

BM327 Microbiology Semester 2 – Lab 5 Handout

TASK 5A: Analysing biofilm formation by *P. aeruginosa* isolates

You should work in groups of four to complete this task (Protocol 5.1), analysing the biofilm formation by four different *P. aeruginosa* strains.

Protocol 5.1: Biofilm formation assay.

1. Plan how you will pipet your samples into the 96-well plate, and enter the well number for each sample in Table 1.
2. Working in triplicate, add 100 µl of sample to each well as follows:
 - a. Pipette 100 µl of medium into each well (e.g., A1, A2, and A3), for your negative control.
 - b. Pipette 100 µl of each of the *Pseudomonas* cultures into each well.
3. Incubate the 96-well plate at 30°C for at least 45 minutes. (Record your final incubation time).
4. Add 25 µl of 1% crystal violet solution to each well.
5. Incubate the plate for a further 15 minutes at room temperature.
6. After this time, rinse the wells thoroughly with water and invert the tray over some blue-roll paper to let the wells drain carefully.
7. Record your observations in Table 1 using qualitative scores to represent the amount of biofilm formation (e.g., 0 = no biofilm formation, + = weak biofilm formation, ++ = strong biofilm formation).

Table 1. Biofilm formation (attachment of bacteria to the wells)

<i>Pseudomonas</i> isolate (name/reference ID)	Repeat (triplicates of each isolate)	Location of wells in 96-well plate	Biofilm estimation (purple ring)
	1		
	2		
	3		
	1		
	2		
	3		
	1		
	2		
	3		
	1		
	2		
	3		

TASK 5A: Analysing antibiotic resistance assay (from TASK 4F)

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 should carefully measure the diameter of each zone of inhibition in millimetres. Record your results in Table 2.

Table 2. Sensitivity of *Pseudomonas aeruginosa* isolates to gentamicin. Inhibition zone diameters should be measured in millimetres.

Isolate	Gentamicin				
	0 µg	4 µg	8 µg	12 µg	16 µg
Culture _____					
Culture _____					
Culture _____					
Culture _____					

TASK 5B: Using differential stain to identify a respiratory tract pathogen

You should perform this task in groups of two, staining your unknown respiratory tract pathogen and the appropriate positive and negative controls using the modified Ziehl-Neelson stain (Protocol 5.2).

Protocol 5.2: Modified Ziehl-Neelson stain.

1. Hold a glass slide with metal forceps and flame thoroughly its operational upper surface using a Bunsen flame. Allow the slide to cool. Flame a loop until red hot and leave it to cool. Place a loopful of sterile water on the upper surface of the slide. Flame the loop again and allow it to cool, then use it to pick up a speck of a colony from the culture provided. 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.
2. Allow the film to dry completely. You may need to do this by warming the slide gently, holding it in forceps well above the Bunsen's flame.

3. Fix the film of cells to the slide by passing the lower surface of the slide through the Bunsen flame slowly twice. The slide will now be hot to the touch; allow it to cool.
4. Place the slide on the staining rack over the basin provided.
5. Cover the slide with carbol fuchsin solution (this contains phenol, so take care to avoid contact with skin. Wash hands immediately if this happens). Allow to stand for 5 min.
6. Rinse with water.
7. *Take great care, wear protective gloves and safety spectacles now to handle the next solution (hydrochloric acid in ethanol) which is corrosive!*
8. Decolourise the slide by covering it with hydrochloric acid in ethanol solution, and allow to stand for 15 - 30 seconds.
9. Immediately remove excess acid by running slide in tap water.
10. Counterstain with malachite green solution for one minute.
11. Remove excess stain with water.
12. Gently blot the slide dry and examine under the microscope. Record your observations in Table 3.

Interpretation: Acid-fast bacilli appear red on a pale green background.

Table 3. Results of the modified Ziehl-Neelson stain

Microorganism	Cellular morphology	Interpretation
Positive control (acid-fast bacterium)		
Negative control (non- acid-fast bacterium)		
Patient sample		

Competency check: streak plate.

If you have not previously passed the streak plate competency check, you will be given another opportunity (you will streak the bacterial strain provided to you on nutrient agar.) One of the lecturers will assess your streak plate (if you have not previously passed this competency check) according to the following criteria:

Streak plate competency check 4:

- ☐ 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: _____

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

Before you leave the lab, one of the lecturers will assess your work (Tables 1-2) for competency, and give you feedback:

Recording results check 4:

- ☐ 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
- ☐ Results table(s) missing key information: _____
- ☐ Other: _____