

# Micro unknown lab report

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| Gram Negative Unknown| Biology 3444-006| | Lena Wallace| 11/7/2011| |

Abstract: The purpose of this lab was to identify an unknown bacteria culture using differential tests. The identification of the unknown culture was accomplished by identifying the bacteria based on its specific metabolic characteristics and morphology.

It is suggested that culture 11 is a sample of *Enterobacter aerogenes*.

Introduction: This experiment was centered on metabolic and biochemical testing procedures. The rationale of performing these tests was to distinguish six different microbes from one another and to compare how their metabolic and biochemical processes differ from species to species to determine the unknown sample. The tests included: Triple sugar iron agar (TSIA), the Sulfide Indole Mobility (SIM) test, Glucose fermentation, the Methyl Red test, the Voges-Proskauer test, Citrate test, the Urease Test, and finally the Gelatin test. The microbes that were tested during this lab were: *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The sample labeled #11 could have been any of the six microbes.

A gram stain was performed to assess the shape and other characteristics of the bacteria, and to ensure that there was no gram positive contamination. Gram positive cells have a thick outer peptidoglycan layer that traps the crystal violet-iodine complex more than gram negative cells. As a result, they are less vulnerable to the de-colorization step with alcohol making them appear purple in color, while the gram bacteria negative appear pink. Triple sugar iron agar slant tests for multiple things: sugar fermentation of glucose,

lactose, and sucrose, and the production carbon dioxide and hydrogen sulfide. The gases are easy to identify.

If any carbon dioxide is produced cracks or bubbles appear inside of the medium, and sometimes enough CO<sub>2</sub> is produced to push the slant up towards the top, this will be reported as +g. The H<sub>2</sub>S is identified by how the gas reacts with an iron compound and makes the agar turn black. There are two possible types of sugar reactions that take place in the areas of the butt and the slant of the medium. The outcome of sugar metabolism will be acid production, so the pH indicator phenol red will turn yellow, and be reported as A. if there is no sugar metabolism, or alkaline by-products are made, will cause the indicator to stay the same color red, and reported as a K. TSIA medium is prepared as a shallow agar with a deep butt, providing for both an aerobic and anaerobic environment.

A TSIA medium must be checked within about 12 hours to see if it ferments glucose, and again after 24 hours to see if it ferments lactose and sucrose. If the slant returns to being red and the butt is still yellow after this time period, the organism ferments glucose but not the other sugars. If it is completely yellow after the time interval, this indicates that the organism ferments all three sugars. SIM Medium is used as differential test of microorganisms on the basis of hydrogen sulfide production, indole production, and motility. The Sulfur reduction test is useful in differentiating enteric organisms, the Indole test is used for differentiating the Enterobacteriaceae, and the Motility test is useful for testing a wide variety of organisms (condalab).

com). Casein is rich in tryptophan which is reduced and produces indole by the enzyme tryptophanase. Ferric ammonium sulfate is the indicator for H<sub>2</sub>S production. Once the medium was done incubating Kovacs' reagent was added to the tube. If the sample was positive the reagent would have a color change to red, if the reagent remained clear, a negative result was reported. Glucose fermentation uses Phenol Red Broth as differential test medium typically used to differentiate based on the color change of the pH indicator.

Phenol red turns yellow below a pH of 6.8, pink above a pH of 7.4, and remains red in between. A Durham tube is used to collect any gas that may be produced, and is reported as (+g) if a bubble appears on the inside and (-) if the organism cannot ferment the glucose and no bubble is trapped inside the tube. If the broth turns yellow, it means that acid was produced and reported as A.

If the organism can break down the amino acids by de-amination and ammonia is produced, this will raise the pH level turning it pink. This alkaline result was reported as K. The Methyl Red test is a differential test for bacterial respiration used to differentiate strains of coliform bacteria capable of performing mixed acid fermentation that will lower the pH despite the phosphate buffer (<http://faculty.deanza.fhda.edu>).

Mixed acid fermentation is confirmed by using methyl red as an indicator. It is red at pH 4.0 and below, yellow at pH 6.2 and above, and orange in between. Red is a positive result reported as (+), yellow is a negative result reported as (-), and orange is negative or inconclusive.

The Voges-Proskauer test to detect organisms that are able to ferment glucose, but convert the products to acetoin and 2, 3-butanediol. This is deduced by the addition of Reagent A and Reagent B, and the observation of the color change thereafter. Reagent A is a solution of  $\alpha$ -naphthol and alcohol. Reagent A catalyzes the conversion of acetoin to diacetyl. Diacetyl then reacts with guanidine-containing compounds from the peptone to form a red color in the presence of  $\alpha$ -naphthol. Reagent B is a solution of potassium hydroxide and water.

It absorbs CO<sub>2</sub> in the medium and acts as an oxidizing agent, catalyzing the reaction that converts acetoin to diacetyl (dalynn.com). After the VP reagents have been added, a red color is observed, this is a positive result reported as (+), if a copper color develops, the result is negative and reported as (-). Citrate test uses Simmons citrate agar to see if the organism is able to utilize citrate as a carbon source. Only bacteria that possess the enzyme citrate-permease can transport citrate inside the cell so it can be converted into pyruvate.

