

AIMS

1. Introduce you to the Gram stain. This is the major aim of this practical
2. Provide further experience in the application of indicator media.
3. Demonstrate some of the characteristics of the yeast (fungus) *Candida albicans*
4. Introduce an approach to the identification of bacteria within a mixture.

PLEASE TURN ON THE HOTPLATE BEFORE STARTING ANY PRACTICAL WORK.

17.1 PLATES FROM PREVIOUS PRACTICAL CLASS: THROAT SWABS

Examine the blood agar plates inoculated last time with the swab taken from your partner's throat (Exercise 16.7). How many different colony types can you see? Discuss the plates with your Demonstrator.

17.2 IDENTIFICATION OF BACTERIA: GRAM'S STAIN

As this is an important simple technique which will help you throughout bacteriology, you will be shown a short video devoted to the method.

This staining procedure divides almost all bacteria into one of two classes: Gram-positive or Gram-negative. In order to stain the bacteria you must first make a thin smear of them on a glass slide and then fix them to the slide.

Materials:

1. Bacteria to be stained: plate cultures of *Escherichia coli* and *Staphylococcus aureus*.
2. Sterile distilled water.
3. Bacteriological loops.
4. Gram's staining reagents.

Procedure:

1. Take three slides and use a wax pencil to label them 1, 2 and 3.
(1= *S. aureus*, 2= *E. coli*, 3 = *S. aureus* and *E. coli* mixture).
2. Transfer a minimum loop of distilled water to the centre of the slide 1. Discard the loop.
3. Use the edge of another loop to pick up part of a colony of *S. aureus*.
4. Using the loop, spread the organisms over a small area of the slide, making an even smear of the bacteria in the distilled water.
When done correctly, the smear will be faintly milky and will dry almost immediately.
The commonest errors are to use too much water, or to make the smear too thick and too large.

Note: films made from liquid media, from swabs, or from body fluids are spread directly without the addition of water.

5. Place the slide on the preheated hotplate and leave until it is dry.
6. Allow the slide to cool.
7. Repeat steps 2 - 6, using slide 2 and slide 3.
8. Place the slides on a staining rack and stain the smears.

GRAM'S STAINING PROCEDURE

1. Flood smear with crystal violet, minimum 30 seconds.
2. Wash off the crystal violet with a sufficient excess of Gram's iodine to remove the precipitate that forms. Leave the iodine on for a minimum of 30 seconds.
3. Wash with tap water.

4. Differentiate with ethanol. Tilt the slide, and apply the ethanol to the slide so that it flows over the smear without dropping directly onto the smear. Continue to apply the ethanol until the blue colour ceases to wash out easily. **This will take from 5 to 10 seconds** (varying with the thickness of the smear). **DO NOT LEAVE ETHANOL ON THE SLIDE FOR LONGER THAN 10 SECONDS.**
5. Wash with tap water.
6. Counterstain with safranin, up to 2 minutes.
7. Wash with tap water.
8. Blot carefully, and allow to dry **completely**.
9. Place a small drop of immersion oil onto the smear.
10. Examine under the oil immersion lens.

Gram-positive = blue/violet; Gram-negative = pink.

| Catalogue Number | Small Image | Image Map | Large Image |
|------------------|--|-----------|--|
| M_BI_ES_28.jpg | E. coli - Gram stain | | E. coli – Gram stain |
| M_BI_SP_22.jpg | S. aureus - Gram stain | | S. aureus – Gram stain |

You should practice the technique of preparing smears and staining with Gram's stain until you can consistently obtain reliable results. (Preparing and/or interpreting Gram's stains is an essential part of the Part IB Practical Examination).

17.3 IDENTIFICATION OF BACTERIA WITHIN A MIXTURE

In this and subsequent practical classes you will be given the opportunity to identify bacteria within a mixture. The Notes which accompany this class sheet suggest a schematic approach which you are advised to follow. It is important that you develop this system not only because it will enable you to correlate the information you are gaining about bacteria but also because an exercise of this kind may well form part of the Practical Examination.

17.4 USING COLONY APPEARANCE AND THE GRAM STAIN: EXAMINATION OF A PLATE CULTURE INOCULATED WITH A CLINICAL SAMPLE

Materials:

A blood agar plate 'A' which was streaked with a sample of pus from a dental abscess.

| Catalogue Number | Small Image | Image Map | Large Image |
|------------------|------------------------------------|-----------|------------------------------------|
| M_BI_MX_19.jpg | Plate A – back lit | | Plate A – back lit |
| M_BI_MX_90.jpg | Plate A – top lit | | Plate A – top lit |

Procedure:

1. Note the type of medium, number of different colony types, appearance of the colonies, including any changes in the surrounding medium.
2. **Make Gram stained smears** of each of the colonies present, and **make notes on the morphology** of the bacteria from each. Can you identify these?
What further steps might be taken to confirm your conclusions?

17.5 USE OF INDICATOR MEDIUM : CULTURE FROM A FAECAL SWAB

A faecal swab from a patient complaining of diarrhoea was used to inoculate a blood agar plate 'B' and a MacConkey plate 'C'. Examine the plates and from your knowledge of the growth of bacteria on the MacConkey agar, can you suggest the probable identity of the different bacteria. Discuss your ideas with a demonstrator.

| Catalogue Number | Small Image | Image Map | Large Image |
|------------------|-------------------------|-----------|-------------------------|
| M_BI_MX_21.jpg | Plate B | | Plate B |
| M_BI_MX_22.jpg | Plate C | | Plate C |

17.6 CANDIDA ALBICANS

The yeast (fungus) *Candida albicans* is a normal commensal component of the body flora, but may also give rise to opportunistic infections. It is dimorphic, growing most frequently as a budding round or oval yeast, but filamentous hyphae are present in invasive lesions. Superficial oral and urinary tract (vaginal) infections with *Candida albicans* (thrush) are commonly encountered. More serious infections may occur in immunosuppressed patients. Like all fungi, *Candida albicans* is a eukaryotic organism.

On many laboratory media, such as the blood agar plate provided, *Candida albicans* grows as a budding yeast which forms small white colonies.

1. Examine the blood agar plate.
2. Gram stain a smear prepared from a sample of one of the colonies.

| Catalogue Number | Small Image | Image Map | Large Image |
|------------------|--|-----------|--|
| M_MY_CA_04.jpg | C. albicans | | C. albicans |
| M_MY_CA_03.jpg | C. albicans – Gram stain | | C. albicans – Gram stain |

Q1 Is the organism Gram positive or Gram negative?

Q2 How does the organism differ from that of the bacteria you have seen?

PLEASE REMEMBER

Return the wooden block if used.

Leave the microscope (covered) on the bench.

Turn off the hotplate.

Wipe the bench top with disinfectant before you leave.

On the way out, wash your hands, turning the taps on and off with your elbows.

Thank You!