

Lecture 9

I. Biochemical testing for Gram positive organisms continued

A. Blood Agar Plates (BAP) review

Review: What three types of hemolysis can be observed on a BAP plate?

B. MSA plate review

Review: What color would an MSA plate be if the organism streaked on it was able to ferment mannitol?

Yellow

C. Taxos testing (antibiotic resistance)

1. _____ is a disk inoculated with _____. This antibiotic is used to differentiate between α -hemolytic strains.

i. Nonpathogenic normal flora, *S. mitis*, shows _____.

ii. Pathogenic *S. pneumonia* is _____ and will show a large zone of inhibition.

2. _____ is a disk inoculated with _____. This is an antibiotic used to differentiate between β -hemolytic strains.

i. *Streptococcus pyogenes* is _____.

ii. *Streptococcus agalactiae* is _____.

II. Gram Negative Organisms

A. _____					
Key characteristics:			Environments		
<ul style="list-style-type: none"> • Straight, Gram-negative rods • _____ (This is the best way to differentiate between <i>Enterobacteriaceae</i> and <i>Pseudomonas</i> spp). • Non-fastidious (simple nutritional requirements) • _____ • If motile, flagella are peritrichous 			Inhabit the gut (“entero” means gut).		
Representative species					
<i>Escherichia coli</i>		<i>Protens mirabilis</i>		Shigella dysenteriae	
Known for	Characteristics	Known for	Characteristics	Known for	Characteristics
<ul style="list-style-type: none"> • Some strains of <i>E. coli</i> can cause _____ _____ _____ _____ 	<ul style="list-style-type: none"> • ferments _____ _____ • reduces nitrate • Produces indole • MR+ 	<ul style="list-style-type: none"> • _____ pathogen causing UTIs 	<ul style="list-style-type: none"> • ferments glucose • swarming motility • _____ _____ • utilizes citrate • hydrolyzes _____ • MR+ 	<ul style="list-style-type: none"> • causes _____ 	<ul style="list-style-type: none"> • ferments glucose, and mannitol • reduces nitrate • motile • produces H₂S • utilize citrate • MR+

<i>Salmonella typhi</i>		<i>Klebsiella pneumoniae</i>	
Known for	Characteristics	Known for	Characteristics
<ul style="list-style-type: none"> • _____ 	<ul style="list-style-type: none"> • ferments _____ • reduces nitrate • MR+ 	<ul style="list-style-type: none"> • normal flora • causes _____ <p>in alcoholics and people with compromised pulmonary function</p>	<ul style="list-style-type: none"> • ferments _____ • reduces nitrate • utilizes citrate • VP+

<i>B. _____</i>			
Key characteristics:		Environments	
<ul style="list-style-type: none"> • Straight, or slightly curved Gram-negative rods • _____ • Non-fermentative • _____ • Motile via polar flagella • Often produce water soluble pigments and colonies appear mucoid 		Generally found _____	
Representative species			
<i>P. aeruginosa</i>		<i>P. fluorescens</i>	
Known for	Characteristics	Known for	Characteristics
<ul style="list-style-type: none"> • _____ that causes disease in immunocompromised (e.g. cystic fibrosis patients, AIDS patients, and burn victims) • produces several enzymes and toxins that account for its virulence. The most important toxin blocks protein synthesis in eukaryotic cells. Even the water-soluble pigment pyocyanin causes tissue damage to a host 	<ul style="list-style-type: none"> • reduces nitrate • _____ 	<ul style="list-style-type: none"> • _____ 	<ul style="list-style-type: none"> • growth temperature below 30°C

III. Biochemical testing for Gram negative organisms

A. Fermentation Broth tubes

1. These tubes contain a simple _____ in addition to a _____, and a _____. A positive reaction will turn the broth _____. The Durham tube will measure _____ by the bacteria (gas produced by an enzyme called _____).

2. Glucose tubes

- In today's lab we will be using a glucose broth tube. If the liquid in the glucose tube is _____, then _____. If the liquid in the glucose tube is _____, then _____.
- A bubble in the Durham tube indicates that gas has been produced. Many members of the family *Enterobacteriaceae* can metabolize _____, which can be converted to _____ by an enzyme called _____.



B. SIM Tube (Sulfur, Indole, Motility)

1. Sulfur: _____ production will turn the agar _____.
 - i. H₂S is produced either through the _____ by an enzyme called cysteine desulfurase or by the _____ during anaerobic respiration.
2. Indole: Adding Kovac's reagent, and _____ determine indole production.
 - i. Indole is produced from the breakdown of the amino acid _____.
3. Motility: Motility is determined by observing the organism's ability to move through the _____.

C. MacConkey agar

1. _____ medium
 - i. Bile salts inhibit the growth of _____ organisms.
 - ii. If the bacteria are able to ferment lactose, the acid produced will cause the pH indicator to _____.

D. Kligler's Iron Agar (KIA)

1. A differential medium that contains two sugars: _____ (low concentration) and _____ (higher concentration).
 - i. Fermenters will typically utilize the _____ and production of acidic fermentation byproducts will cause the entire tube to turn _____. However, the glucose is in short supply and, after the first few hours of growth, will all be used up.
 - ii. After the glucose is gone, bacteria that are capable of fermenting lactose will do so. Production of acidic byproducts will continue and the medium will _____.
 - iii. Gas produced as a product of fermentation will form _____ or completely _____ of the tube.
 - iv. Bacteria that are incapable of fermenting lactose have to utilize the _____ in the medium. This produces NH₃ (a weak base), which _____ and turns the slant red. The butt remains yellow.
 - v. Non-fermenters will utilize only the amino acids and proteins in the medium. The slant will be _____ and the butt will remain the original red/orange color.
 - vi. The production of H₂S results in a _____. If the black precipitate obscures the medium color, it is _____.

