Gram Negative Enteric Rods: Test Battery

Identifying an Enteric Gram Negative Rod Using a Test Battery. This is a group of tests that are performed all at the same time and whose purpose is to **IDENTIFY AN ENTERIC GRAM-NEGATIVE ROD.** Enterics includes all of the Enterobacteriaceae family. These are all Oxidase negative, nitrate positive, glucose fermenting Gram-negative rods. Refer to your atlas and text to complete the table below.

Test		Principle
TSI Triple Sugar Iron Agar Slant		
SIM Sulfide Indole Motility Deep		
UREASE Agar Slant		
CITRATE Slant		
PAD Phenylalanine Deaminase Agar Slant		
MOELLER DECARBOXYLASE Broth Lysine and Base		
MIO Deep	**Motility**	
	Indole	
	Ornithine	
DNAse Plate		

Enteric Gram Negative Rod Test Battery

Lab Procedure:

Do an Oxidase and set up the test battery for the oxidase-negative Gram Negative Rods. The instructor will demonstrate this for you. You will be given 4 unknowns to identify using the following tests. This is a group activity to be done in pairs, and the results will be posted and discussed in lab.

Enteric lab - Day 1

MEDIA	PROCEDURE
TSI slants	Using needle, stab to the bottom of the tube, withdraw needle, streak slant with fishtail movement. Incubate at 37° for 24 hours.
SIM Deeps	Using a needle, stab in the center of the meduim to the bottom of the tube. Incubate at 37 C
Urease broth or Slant	Using the loop, **HEAVILY** inoculate (fishtail on slant). Incubate at 37° for 24 hours.
Simmon's Citrate	Using a loop, inoculate slant with one colony. Use a fishtail movement. Inc. at 37ºC for 24 hrs. If negative, reincubate up to 72 hours.
Phenylalanine deaminase slants (PAD,PA)	Inoculate with a loop. Incubate at 37° for 24 hours.
Moeller Decarboxylase Broths (Lysine and Base)	Inoculate each organism into each of the broths using a loopful of culture. Overlay each tube with 1.0 ml sterile mineral oil. Incubate at 37° for 24 hours. **DO NOT FORGET THE OIL, OTHERWISE IT WILL NOT WORK.**
MIO (Motility, Indole, Ornithine)	Use the inoculating needle. **Stab straight down.** Do not wiggle while inoculating the tube. Incubate at 37ºC for 24 hours.
DNAse	With an inoculating needle and heavy inoculum make a band at least 1 in. long from rim to center of the plate. Incubate at 37° for 24 hours.

REMEMBER, THIS BATTERY OF TESTS IS FOR OXIDASE NEGATIVE ORGANISMS, TO IDENTIFY ENTERIC GRAM-NEGATIVE BACILLI.

Observe all the tubes in the battery and complete the tables showing all results and conclusions (include the identification of each organism). Refer to your atlas and Exercise 12 on the Selected Biochemical Reactions Used to Identify the Physiological Characteristics of Bacteria.

Enteric lab - Day 2

MEDIA	PROCEDURE
TSI slants	**Observe** slants for (1) glucose fermentation, (2) lactose and/or sucrose fermentation, and (3) H_2S production. **Interpretation**: Red slant/yellow butt - Alkaline/acid (glucose fermented only). Yellow slant/yellow butt - Acid/acid (glucose *and* lactose and/or sucrose fermented) Red slant/back butt - Alkaline/acid + H_2S (glucose fermented with H_2S production Yellow slant/black butt - Acid/acid + H_2S (glucose *and lactose and/or sucrose fermented with* H_2S production) Red slant/red or orange butt- Alkaline/No change (peptones utilized only).
SIM Deeps	**Sulfide:** Look at the color of the medium. A positive sulfide test is indicated by a black color. **Motility:** Look at the central stab path. Look for turbidity of the medium away from the stab. **Indole:** Add a few drops of Kovac's reagent to tube (one half-dropperful). Wear gloves. Observe color. Positive test: pink color at liquid interface.
Urease slant or broth	Observe formation of intense pink color. Positive test (pink color); negative test (no color change, reddish-orange)
Simmon's Citrate	**Observe** slant for color development. Positive test (blue color), negative test (no color change, green). If negative, reincubate up to 72 hours.
(PAD) **Phenylalanine deaminase slants**	Add 5 drops of 10% $FeCl_3$ to the slant. Rotate the slant so as to expose all of the growth to the ferric chloride. Observe for color formation in 1-5 minutes. (After 5 minutes green (positive) color begins to fade.)
Moeller Decarbo-xylase Broths (Lysine and Base)	Observe color. Positive test (purple); negative test (yellow). Base should be yellow.
MIO **(Motility, Indole, Ornithine)**	**Motility:** look at organism stab. Look for turbidity of the medium away from the stab. **Indole:** Add a few drops of Kovac's reagent to tube (one half-dropperful). Wear gloves. Observe color. Positive test: pink color at liquid interface. **Ornithine:** Initially the tube is purple. It first turns yellow if it metabolizes glucose, then it turns purple if it is metabolizes ornithine.
DNAse	Look for a distinct clear zone around bacterial streak in otherwise green agar. Clear zone best observed against a **white background.**

Identifying Enteric Gram Negative Rods (Enterobacteriaceae)

Tests	*ORG #1*		*ORG #2*		*ORG #3*		*ORG #4*	
	Observation	***Conclusion***	***Observation***	***Conclusion***	***Observation***	***Conclusion***	***Observation***	***Conclusion***
TSI SLANT								
SIM Deep								
Sulfide								
Indole								
Motility								
UREASE SLANT								
CITRATE SLANT								
PAD SLANT								
MOELLER								
Lysine								
Base								
MIO								
Motility								
Indole								
Ornithine								
DNAse PLATE								
IDENTIFICATION								