Simmons citrate agar utilizes sodium citrate as its only carbon source and ammonium phosphate as the nitrogen source. The pH indicator bromthymol blue dye is green at a pH of 6.9 and blue at pH of 7.6. Bacteria that can survive on the agar and utilize the citrate, alkalize the agar by breaking down the ammonium phosphate to ammonia and ammonium hydroxide, both increase the pH. Any change to a blue color is a positive result reported as (+), and if there is no change and the agar remains green the result is negative and reported as (-).

The Urea hydrolysis is catalyzed by the enzyme urease. Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia using water. A urea broth is used that contains yeast extract as its only nutrient source, buffers to inhibit alkalization of the medium, and phenol red as a pH indicator. Phenol red in this solution will be yellow or orange below pH 8. and pink above, to show any increase in pH. A pink color in the both indicates a positive result and reported as (+), and an orange or yellow appearance the result is negative and reported as (-).

The Gelatin test is used to see if the microbe produces the enzyme gelatinase. Gelatin is a protein made from collagen, made from animal connective tissue. Gelatinase is an extracellular proteolytic enzyme that aids in the breakdown of protein into amino acids (Harisha 244). Gelatin is used as the medium, which can liquid at room temperature but solidifies at about 4°C. Since the gelatinase enzyme can be quite slow, an incubation time of one week is needed. A positive test result will be reported if the sample remains a liquid after it is placed in the cold room, and a negative result will be reported if it re-solidifies.

Experimental Procedures: The tests performed provided key information about the unknown bacteria and how it carries out its metabolic functions. The visualization of bacteria at the microscopic level is made possible by the use of various stains, which react with elements present in some cells but not others. The Gram stain was utilized in this procedure in four essential steps: apply the primary stain crystal violet, fix with iodine, decolorize with 95% ethyl alcohol to wash out the crystal violet-iodine complex, and the counter-stain Safarin was added. TSIA medium was inoculated using an <https://assignbuster.com/micro-unknown-lab-report/>

inoculating needle by stabbing the agar through the butt, and then the needle was pulled out and a streak was made up the slant. The TSIA medium was incubated at 34°C and checked after 18 and 24 hours for a change in color.

TSI contains the three carbohydrates glucose, sucrose, and lactose. The medium also contains animal and yeast extract, and peptones as the sources of nitrogen, vitamins and minerals, and ferrous ammonium sulfate as the indicator for H<sub>2</sub>S. Phenol red is the pH indicator. (microbelibrary. org) The SIM medium contains casein digest and animal digest to provide peptones to provide nutrients, vitamins, and minerals that are essential for growth. The SIM medium was inoculated by stabbing the medium with an inoculating needle, and incubated at 34°C for 24 to 48 hours.

Once the medium was done incubating Kovacs' reagent was added to the tube to check for indole production. Phenol Red Broth, used for glucose fermentation, contains peptone, phenol red (a pH indicator), a Durham tube, and glucose. The broth is inoculated with the inoculating loop, and incubated at 34°C for 48 hours. The Methyl Red broth contains peptone, glucose, and a phosphate buffer. The broth is inoculated with the inoculating loop, and incubated at 34°C for 48 hours.

Once the sample is done incubating, a 1.0 mL aliquot is taken and three drops of the Methyl red indicator is added. The results of a red color can be observed immediately if it is positive, otherwise it is a negative result. The Voges-Proskauer broth contains peptone, glucose, and a phosphate buffer

just as in the MR broth. The broth is inoculated with the inoculating loop, and incubated at 34°C for 48 hours.

Once the sample is done incubating, a 1.0 mL aliquot is taken and 15 drops of Reagent A is added along with 5 drops of Reagent B. The result is monitored at ten minute intervals for 1 hour. The results of a red color can be observed if it is positive, otherwise it is a negative result if there is no color change. The Citrate test was lightly inoculated using an inoculating needle by streaking the slants with the unknown, incubated at 34°C for 48 hours, and read for a color change.

The Urea hydrolysis uses Rustigian and Stuart's broth that contains yeast extract, monobasic potassium phosphate, dibasic potassium phosphate, urea, and phenol red. The broth was heavily inoculated with the inoculating loop and incubated at 34°C for 24 hours. The Gelatin test uses gelatin agar that also contains beef extract and peptone. The medium is stab inoculated with an inoculating needle and incubated at 34°C for up to 7 days. The sample is then placed in the cold room to check for re-solidification. Results: The gram stain procedure showed to be all gram negative pink, straight rods.

They had no particular arrangement or clustering. TSAI SIM test Glucose fermentation The Methyl Red test The Voges-Proskauer test Citrate test The Urease Test Gelatin test Conclusion: *Enterobacter aerogenes* Material ; Methods Gram negative cells have a thinner peptidoglycan layer and a lipid membrane external to the cell wall...Gram-positive bacteria appear dark blue or violet due to the crystal violet stain following the Gram stain procedure;



Gram-negative bacteria, which cannot retain the crystal violet stain, appear red or pink due to the counterstain (usually safranin).

References: [http://www. microbelibrary. org/component/resource/laboratory-test/2842-triple-sugar-iron-agar-protocols](http://www.microbelibrary.org/component/resource/laboratory-test/2842-triple-sugar-iron-agar-protocols) <http://www. condalab. com/pdf/1514. pdf> [http://faculty. deanza. fhda. edu/gilleskay/stories/storyReader\\$80](http://faculty. deanza. fhda. edu/gilleskay/stories/storyReader$80)

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