**Identification of an Unknown Bacterial Species**

**MCB2010L**

**INTRODUCTION:**

Numerous times a day, medical professionals apply the scientific method to diagnose and treat patients. When a patient is admitted to the hospital, the doctors and nurses must gather quantitative and qualitative data about the patient’s condition in order to treat the disease and have the best outcome for the patient. Examples of this data include qualitative signs such as fatigue, nausea, pain, and qualitative symptoms such as body temperature, heart rate, and blood pressure. As a future medical professional, you must become adept at gathering qualitative and quantitative data, evaluating that data and results of test, and making decisions about how best to treat a patient – all aspects of the scientific method.

In this exercise, you will use the scientific method – specifically performing tests, gathering and interpreting qualitative data, making conclusions, and deciding the appropriate next steps – to correctly identify a bacterial specimen. The skills of making observations, using those observations to interpret results of tests, using the results of tests to make a conclusion, and evaluating the conclusion to identify appropriate next steps, are directly transferrable skills you will use in your career as medical professionals.

Consider this scenario:

A patient presents with a persistent cough, fever, and malaise. How do you treat the patient?

You might think the patient has pneumonia, so you prescribe an antibiotic and discharge the patient. However, there are over a dozen microbes that can cause the symptoms listed above, and some require specialized antibiotics. The better course of action would be to collect a specimen of sputum and send the specimen to a clinical lab for analysis. The lab will use a series of experiments to identify the bacteria in the specimen and will test the specimen for antibiotic sensitivity. When you get the results, you will know exactly what antibiotics to prescribe to the patient to treat their disease.

To identify the bacteria in the specimen, the clinical lab uses a variety of tests, some of which we have used in this course. In this exercise, you will use a series of four tests, all of which you have performed previously, to positively identify a bacterial specimen. You have the option of performing, or evaluating the results of, seven different experiments: the Gram stain, Acid-Fast stain, SIM agar motility assay, catalase test, Staphyloside test, UV sensitivity, and Enteropluri Tube test. You will follow a flow chart to determine when/if you use the test.

In this course, we have used different types of tests to identify and characterize microorganisms. Morphological tests distinguish microorganisms based on physical characteristics. Differential and specialized stains are a perfect example of morphological tests. There are capsule stains, flagella stains, and endospore stains. In this course, we used the Gram stain and the Acid-Fast stain to differentiate between the structure and components of the cell wall. Other morphological tests include motility assays and endospore tests, like UV sensitivity.

There are also immunological tests that distinguish organisms based on antigens found on the cell surface. These tests employ antibodies that specifically recognize the antigens that are characteristic of the particular organism. These tests, unlike morphological tests, can distinguish between closely related organisms. Immunological tests include the Streptocard test which uses antibodies to distinguish between different types of Streptococci and the Staphyloside test which allows for the positive identification of *Staphylococcus aureus* based on the presence of the antigen, Protein A.

Finally, there are biochemical tests, which distinguish organisms based on their ability to utilize specific metabolites due to the presence of specific metabolic pathways and/or enzymes. Examples of biochemical tests include the Enteropluri Tube, which has 16 different biochemical tests, that is used to positively identify enteric bacteria, and the catalase test, which positively identifies aerobic bacteria due to the presence of the enzyme catalase.

Fill out the following chart to prepare for the experiments in the unknown analysis.

|  |  |  |  |
| --- | --- | --- | --- |
| **Test** | **Positive Result** | **Negative Result** | **Characteristic** |
| Gram stain |  |  |  |
| Acid-Fast stain |  |  |  |
| SIM agar Motility Assay |  |  |  |
| Catalase Test |  |  |  |
| UV Sensitivity |  |  |  |
| Staphyloside Test |  |  |  |

\*Enteropluri Tube can also positively identify enteric bacteria based on 16 biochemical tests

Every bacterium can be classified as either Gram positive or Gram negative based on the structure of the cell wall. In a clinical lab, this is usually the first test performed to start to identify a bacterial specimen, and this is where we will start. From there, you will perform three more tests to be able to positively identify your bacteria out of seven other possible candidates. To aid you in determining which tests to perform, you have been provided with a flow chart .

**PROCEDURES:**

**Modified Smear Procedure for Gram and Acid-Fast stain**

In order to determine if your stain was performed correctly, you need to have a control that you know the identity and Gram or Acid-Fast staining results for. We will use *Staphylococcus aureus* as the control in both the Gram and Acid-fast stain. It must be on the same slide as the unknown sample, but not mixed with it. *S. aureus* will stain purple in the Gram stain and blue in the Acid-Fast stain. If you see these results, you know the results for your unknown are correct and valid.

1. Obtain one slide for each stain (Gram and Acid-Fast)
2. Label the left side of the slide with a “C” for control
3. Label the right side of the slide with a “?” for unknown
4. Sterilize the loop
5. Place a loopful of water on each side of the slide
6. Sterilize the loop
7. Add *Staphylococcus aureus* to the left droplet and spread it around the left half of the slide.
8. Add the unknown specimen to the right droplet and spread it around the right half of the slide. DO NOT MIX it with *Staph aureus*.
9. Air dry.
10. Heat fix the slide.
11. Follow the procedure for the Gram or Acid-Fast stain.

**Gram Stain Procedure**

1. Flood the slide with Crystal Violet for 1 min. Rinse with water.
2. Flood the slide with Iodine for 1 min. Rinse with water.
3. Drip Gram decolorizer (95% alcohol) on the slide for 2-3 seconds, rinse with water.
4. Flood slide with Safranin for 1 min. Rinse with water.
5. Blot dry and view each smear under oil immersion.

