

Lab Procedures Narrative

Day One - Monday April 13, 2009

Today I performed the gram stain test for unknown tube #35 and determined that my unknown tube contained a Gram (+) rod and a Gram (-) rod. To prepare for day two of this unknown project, I inoculated my unknown on an EMB plate (to isolate for the Gram (-) rods) and a PEA plate (to isolate for the Gram (+) rods). The EMB and PEA plates were both placed in an incubator set at 37°C, while my unknown tube was placed in the refrigerator.

Day Two - Tuesday April 15, 2009

Today I retrieved my EMB and PEA plates from the incubator. The Gram (-) colonies that had grown on my EMB plate were metallic green/black, which suggested that my unknown is a probable coliform that ferments lactose/sucrose and produces acid. Therefore, my unknown Gram (-) organism is *C. freundii*, *E. coli*, or *E. aerogenes*. To differentiate between these three organisms, I will perform the Methyl-Red test.

The Gram (+) organism that had grown on my PEA plate was made of opaque and off-white colored colonies. Their appearance suggests that the organism is not inhibited by phenylethyl alcohol (and is thus gram positive). The only two Gram (+) rods that we have studied in lab includes *B. megaterium* and *B. subtilis*. To differentiate between these two organisms, I will perform the BA Hemolysis test.

First I streaked one NA plate with a Gram (+) sample from my PEA plate and another NA plate with a Gram (-) sample from my EMB plate. These NA plates should ensure growth of pure Gram (+) and pure Gram (-) colonies, and both were placed into the 37°C incubator.

Second, I inoculated a Methyl Red - Voges Proskauer media tube with a Gram (-) colony sample from my EMB plate. I also streaked a PEA plate Gram (+) colony sample onto a plate of Blood Agar (BA). Both the MR-VP tube and the BA plate were placed in the incubator at 37°C, while the EMB and PEA plates were placed in the refrigerator.

Day Three - Monday April 20, 2009

Today I retrieved my BA plate and Methyl Red tube from the refrigerator. My BA plate exhibited good growth, yet there was no change in media color. This suggested my Gram (+) unknown does not hemolyze RBCs, and according to my flow chart should be *B. megaterium*.

I added three drops of Methyl Red to my MR-VP culture, and the contents immediately turned red indicating a positive result. This meant that my Gram (-) unknown is either *C. freundii* or *E. coli*. To differentiate between these two organisms, I will use the Simmons Citrate test. I inoculated a citrate slant using a colony sample from my Gram (-) NA plate, and placed the slant into the 37°C incubator and the NA plate back into the refrigerator.

Day Four - Wednesday April 22, 2009

My Simmons Citrate slant was blue, meaning that my unknown organism can utilize citrate. Of my two remaining possible unknowns, only *C. freundii* yields citrate positive results.

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Narrative of Lab Procedures for Test Tube #41

For my Microbiology unknown project I choose test tube #41 which I then performed a gram stain test on to figure out the cell morphology of my organisms and to identify which organism was Gram-negative and which was Gram-positive. After staining my organisms and observing them after oil immersion with the microscope I found out that I had Gram-negative rods and Gram-positive rods. For my next test I cultured the organisms on Eosin Methylene Blue to select for growth of Gram-negative bacteria while inhibiting growth Gram-positive bacteria. I also cultured my organisms on Phenyl Ethyl Agar to select for growth of Gram-positive bacteria while inhibiting the growth of Gram-negative bacteria. My Gram-negative culture on Eosin Methylene blue resulted in growth that was pink meaning it was a possible coliform. This narrowed down the possibilities of what my Gram-negative bacteria could be to either: *Proteus vulgaris*, *Psuedomonas aeruginosa*, *Proteus mirabilis*, or *Serratia marcescens*. The next test I did was a MRVP test to identify my Gram-negative bacteria out of these four. The MR test came out negative which meant that my Gram-negative bacteria were either *Serratia marcescens* or *Psuedomonas aeruginosa*. Finally I preformed a VP test which was positive and I identified my unknown Gram-negative bacteria as *Serratia marcescens*. For my Gram-positive bacteria after I identified them as Gram-positive bacilli I knew that it could only be either *Bacillus subtilis* or *Bacillus megaterium*. Therefore I cultured the Gram positive bacteria on Blood Agar to differentiate my organism from my two choices. The result from my Blood Agar test was that my unknown Gram-positive bacteria preformed β -hemolysis which meant that my unknown Gram-positive bacterium was *Bacillus subtilis*.

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