TASK 3A: Analysing blood agar plates (from TASK 2C)

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. Observe your blood agar plates from the last lab. You should hold the plate up to the light to observe any haemolysis (with transmitted light coming through the plate).

Record the results in Table 1. Be sure to use the appropriate terminology (colony morphology descriptors) to describe your results (refer to the handouts if needed).

Table 1. Observation of haemolysis on blood agar plates.

Microorganism	Colony morphology	Haemolysis
Enterococcus faecalis		
Streptococcus pneumoniae		
Streptococcus pyogenes		
Your unknown #_____		

Competency check: streak plate.

One of the lecturers will assess your streak plate for competency according to the following criteria.

Streak plate competency check 2:
- ☐ 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: _____

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.

TASK 3B: Using selective and differential media for the cultivation and identification of *Escherichia, Salmonella,* and *Shigella* sp.

Working as a group of four, you will be streaking your unknown ("unknown spinach" from Case Study 3), as well as positive and negative controls, on MacConkey agar.

Protocol 3.1: Streaking for single colonies on MacConkey agar plates

1.   Using a flamed loop, take a colony from the agar plate provided containing your unknown spinach strain.

2.   Use this to streak a MacConkey agar plate for single colonies, using good aseptic technique.

3.   Repeat this exercise on fresh MacConkey agar plates for the positive and negative controls (*Escherichia* and *Salmonella*).

4.   Your plates will be incubated at 37°C overnight for you. You will observe and record your results in laboratory session 4.

TASK 3C: Biochemical assays to identify pathogens of the intestinal tract

Working in a group of two, you should perform the catalase (Protocol 3.2) and oxidase (Protocol 3.3) tests on your unknown ("clinical unknown") as well as the appropriate positive and negative controls for each test.

Protocol 3.2: Testing for catalase activity.

1.   With a flamed loop that has been allowed to cool, transfer cells from the centre of an isolated colony onto the surface of a glass slide.

2.   Add 1 or 2 drops of 3% hydrogen peroxide to the cells.

3.   Observe the slide and record your results in Table 1.

4.   Repeat the assay for your other samples. You should assay your positive and negative controls as well as your unknown microbe.

Interpretation

Rapid appearance and sustained production of gas (oxygen) bubbles is indicative of a positive test. A few tiny bubbles after 20 – 30 seconds are not considered positive.

Table 2. Catalase activity assay results

Microorganism	Catalase test result – carefully record what you observe	Catalase test result – interpretation
Positive control (*Escherichia coli*)		
Negative control (*Streptococcus* sp.)		
Your unknown (<u>clinical unknown</u>)		

Protocol 3.3: Testing for oxidase activity.

1. Apply the oxidase strip onto an isolated colony.

2. Record any colour changes in Table 2.

3. Repeat the assay for your other samples. You should assay your positive and negative controls as well as your unknown microbe.

Interpretation

If the test microorganism is oxidase-positive, then an intense deep blue colour will develop within seconds.

Table 3. Oxidase activity assay results.

Microorganism	Oxidase test result – carefully record what you observe	Oxidase test result – interpretation
Positive control (*Pseudomonas aeruginosa)*		
Negative control (*Escherichia coli*)		
Your unknown (unknown "spinach")		

TASK 3D: Determining whether an unknown microorganism requires oxygen for growth

Working in a group of four, you should inoculate your unknown ("clinical" unknown from Case Study 4) in duplicate, and the appropriate controls (*E. coli* and *Clostridium*) in fluid thioglycolate medium (Protocol 3.4). You will also inoculate your unknown and the control (*Clostridium*) on two stiff blood agar plates (Protocol 3.5). One of the stiff blood agar plates will be incubated anoxically using the GasPak system, while the other will be incubated in the presence of ambient oxygen levels.

Protocol 3.4: Anaerobic growth of microorganisms using fluid thioglycollate medium.

1. Label the culture tube with your name, today's date, and the name of the inoculum.

2. Flame your inoculating loop, making sure to sterilize the <u>entire</u> length of the platinum wire.

3. Lift the lid of the Petri dish slightly.

4. Cool the loop on an empty area of agar. (If it is too hot, you will kill the bacteria in the colony you choose.)

5. Gently pick up a single colony with the loop.

6. Close the lid of the Petri dish.

7. Using good aseptic technique, open the culture tube containing thioglycollate medium.

8. Put your loop into the broth and shake it gently. Be sure to innoculate the depths of the culture media.

9. Close the culture tube. Flame your loop before setting it down.

10. Repeat the procedure for each sample; you should inoculate your controls (*E. coli* and *Clostridium*) as well as your unknown organism into thioglycollate medium.

Your cultures will be incubated 37°C overnight for you and you will record your results in the next laboratory session.

Protocol 3.5: Anaerobic growth of microorganisms using the Gas-Pak system.

1. Label the base of the agar plate with your group name, today's date, and label each plate +O$_2$ or -O$_2$ as appropriate. Divide the plate in half and label each half with the name of the organism you will streak on it.

2. Using a flamed loop and proper aseptic technique, take a colony from the agar plate provided containing your unknown clinical specimen.

3. Use this to streak for single colonies on **half** of each of your <u>two</u> stiff blood agar plates.

4. Repeat steps 2 - 3 for your control (*Clostridium*) on the other half of the plate.

5. Keep one plate on your bench for incubation +O_2 at 31°C for 48 - 72 hours. Deposit the other plate in the anaerobic jar for incubation -O_2 at 31°C for 48 - 72 hours. You will observe your cultures and record your results in the next laboratory session.

TASK 3E: *Staphylococcus aureus* inoculation on Mannitol Salt Agar

Working as a group of four, you will streak your unknown organism ("unknown 3E") on mannitol salt agar (MSA). (The person who did not streak a plate in Task 3B should do this.)

Protocol 3.6: Streaking for single colonies on MSA agar.

1. Using a flamed loop, take a colony from the agar plate provided containing your unknown 3E sample.

2. Use this to streak a Mannitol Salt Agar plate (see Protocol 1.3 for a more detailed explanation if necessary).

3. Your plate will be incubated at 31°C for 48 - 72 hours for you. You will observe your cultures and record your results in 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 1-3) for competency, and give you feedback:

> Recording results check 2:
> ☐ 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: _____