

# Unknown paper

[Science](#), [Biology](#)



The objective of these “ unknown” experiments was to take a mixed culture, which contains two unknown species, and identify those species through a series of tests. The group was informed that one species of bacteria would be a gram-negative bacillus and the other would be a gram positive coccus. The tests to be conducted ranged from streak plate isolation to biochemical tests. Each test to be conducted was discussed and agreed upon by all group members. The results of each test were analyzed by the group and led to selection of the next test that would further narrow the possible identity of the unknown species.

On September 16, 2010, our group was given a mixed culture in which we were to identify two organisms within the mixture, by running several biochemical tests. On this day our objective was to prepare the specimen of the mixed culture into discrete colonies. Each member of our group then conducted a streak plate and we would later pick the best plate of isolated colonies. To perform a streak plate, aseptic technique was required. We had our mixed culture in the form of a broth therefore our inoculating instrument would be a loop.

We also needed our agar plates each marked into four quadrants and a Bunsen burner. We then proceeded to transfer the mixed culture to the plates aseptically. In preparation for the transfer of the mix culture to a plate we placed the tube of broth in our non-dominant hand. The loop was sterilized by placing it into the fire of the Bunsen burner until the entire wire became red hot, “ red is dead”. The tube was uncapped facing the cap downward along with the inoculated loop in the dominant hand.

We then passed the tube through the flame of the Bunsen burner briefly to burn off any contaminants that may be present at the opening of the tube. The inoculated loop was then inserted into the broth of the mixed culture to obtain the organisms to be transferred to the plate. The tube was then passed through the Bunsen burner again, capped, and put aside. With the sterilized loop containing the organism we proceeded to transfer the organism to the plate of quadrant I in a zigzag movement. We then re-flamed the loop till red and cooled the instrument to the side of quadrant II.

Then from quadrant I we made four lines crossing into quadrant II. We re-flamed the loop till red and then cooled the instrument again to the side of quadrant III. From quadrant II we made four lines crossing into quadrant III. From quadrant III we continued making four more lines crossing into quadrant IV. We inoculated our loop once more, freeing the instrument of any organism by re-flaming till red. Once we each completed a streak plate, the plates were taped and marked with the date, initials, and group number. On September 23, 2010, we obtained our plates made from September 16.

We identified discrete colonies into two organisms that we named yellow and beige. The yellow organism was an obvious yellow pigmentation, moderate in size, entire, circular, raised colony and the beige was an off-white pigmentation, small, entire, circular, umbonate colony. We next chose the best representative colony of each organism to be transferred to a nutrient agar slant. Again we aseptically transferred the organisms, yellow and beige, into individual agar slants. Our instrument that we used was a loop along with two slant tubes and a Bunsen burner.

With our selected plate ready and available, the slant in the least dominated hand, we inoculated the loop till red, uncapped the tube, flamed the tubes, obtained the yellow organism from the plate, and transferred it to the slant in a zigzag motion. We then re-flamed the tube, capped the test tube, and flamed the loop. Then we proceeded with the same procedures for the beige organism. The purpose of transferring the organisms was to evaluate the abundance of growth, pigmentation, optical characteristics, form (not applied due to the use of a zigzag rather than a straight line), and consistency.

On October 7, 2010 our third day of our Unknown's project we conducted a Gram stain procedure. From last week's test, we achieved pure cultural characteristics from the two slants we made. The growth we saw on the agar slant that contained the yellow specimen was a soft, smooth, yellow growth. The growth we saw on the beige specimen was a thin, even, beige growth. Both cultural characteristics were achieved in the appropriate categories. The categories we were looking for contained abundance of growth, pigmentation, optical characteristics, and consistency.

Today we will be preparing two bacterial smears from each specimen and Gram staining them. The reason we are conducting this test is to differentiate between two principle groups, gram positive and gram negative and to further know if a pure culture from both organisms was achieved. This is important for classification and differentiation of microorganisms. The Gram stain reaction will help us tell the difference of the chemical composition of bacterial cell walls. The Gram stain procedure uses four different reagents such as crystal violet, gram's iodine, ethyl alcohol, and safranin.

Before the Gram stain is performed we must make two bacterial smears of the two specimens. We placed one loop of distilled water on a clean slide aseptically. He transferred the specimen from the agar slant that contained the yellow growth and placed it on the slide with the water and gently mixed it together in a circular motion approximately the size of a nickel. He let the smear air dry for one minute and gently heat fixed it by quickly passing the slide through the flame 3-5 times with a clothes pin. The same aseptic transfer and Gram stain procedure was performed on the agar slant that contained the beige specimen.

