

## Lab Exam #2

### Slide #1

Which is a positive test for MR and which is negative? What does a positive test for MR mean?

**Glucose fermentation**

Would you normally have a positive MR and positive VP test? **NO**

### Slide #2

Can you have gas byproducts in this test reading? Why? **NO. This reads as negative for sugar fermentation, therefore cannot have gas products**

What would this PR Durham tube result indicate? **Negative for sugar fermentation... used protein degradation**

### Slide #3

How do these two results differ? How are they similar? **The tube on the left has gaseous products indicating more vigorous fermentation which allowed gaseous products. They are similar as sugar fermentation released acidic byproducts to decrease the pH**

### Slide #4

What is the name of the reagent used in this test? **Kovac's reagent**

What is the name of the enzyme which breaks down tryptophan into indole? **Tryptophanase**

What are the three products from tryptophan breakdown? **Indole, pyruvate, ammonia**

### Slide #5

What do these three tests have in common? What are they testing for? **Enzymatic activity to breakdown components of agar.**

Which test is which... and what enzyme is being used for each? **Casein:casease, starch:amylase, lipids:lipase**

### Slide #6

What is this test? **MSA Salt Agar**

What part of the media is selective? part is differential? **salt = selective; mannitol = differential**

### Slide #7

Would this test result indicate anaerobic or aerobic bacteria? **Aerobic... contains catalase to convert toxic byproducts**

What reaction occurs in this test?  **$H_2O_2 \rightarrow 2H_2O + O_2$**

### **Slide #8**

Which is a positive test for oxidase? Which is negative? **Left = positive, right = negative**

How does this enzyme act in the cell? What part of cellular respiration does this enzyme act?

**Acts in electron transport chain to speed up reaction of reducing O<sub>2</sub> as final electron acceptor.**

### **Slide #9**

Does the color change to blue occur due to acidic or alkaline conditions? **Alkaline; organisms which utilize the sodium citrate must also use the ammonium for nitrogen source to increase pH.**

What is the source of carbon in this media? Nitrogen source? **Sodium citrate; ammonium phosphate**

What would growth with no color change in this test indicate? **Citrate cannot be used as sole source of carbon**

### **Slide #10**

What bacteria is this test selective for? What is it differential for? **Selective for gram (-) bacteria; differential for enterobacteria**

What does this test result indicate in terms of fermentation of the media? **Positive for vigorous lactose fermentation; ie mixed acid fermentation**

Which organism would be a mixed acid fermenter? **Bacteria #1**

### **Slide #11**

Which test is positive? **Left**

What does the positive bacteria contain that causes the color change? **Exoenzyme urease to breakdown urea.**

How does this test result in a color change? **Breakdown of urea releases ammonia that increases the pH**

### **Slide #12**

What would this result indicate? **High lactose/sucrose fermentation.**

What test would you do after to determine which sugar the bacteria uses? **PR durham tubes**

### **Slide #13**

Does this bacteria utilize other sugars in the media or another source? What is that source?  
**Other source... beef extract/peptones.**

Explain what causes the reversion of the slant in this media. **The organism is only able to use the limited amount of glucose (0.1%) in the media. After, it cannot continue using the lactose/sucrose and must use the peptones. The fermentation of peptones causes reversion of slant to alkaline pH due to release of ammonia.**

#### **Slide #14**

What color would you expect the butt to be? **Yellow**

What causes the production of the black gas? What is the name of this gas? **Hydrogen sulfide gas (H<sub>2</sub>S) interacts with ferrous sulfate. Forms ferric sulfate (FeS)**

How does this reaction occur? What enzyme must the organism contain? **If organism has cysteine desulferase it can breakdown the cysteine in the media to combine with the ferrous salts of the KIA media.**

#### **Slide #15**

If you had this result after the addition of Reagents A & B, was nitrate reduced? What was it reduced to? **Yes, nitrate was reduced to nitrite.**

How does this denitrification process occur? How is nitrate reduced in terms of metabolic pathways? **Nitrate acts at the final electron acceptor over oxygen. Through anaerobic respiration, nitrate receives electrons to reduce to nitrite.**

If zinc were added to the broth and this was the result, what would this indicate? **Indicates a lack of reduction because the zinc will interact with nitrate to reduce it to nitrite. If the solution remains clear, it would indicate nitrate was reduced to other end products besides nitrite.**

#### **Slide #16**

What would this result indicate after reagents A&B AND zinc were added? **This would indicate nitrate was reduced beyond nitrate to other end products such as ammonia.**

#### **Slide #17**

Which plate would you expect to have the greatest serial dilution? Why? **Plate #3 as there are the least amount of plaques**

In terms of number of plaques, what is the maximum and minimum number outside of which you would indicate too many or too few to count? **25-250 plaques.**

#### **Slide #18**

Which had the greatest zone of inhibition? #3

Which had the greatest resistance overall? #3

### **Slide #19**

In this test, which would indicate positive for serum antibody presence. #2

Explain how a primary ELISA test acts in terms of antibodies and antigens (no washing steps). **An antigen is added to the bottom of the well. Patient serum, which may or may not contain the antibodies are added to the well. A secondary antibody is then added to the well. If a patient has an antibody that is bound to the initial antigen, the secondary antibody binds. An enzyme that is attached to the secondary antibody will cause a color change indicating positive ELISA.**

### **Slide #20**

What type of stain was this testing for? **Endospores.**

What two genus are known for creating endospores? **Bacillus & Clostridia**

How will an endospore eventually release? What happens when it is released? **If favorable conditions are met again. The organism is released and continues to germinate and maturation.**

What stain corresponds to the part of the cell and endospore? **Malachite green stains the endospore green. Safranin stains the vegetative spore red/pink.**

### **Slide #21**

List two ways this staining process differs from others: **This process stains the background of the slide rather than the capsule itself. You do NOT heat fix the slide b/c it can cause the capsule to shrink.**

What do capsules tend to be made of? **Proteins or polysaccharides**

### **Slide #22**

What component of the cell wall do organisms with a positive result for this stain contain? **Mycolic acid.**

What is a disease caused by one acid-fast organism? **Tuberculosis caused by Mycobacterium tuberculosis.**

Which would be considered acid-fast positive? What is a common genus for acid-fast organisms? **The red-color. Mycobacterium.**