

## BM327 Microbiology Semester 2 – Lab 2 Handout

### TASK 2A: Observation of macroscopic characteristics

You should work individually to complete this task (make independent observations of each plate), though you may wish to compare your results with your neighbours.

You have previously inoculated a number of nutrient agar plates with 5 different bacterial species (Lab 1), and these were incubated for you for 24 hours at 37°C. Examine the colonies, ensuring that you are examining **well-separated, individual colonies** (Protocol 2.1). Record your descriptions of the colonies in Table 1. Use of a hand lens will allow you to observe individual colonies in greater detail and a ruler will be useful in providing accurate colony size measurements.

#### *Protocol 2.1: Observation of bacterial colony morphology*

1. Measure the diameter of a representative colony in millimetres.
2. Describe the pigmentation (distinguishing between pigmented colonies and those secreting diffusible pigments) and record in Table 1.
3. Describe the form, elevation, and margin of a representative colony (using the correct terminology as indicated in the handout). Also indicate whether the colonies are smooth (shiny glistening surface), rough (dull, bumpy, granular, or matte surface), or mucoid (slimy or gummy appearance) and record in Table 1.
4. Record the opacity of the colonies (transparent, translucent, or opaque) in Table 1.

#### Competency check: streak plate.

One of the lecturers will assess your streak plate for competency according to the following criteria. You should use the feedback provided by the lecturer on your streak plate to improve your technique. You will be given several more opportunities to pass the streak plate competency test over the course of this module.

##### Streak plate competency check 1:

- ☐ Well-streaked plate, with several well-separated and distinct individual colonies
- ☐ Streaking technique needs improvement: does not make good use of the space available on the plate
- ☐ Streaking technique needs improvement: no/few single colonies obtained
- ☐ Streaking technique needs improvement: probable faults in aseptic technique
- ☐ Streaking technique needs improvement: other \_\_\_\_\_

##### Labelling technique:

- ☐ Clear, legible, complete label
- ☐ Labelling is illegible/unclear
- ☐ Label placed incorrectly: \_\_\_\_\_
- ☐ Label is missing key information: \_\_\_\_\_

Table 1. Macroscopic characteristics of the colonies of typical bacteria grown on nutrient agar.

Bacterial species	Characteristic			
	Size range (mm)	Shape	Colour	Odour
<i>Escherichia coli</i>				
<i>Staphylococcus aureus</i>				
<i>Bacillus cereus</i>				
<i>Proteus species</i>				
<i>Pseudomonas aeruginosa</i>				

### ***Fungal colonies on solid media***

You are supplied with a culture of a yeast (unicellular fungus) and moulds (multicellular fungi) that has been grown on the surface of Sabouraud/dextrose agar plates for 72 hours at 25°C. Examine the colonies and record your descriptions in Table 2.

Table 2. Macroscopic characteristics of the colonies of fungi grown on Sabouraud/dextrose agar.

Characteristic	Microorganism		
	<i>Saccharomyces cerevisiae</i>	<i>Penicillium notatum</i>	<i>Aspergillus niger</i>
Size range (mm)			
Shape			
Colour			

## TASK 2B: Observation of microscopic characteristics

Working as a group of two, you should prepare Gram-stained slides for each of the five bacterial cultures from Task 1A/Task 2A.

**Please note, you should wear safety gloves when handling staining reagents. However, gloves must be removed when working near the Bunsen burner.**

### Protocol 2.2: Preparing Gram-stained bacterial cells.

1. Hold a glass slide with metal forceps and flame thoroughly its upper surface using a Bunsen flame (this degreases the slide and allows even spreading of the film of bacterial cells). Allow the slide to cool. **Note**, take care when doing this – holding the glass slide in the flame too long can cause the glass to shatter!
2. Flame a loop until red hot and allow it to cool (hold it steady, do not move around or lay it on the bench). Once cool, place a loopful of sterile water on the upper surface of the slide. Note, do not use too much water or it will take a very long time for your slide to dry!
3. Flame the loop again and allow it to cool, then use it to pick up a speck of a bacterial colony (ideally this would be from an isolated single bacterial colony – **not** from a lawn or confluent smear). Mix this speck thoroughly into the drop of water to form a dilute milky suspension covering an area of about the size of a 20p coin (the aim is to provide a film that contains a single layer of cells. Too high a density of cells applied to the slide results in loss of detail as the cells are too closely packed together to visualise under the microscope or even too thickly packed for the stains to penetrate. The amount of culture necessary to produce a good film is learned from experience).

4. If labelling the samples on a slide using a permanent marker, use caution to ensure that the label is not destroyed during the ethanol wash.
5. Dry the film completely by warming the slide gently. Hold the slide well above the Bunsen flame.
6. Fix the film of cells to the slide by passing the lower surface of the slide through the Bunsen flame slowly twice. Allow the slide to cool.
7. Stain the film with 0.5% w/v crystal violet for 30 seconds. (Reminder – at this point you should have turned off your Bunsen burner and should be wearing gloves to work with the stains.)
8. Drain the crystal violet, rinse off the remainder with a solution of iodine (1% w/v iodine in 2% w/v potassium iodide). Cover the film with iodine for one minute.
9. Drain the iodine, rinse off the remainder with alcohol. Rock the alcohol from side to side until no more crystal violet is removed. This process should take no longer than 45 seconds.
10. Drain off the alcohol remaining and gently blot the slide dry.
11. Rinse with water.
12. Cover the film with 1% w/v safranin for 30 seconds. Wash the safranin off the slide with water and allow the slide to dry.
13. Observe your slides under the microscope, using a low power objective lens (x4) first [note: you do not need to use a coverslip]. Then, work your way up through the lenses until you reach the oil-immersion lens (x100).

