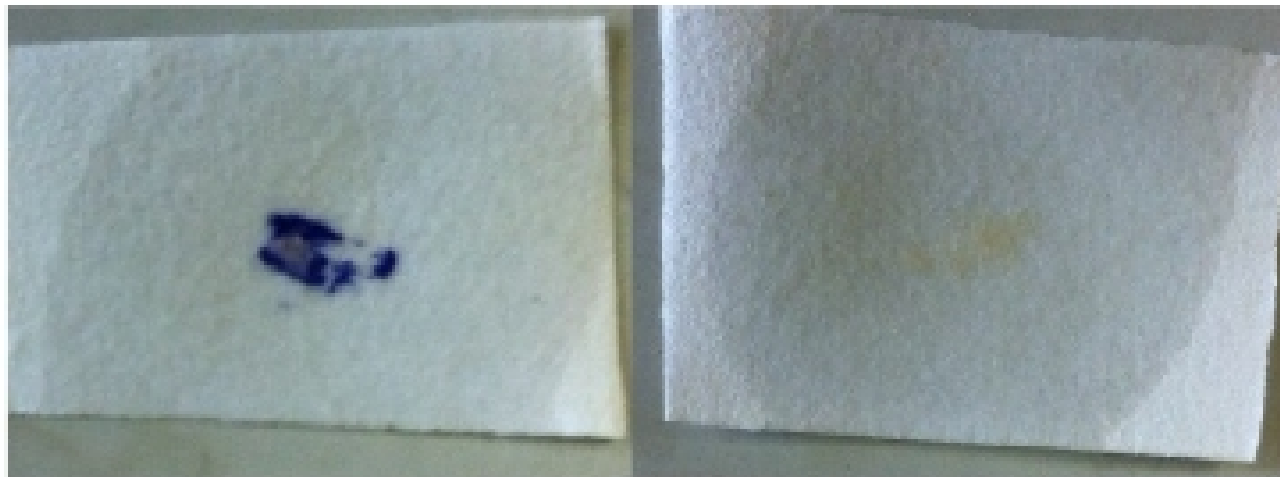


MICR 351 Lab Study Guide

It is important to know and understand the procedure, purpose, materials used, and biochemical reactions for all of these tests.

1. Oxidase:



Positive Oxidase Test

Negative Oxidase Test
(Read within 30 seconds)

- Reagent used: Oxidase
 - Indicator is Tetramethyl-p-phenylenediamine
- Why is the purpose of the oxidase test?
 - To identify the presence of cytochrome c oxidase
- How is the oxidase test performed?
 - Transfer a bacterial colony to filter paper. Add a few drops of oxidase reagent to and observe for a color change within 20 seconds.
- Why should you read the results within 30 seconds?
 - The reagents used for this test are unstable and may oxidize independently shortly after they become moist.
- Be able to recognize a positive and negative reaction.
 - Positive = color change to blue/purple in 20 seconds
 - Negative = no color change in 20 seconds

2. Catalase:



Positive Catalase Test

Negative Catalase Test
Bacterial smear shown with no bubbles produced

- Reagent used: Hydrogen peroxide
- Why is the catalase test used?

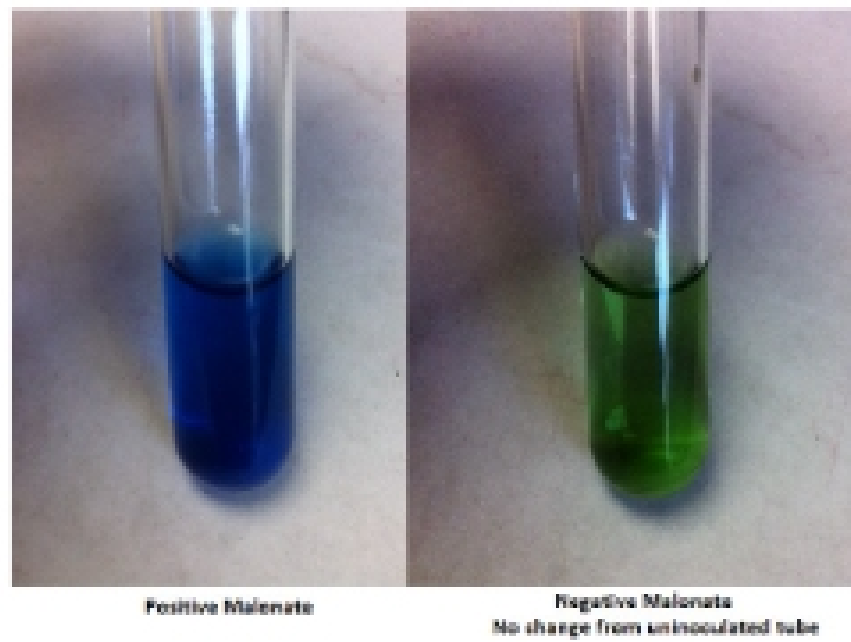
- o To identify bacteria that produce catalase.
- How is the catalase test performed?
 - o Bacteria are added to a microscope slide and one or two drops of hydrogen peroxide is added onto the bacteria.
- Be able to recognize a positive and negative reaction.
 - o Positive = bubbles
 - o Negative = no bubbles

