

PRE-LAB 6: STREAKS

Please complete the table below before lab. We will be using the following selective and differential media in lab this week:

Phenyl ethyl alcohol agar (PEA)

Mannitol salt agar (MSA)

MacConkey's Agar (MAC)

Eosin methylene blue agar (EMB)

TSA+5% sheep's blood (Blood)

Triple sugar iron agar (TSIA)

Please fill out the following table. Access the posted protocols on FOL for the media to read about the reagents. You will need to know this information to correctly interpret the test results.

Medium	Reagent(s)	Purpose of reagent: If differential, what is the reagent testing? If selective, what is inhibited?	Appearance of test result	
			Positive	Negative
PEA	Phenylethyl alcohol			
MSA	Mannitol + phenol red			
MSA	NaCl			
MAC	Bile salts, crystal violet		Gram pos:	Gram neg:
MAC	Lactose + neutral red			
EMB	Eosin Y			

EMB	Methylene blue			
Blood	Sheep's blood			
TSIA	Ferrous sulfate+thiosulfate			
TSIA	Sugars+phenol red			

LAB 6 WORKSHEET

This lab report summarizes the experiments you did to isolate your environmental strain (Part 1), determine its cellular morphology and gram reaction (Part 2), and describe its metabolism (Part 3).

Part 1: Isolation of Environmental Strain

Aseptic technique exercise: **3 marks**

- **Figure 1:** Make a figure with a descriptive legend, that shows the mixed culture of *Staphylococcus aureus* and *Salmonella typhimurium* on a streak plate. **2 marks**
 - Write a sentence to discuss whether your streak plate was successful. **1 mark**

Isolation of environmental strain (Swabbing experiment): **5 marks**

- **Figure 2:** Images of your blood agar swab plate and streak plate of the environmental strain. **2 marks**
- Discuss if you successfully isolated a pure culture of a strain of environmental bacteria. **2 marks**

Part 2: Cell and colony morphology, Gram reaction of Environmental strain

Table 1: Cell morphology and Gram reaction of environmental strain **9 marks**

Species	Gram reaction		Cell morphology (shape and arrangement)	Gram result from KOH test	
	colour	reaction		Strings (y/n)	Gram reaction
Positive Control 1: _____					
Negative Control 2: _____					

Environmental isolate			
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Figure 3: Make a figure showing the images of Gram stained cells under the microscope. The figure should show the three strains: environmental strain, positive and negative controls. Include a descriptive legend with the total magnification indicated. **4 marks**

Question 1: Can you interpret the Gram stain result for the environmental isolate based on the results of the controls (*E. coli* and *S. aureus*)? Explain. **2 marks**

Question 2: Do the environmental isolate Gram staining and KOH string test results agree with each other? **1 mark**

Table 2: Colony morphology of environmental strain. **4 marks**

Species	Colony opacity	Colony pigmentation	Colony description (4 terms)	Colony texture
Environmental isolate				

Part 3: Metabolism of environmental isolate

Insert your plate images in column 1, 'culture media'. You can label the strains with textboxes directly on the image. You can use shorthand: pos for positive control, neg for negative control and two letters for the species name of the test (Sa, St, Ec, Se, Bs)

Table 3: Selective and differential media results **15 marks**

Culture medium image	Positive control	Negative control	Test strain: environmental isolate
Blood	Species: Observation:	Species: Observation:	Observation:

	Reaction:	Reaction:	Reaction:
PEA	Species: Observation: Reaction:	Species: Observation: Reaction:	Observation: Reaction:
MSA	Species: Observation: Reaction:	Species: Observation: Reaction:	Observation: Reaction:
MAC	Species: Observation: Reaction:	Species: Observation: Reaction:	Observation: Reaction:
EMB	Species: Observation: Reaction:	Species: Observation: Reaction:	Observation: Reaction:

Notes: Blood agar results are indicated with α (alpha), β (beta), or γ (gamma)

Observation: colour change of colony or agar (as appropriate for media) or NG (for 'no growth')

Reaction: Mark with + or -. If strain was 'NG', leave this blank.

You will lose a mark for incorrect interpretation of a test result.

Note if the control didn't work, as this limits your ability to interpret the test strain.

Indicate the test results for the organisms you tested by using "+" or "-".

You will lose a mark for incorrect interpretation (if the observation doesn't match your interpretation). Observations can be an image of your tube inserted into the observations column, or words describing the tube. **16 marks**

Table 4: TSIA test results

	TSIA				
Species	Glucose fermentation	Sucrose/lactose fermentation	CO ₂	H ₂ S	Observations (colour, gas cracks)
<i>E. coli</i>					Butt: Slant: Other:
<i>P. vulgaris</i>					Butt: Slant: Other:
<i>S. marcescens</i>					Butt: Slant: Other:
Your isolate					Butt: Slant: Other:

Question 3: Based on the results of *all of the media* in part 3 of the lab, interpret the results for your environmental organism (and reference the media you used to determine this in parentheses): **3 marks**

a) Sugars fermented: _____ based on the media: _____.

b) Gram reaction: _____ based on the media: _____.

Does this agree with your Gram stain and KOH results from Part 2? **1 mark**