After we successfully performed the bacterial smear, we started the Gram Stain procedure. The first step in the Gram stain procedure is flooding the bacterial smear with crystal violet and letting it sit for one minute. After the crystal violet has set we rinsed the reagent off with distilled water. Next, we flooded the bacterial smear with Gram's Iodine for one minute. After we let the Gram's Iodine set we rinsed the Gram's Iodine off of the slide gently with distilled water. The next step in the Gram stain procedure contained 95% Ethyl alcohol.

Drop by drop we let the alcohol run onto the stain until the color of the stain was almost clear. After this step we rinsed off the alcohol with distilled water once again. The next step in finalizing the Gram stain procedure is counterstaining the smear with safranin for 45 seconds. Once the counterstain has set we rinsed the stain gently one last time with distilled water and used bibulous paper to blot dry the stain. After we completed the Gram stain procedure we looked at both Gram stain's under a light

microscope at 100X with immersion oil. The steps in preparing the light microscope are very simple.

First we plugged in the microscope and turned it on, second we made sure the light intensity has been adjusted and the stage is all the way down. Then we placed the slide on the stage and clipped it into place and raised the stage all the way up with the course adjustment knob. We made sure the objective lens is started at 4X also known as the scanning objective. While we were looking through the oculars we slowly lowered the stage until we could see our specimen. It was not clear so with the fine adjustment knob we turned the knob away from us and fine focused the specimen until we could see it much clearer.

Then we change the objective lens to 10X and again turned the fine adjustment knob away from us until the specimen became clearer. We remembered to not touch the course adjustment knob once we have moved away from the scanning objective lens or we would lose our specimen. After we saw our specimen clear under 10X, we turned the objective lens to 40X and turned the fine adjustment knob until we once again saw a clear specimen through the oculars. Once we saw the specimen under 40X we turned the objective lens between 40X and 100X, this is where we used immersion oil only.

We did not lower the stage to put oil immersion on the stage or our specimen would be gone. The reason we used oil immersion is so there was way for light to escape through the slide, and the 100X objective lens. It is used as a piece of glass that does not let the light bend and refract, so the image of our specimen is seen even clearer than before. We place two drops

of immersion oil on the slide and turned the objective lens all the way to 100X and slid the objective back and forth a couple of times through the oil that way it is covered completely and there were no air bubbles.

Using the fine adjustment knob we found our specimen once again and it was clearer than ever. We have found your specimen. Under the microscope the yellow specimen we stained was a purple gram positive stain with a tetrad arrangement. The beige organism we Gram stained was a pink gram negative stain with no arrangement. Once we were done with this part of the experiment we decided as a group that the next test we needed to run was the Carbohydrate Fermentation test. The reason for choosing this test was so we would be able to determine if the organism is able to degrade and ferment carbohydrates with the production of acid and gas.

After finding our specimens we lowered the stage and took the slide off of the stage and cleaned the 100X oil objective lens with Kym wipes. We turned the objective lens back to 4X, the scanning objective, and turned the microscope off. On October 21, 2010 the Lactose Carbohydrate Fermentation test was previously selected and prepared for the week prior in order to reduce the probability of our organisms. We performed aseptic technique when transferring our unknown organisms which consisted of performing these previously perfected steps to ensure that our tests be inoculated properly.

When performing aseptic technique you need to have all the proper materials necessary to ensure the highest level of sterility while maintaining safety. In sequential order these are the steps that we used to perform the sub culturing for our unknowns assignment. In preparation for the transfer,  
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the stock tube and the tube to be inoculated both had caps loosened and properly placed in the non-dominant hand in a V formation separated by the third digit. An inoculating loop was apprehended and sterilized by placing it to the fire until the entire wire became red hot (“red is dead”).

We uncapped the tubes simultaneously with the inoculating loop still in the dominant hand and immediately passed the two tubes through the flame of the Bunsen burner briefly. The inoculating loop then was inserted into the subculture tube and the inoculum was obtained and transferred to the tube that needed to be inoculated. Following the proper inoculation and removal of the loop from the tube, the necks of both tubes were then again passed through the Bunsen burner, and then the caps were replaced on the proper tube. Lastly the inoculating loop was again flamed to destroy the remaining organisms on the instrument.