3. MAC- MacConkey:



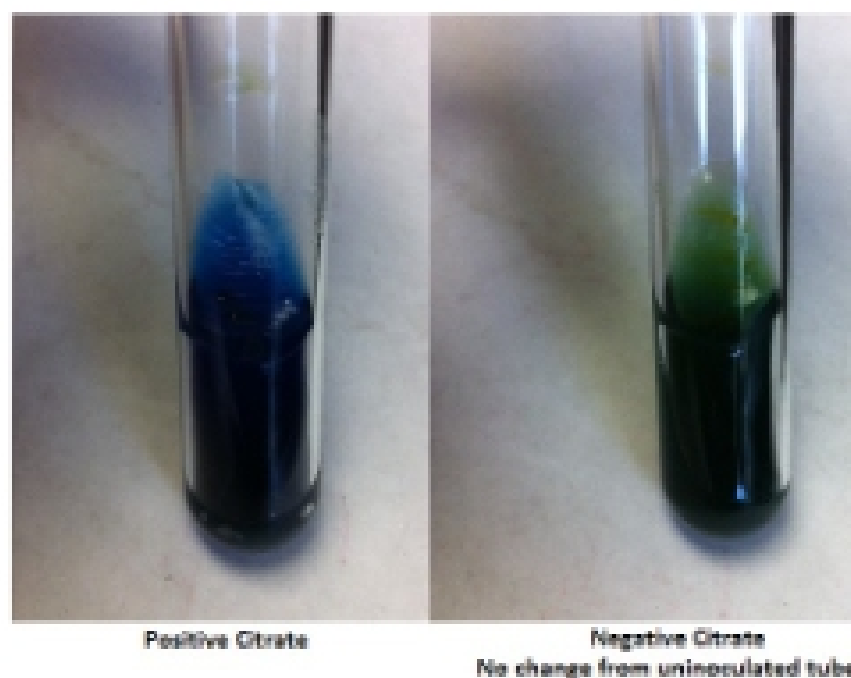
- Why is MAC used?
 - o MAC is used to isolate and differentiate members of the *Enterobacteriaceae* based on the ability to ferment lactose. It also selects against Gram-positive bacteria.
- What does the bacteria look like on the media?
 - o Gram-Positive = Poor growth or no growth → Organism is inhibited by crystal violet and/or bile
 - o Gram-Negative = Good growth → Organism is not inhibited by crystal violet or bile
 - o Probable coliform = Pink to red growth with or without bile precipitate → Organism produces acid from lactose fermentation
 - o Noncoliform = Growth is "colorless" (not pink or red) → Organism does not ferment lactose.
- What does MAC do?
 - o
- Is this test selective or differential? Why?
 - o Both. The bile salts and crystal violet inhibit the growth of Gram-positive bacteria (selects against) so only Gram-negative bacteria will grow (selects for). It is differential because it differentiates lactose versus non-lactose fermenting bacteria. The lactose fermenting bacteria will lower the pH and appear red. The non-lactose fermenting bacteria will have a higher pH and will be colorless.

4. Malonate:



- Be able to recognize a positive and negative reaction.
 - Positive = dark blue
 - Negative = No color change or slightly yellow
- Why is this test used?
 - Originally designed to differentiate between *Escherichia*, which will not grow in the medium, and *Enterobacter*. Its use as a differential medium has now broadened to include other members of *Enterobacteriaceae*.
- Determine the chemical reaction that takes place.
 - Figure 5.34 on page 345
- What causes the color change?
 - Bromothymol blue dye is added to indicate any shift in pH. If an organism cannot utilize malonate but manages to ferment a small amount of glucose, it may turn the medium slightly yellow or produce no color change at all. These results are negative. If the organism utilizes malonate, it will alkalize the medium and change the indicator from green to deep blue.
 - Malonate is a competitive inhibitor of succinate dehydrogenase. In combination with the subsequent buildup of succinate in the cell, it shuts down the citric acid cycle and will kill the organisms unless it can ferment or utilize malonate as its sole remaining carbon source.

5. Simmon's Citrate:



- Be able to recognize a positive and negative reaction.
 - Positive = blue