To use the oil-immersion lens, only a **very small** amount of immersion oil should be used. Do not use the oil with any other lenses! Using **lens paper** only (do not use any other tissue/cloth!), be sure to carefully clean the oil off the x100 lens when you are finished.

14. First record your raw data (observations, e.g. colour observed, any notable features observed on the slide). Make accurate drawings of the cells of the five bacteria to show clearly the shapes of the individual cells, and the groupings of the individual cells (Table 3). Be sure to note the magnification used to observe the cells that you sketched. Then, record your interpretation of these observations (indicate whether each organism is Gram-positive or Gram-negative and its morphology – e.g., Gram-negative coccus, Gram-positive filamentous).

### ***Interpretation***

**Gram-positive** organisms are stained **blue/violet**.

**Gram-negative** organisms are stained **red/pink**.

Table 3. Microscopic characteristics of bacterial colonies isolated from solid nutrient agar following application of Gram stain.

Microorganism	Reaction to Gram stain	Sketch of cellular morphology and arrangement	Interpretation
<i>Escherichia coli</i>			
<i>Staphylococcus aureus</i>			
<i>Proteus</i> species			
<i>Bacillus cereus</i>			
<i>Pseudomonas aeruginosa</i>			

Working as a group of two, you will examine the three different fungal species provided for TASK 2A under the microscope (Protocol 2.3). Please note that you should take care when opening the Petri dishes containing the fungal cultures, as you do not want to disperse spores throughout the lab (or inhale them) – **open each plate narrowly, and for the minimum amount of time required to take a sample of the culture.**

*Protocol 2.3: Microscopic examination of fungi.*

1. Place a drop of iodine solution on the surface of a glass slide.
2. Using a flamed and cooled loop, pick up a small amount of a colony of a fungus and tease it out in the drop of iodine.

3. Place a cover slip on top of the mounted material and examine the fungi using first the low power (x4) and then the high power (x40) objective lenses of the microscope. Make accurate drawings of the three fungi showing their *mycelia*, hyphal structure (whether septate or non-septate), *conidiophores* and spores (Table 4).

Table 4. Microscopic characteristics of fungi isolated from solid Sabouraud/dextrose agar following addition to iodine solution.

Microorganism	Sketch of cellular morphology and arrangement
<i>Penicillium notatum</i>	
<i>Aspergillus niger</i>	
<i>Saccharomyces cerevisiae</i>	

## TASK 2C: Differential media - use of blood agar to differentiate bacteria based on their haemolytic activity

Working as a group of four, you will be given an unknown *Streptococcus* sp. to identify based on its haemolytic activity, as well as a set of controls (strains with known alpha, beta, and gamma haemolytic activities). (Each group member should follow Protocol 2.4 to prepare one streak plate.)

### Protocol 2.4: Streaking for single colonies on blood agar plates.

Make sure that all agar plates are clearly labelled with your name, the sample name or number, your bench number, and the date. You should label the bottom of the plate (not the top – which may become separated from the bottom!). It is best to write in a circle around the edge of the plate, so that your writing will not obscure observation of the growth on the plate.

1. Flame a loop, let it cool slightly and then touch it to the bacterial colony you have chosen to streak on a new plate.
2. Pick up the bottom of the new plate (leaving the lid on the bench) with your other hand, turn it over and gently streak the loop back and forth over a small area of the plate. Use light pressure so you don't gouge the agar.
3. Return the plate to its lid so as to protect it from contaminants in the air while you re-flame the loop. Allow the loop to cool.
4. Pick up the plate again and touch the cooled loop onto an area of the agar that has not previously been streaked. (This step helps to quench the heat from the loop and so prevents it from killing any bacteria it subsequently comes into contact with.)
5. Drag the loop once through the previous streaked area and streak it out across a new area of agar. As the loop drags across the previous streak it will pick up some bacteria and distribute them across the new area of agar.
6. Repeat steps 4 - 5 two or three times as necessary to cover the entire surface of the plate (Be sure to use your feedback from Task 2A to improve your technique).

Your plates will be incubated at 37°C for you. You will observe them and record your results during the next laboratory session.

**Competency check:** documenting and reporting on experimental results and conclusions.

Before you leave the lab, one of the lecturers will assess your work (Tables 1-4) for competency, and give you feedback:

Recording results check 1:

- ☐ Results and interpretation clearly and concisely recorded
- ☐ Poor sketch(es), lacking adequate detail
- ☐ Sketches/results illegible/unclear
- ☐ Lack of separation between results (raw data) and interpretation
- ☐ Failure to use accurate/precise terminology
- ☐ Failure to record magnification used
- ☐ Results table(s) missing key information: \_\_\_\_\_
- ☐ Other: \_\_\_\_\_