillary tube.

### Procedure:

1. Use a capillary tube to flood a **small area** of the plate with the oxidase reagent.
2. *Neisseria* colonies first turn purple and then black.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_NS_07.jpg	<a href="#">Neisseria gonorrhoeae</a>		<a href="#">Neisseria gonorrhoeae</a>
M_BI_NS_09.jpg	<a href="#">Neisseria pharyngis</a>		<a href="#">Neisseria pharyngis</a>
M_BI_NS_10.jpg	<a href="#">Neisseria phayngis - oxidase</a>		<a href="#">Neisseria phayngis - oxidase</a>
M_BI_NS_02.jpg	<a href="#">N. gonorrhoeae – Gram's stain</a>		<a href="#">N. gonorrhoeae – Gram's stain</a>
M_BI_NS_08.jpg	<a href="#">N. pharyngis – Gram's stain</a>		<a href="#">N. pharyngis – Gram's stain</a>

## NEISSERIA GONORRHOEAE AND VENEREAL DISEASE:

### 20.6 URETHRAL SMEAR FROM A PATIENT WITH GONORRHOEA 83.681

A purulent urethral discharge stained by Gram's method. The slide shows a large number of Gram-negative diplococci present in a few of the pus cells (polymorph neutrophil leukocytes). A similar appearance is seen in cerebrospinal fluid smears in meningococcal meningitis.

***N.B. This slide is part of a slide collection, please do not discard it.***

Catalogue Number	Small Image	Image Map	Large Image
N_CS_NU_02.jpg	<a href="#">Urethral smear</a>		<a href="#">Urethral smear</a>

## 20.7 IDENTIFICATION OF ORGANISMS ISOLATED FROM A THROAT SWAB

In this exercise, you are provided with a blood agar plate (**I**) that has been inoculated with a swab taken from a patient who complained of a sore throat and a photograph labelled Plate I. You are asked to identify the bacteria present on the plate.

### Procedure:

Examine the plate and tentatively identify the colonies by their colonial appearance and their effect on the blood agar. The photograph shows the results of the Gram stain of each colony type. You may investigate further if you wish by additional gram staining.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_MX_28.jpg	<a href="#">Plate I - top and back lit</a>		<a href="#">Plate I - top and back lit</a>

## 20.8 IDENTIFICATION OF ORGANISMS ISOLATED FROM A PATIENT WITH A URINARY TRACT INFECTION

The blood agar plate (**J**) and CLED plate (**K**) provided were inoculated with a sample of urine from a patient with a suspected urinary tract infection. Examine the plates and photographs provided. Identify the colonies on the blood agar plates and photographs. The photographs show the results of the Gram stain of each colony type. You may investigate further if you wish by additional gram staining.

Catalogue Number	Small Image	Image Map	Large Image
M_BI_MX_31.jpg	<a href="#">Plate J</a>		<a href="#">Plate J</a>
M_BI_MX_32.jpg	<a href="#">Plate K</a>		<a href="#">Plate K</a>

### **Please Note:**

***Disinfect your bench area  
Cover and put your microscope away.  
Wash your hands as you leave.***

***Thank you!***