After successfully transferring the broth culture with the organism to phenol red test tubes with Durham tubes in each, we reviewed our possible results to ensure we understood what was expected. “Anaerobic use of sugars produces pyruvic acid from glycolysis, and eventually lactic acid or lactic acid and carbon dioxide through the fermentation pathways.” Results after incubation should show negative or positive results. A negative result for sugar fermentation is shown as no color change and no gas in Durham tube. A positive result for carbohydrate fermentation is shown as color changed to yellow indicating acid production.

Gas bubble in Durham tube indicates carbon dioxide production. Preparation for Nitrate reduction test was performed and it consisted of us maintaining our initial broth of our G -unknown. The reason we did the Nitrate reduction



test on our Gram – specimen was because according to our chart getting a positive or negative result would eliminate several organisms and would bring us closer to finding out which specimen we had. Some organisms have the ability to reduce  $\text{NO}_3^-$  to Nitrite  $\text{NO}_2^-$  or  $\text{N}_2$  or ammonia. \*\*\*Javance can you type out the procedure that was done for the Nitrate reduction test right here where this note is.

We only did this test in our Gram negative organism. I found out where I made that mistake in my notes. So I fixed it for you. Just type out our transfer from our original broth to the tryptic nitrate broth step by step like you did for our carbohydrate fermentation test\*\*\* We added 5 drops of Nitrate A and 5 drops of Nitrate B to our Nitrate broth which contained our G-specimen and also had a Durham tube in it and incubated for one week. On October 28, 2010, after a week has passed of incubation, we reviewed our results from our two previous tests; the Carbohydrate Fermentation and Nitrate reduction test.

Carbohydrate fermentation test results were for the yellow G+, no gas bubble was present and the color did not change, it remained red which shows negative fermentation. For beige G-, no gas bubble or color change was seen so it also showed negative fermentation. According to our charts, our G+ specimen was the only one in our chart that had G+ cocci and negative fermentation so our result indicated our first specimen was *M. Luteus*. Our Nitrate reduction test result was our G- organism turned red which indicated a positive nitrate reduction to nitrite. We then prepared our Gram-negative organism for a Hydrogen Sulfide Test ( $\text{H}_2\text{S}$ ).

We chose the H<sub>2</sub>S test because we found that it would illuminate our organism into a possibility of either two positive results or three negative results. In the H<sub>2</sub>S test we used a SIM agar deep test tube as our medium and aseptically transferred our Gram-negative organism from a broth using a needle to stab into the test tube. \*\*\*Veda can you type step by step right here the procedure done for the H<sub>2</sub>S test into the SIM agar deep, not just that we stabbed it\*\*\*This medium contains peptone and sodium thiosulfate as the sulfur substrate; ferrous sulfate behaves as a H<sub>2</sub>S indicator and enhances anaerobic respiration.

Since Hydrogen sulfide is a colorless and invisible gas, the ferrous ammonium sulfate in the medium combines with the gas producing FeS, which produces a black precipitate. After a week of incubation On November 4, 2010, we observed black precipitate leading to a positive result for H<sub>2</sub>S gas production, if there was no color change, then that would have indicated a negative result for H<sub>2</sub>S gas production. With a positive result of our findings, our Gram negative organism concluded a possibility of two organisms of *Salmonella typhimurium* or *Proteus vulgaris*.

After we analyzed our findings, we figured the next test that would reveal our organism would be through the Indole Production Test. An Indole Production test also uses a SIM agar deep medium and with the Kovac's reagent we would get immediate results by adding ten drops of Kovac's reagent. By adding the Kovac's reagent, a negative result for indole production by giving off a yellow or brown color. A positive result would show if there was a red pigmentation in the reagent layer.

With gloves on we added 10 drops of Kovac's reagent to our specimen and immediately observe a yellow/brown reagent layer, concluding a negative result. We finally discover that our Gram-Negative organism is *Salmonella typhimurium*. Based on the results of our biochemical tests, our two species of Unknown bacteria were found to be *Micrococcus luteus* and *Salmonella typhimurium*. *M. Luteus* was determined to be our Gram positive organism after a negative result on the Lactose fermentation test.

This test ruled out *L. lactis* and *S. aureus*. To determine that *Salmonella typhimurium* was our Gram negative organism we needed to conduct a few more tests. The lactose test yielded a negative result which led us to conduct a Nitrate Reduction test that yielded a positive result. At that point we conducted a Hydrogen Sulfide test and it yielded a positive result and that allowed our group to conduct an Indole test. This finalized our identity for the Gram negative organism *Salmonella typhimurium*.