**Acid-Fast Stain Procedure**

1. Flood the slide with Carbolfuchsin for 5 min. Rinse with water.
2. Drip TB decolorizer (acid-alcohol) on the slide for 30 seconds. Rinse with water.
3. Flood slide with Methylene Blue for 1 min. Rinse with water.
4. Blot dry and view under oil immersion

**SIM agar Motility Assay**

1. Obtain a SIM agar tube and label it appropriately
2. Sterilize the inoculating needle
3. Dip needle into the broth culture of your unknown
4. Stab the SIM agar with the inoculation needle, going in an out as straight as possible.
5. Sterilize your needle.

**Catalase Test**

1. Using the loop, pick a colony of your unknown off of the agar plate and spread it on a clean slide.
2. Place one drop of hydrogen peroxide on the slide, where you spread the bacteria.
3. Look for the production of bubbles.

**Staphyloside Test**

1. Drop one drop of the test latex onto one of the circles on the reaction card.
2. Use a sterile toothpick, or sterilized loop, to pick one colony from your unknown agar plate and mix into the test latex.
3. Gently rock the card and look for agglutination.

**UV Sensitivity**

This is a DEMO – the experiment has been done for you. You just need to interpret the results.

1. Locate the UV plate that corresponds to your unknown number.
2. It has been exposed to UV light for 2 minutes.
3. Evaluate the growth on the exposed (not covered by an index card) side of the plate and determine whether or not this bacteria produces endospores (based on growth or no growth)

**Enteropluri Tube Test**

This is a DEMO – the experiment has been done for you. You just need to interpret the results.

1. Locate the Enteropluri Tube that corresponds to your unknown number.
2. Determine whether each compartment in the test is positive or negative and use the key to code the test.
3. Look up the code you generated and identify your bacteria.’

**HOW YOU WILL BE TESTED:**

There are two assessments in the unknown analysis. Together these assessments are worth as much as a practical grade. This exercise has the ability to significantly help your grade, or significantly hurt your grade, depending on how seriously you take the exercise, how well you perform the experiments, make observations, and analyze results, and whether you can correctly identify your unknown specimen.

The first assessment, worth 50 points, is an open book/open note test to be taken at home on Blackboard. This test will assess your fundamental knowledge about the experiments you will perform, what results you should expect, and how to interpret those results.

Sample questions:

What morphological feature of the bacterial cell does the Gram stain distinguish between?

Which shape of bacteria is always non-motile?

What organisms are acid-fast positive?

A positive catalase test will be indicated by the presence of what?

Which organism expresses Protein A?

The second assessment, also worth 50 points, is a written assignment that will also be submitted and graded on Blackboard. This assessment tests your ability to perform the experiments and interpret the results of each experiment. The assignment asks you to write down your observations of each test, interpret those observations as a positive or negative result, and decide what to do next. The last question asks you to identify your unknown. The assignment is replicated below in the worksheet. It is highly recommended that you complete the worksheet in lab while you are doing the experiments, and then use the worksheet to complete the Blackboard assessment. There is also a completed sample of this worksheet on Blackboard so you know how much detail should be included in your responses.

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**Unknown Analysis Assessment – Part 2**

**Please complete this worksheet as you complete the unknown analysis in lab. You will later enter your answers into Blackboard to be graded by your instructor. Total Points Possible: 50/50]**

1. What is the number of the unknown bacterial specimen you were given? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. What question are you addressing in the unknown analysis? (What are you trying to accomplish?)
3. Why is it important to be able to identify the types of bacteria in a clinical sample? How are you able to help the patient with this information?
4. What is the first test you performed?
   1. Gram stain
   2. Acid-fast stain
   3. SIM motility assay
   4. Catalase test
   5. UV Sensitivity
   6. Staphyloside test
   7. Enteropluri Tube
5. What did you observe in the test? *(i.e. color of cells, shape of cells, bubbles, inhibition of growth, agglutination, etc.)*
6. From your observations, what did you conclude? *(i.e. Gram positive/negative, Acid-fast positive/negative, motile/non-motile, presence/absence of catalase, presence/absence of endospores, presence/absence of Protein A, identity of bacteria based on metabolic activity)*
7. Using your conclusion about the first test and the flow chart, circle all possible bacteria that could be in your sample.
   1. *M. smegmatis*
   2. *B. subtillis*
   3. *Ps. aeruginosa*
   4. *S. aureus*
   5. *S. epidermidis*
   6. *E. coli*
   7. *S. marcescens*
   8. *K. pneumonia*
8. What is the second test you performed?
   1. Gram stain
   2. Acid-fast stain
   3. SIM motility assay
   4. Catalase test
   5. UV Sensitivity
   6. Staphyloside test
   7. Enteropluri Tube
9. What did you observe in the test? *(i.e. color of cells, shape of cells, bubbles, inhibition of growth, agglutination, etc.)*
10. From your observations, what did you conclude? *(i.e. Gram positive/negative, Acid-fast positive/negative, motile/non-motile, presence/absence of catalase, presence/absence of endospores, presence/absence of Protein A, identity of bacteria based on metabolic activity)*
11. Using your conclusion about the second test, and the flow chart, circle all possible bacteria that could be in your sample.
    1. *M. smegmatis*
    2. *B. subtillis*
    3. *Ps. aeruginosa*
    4. *S. aureus*
    5. *S. epidermidis*
    6. *E. coli*
    7. *S. marcescens*
    8. *K. pneumonia*
12. What is the third test you performed?
    1. Gram stain
    2. Acid-fast stain
    3. SIM motility assay
    4. Catalase test
    5. UV Sensitivity
    6. Staphyloside test
    7. Enteropluri Tube
13. What did you observe in the test? *(i.e. color of cells, shape of cells, bubbles, inhibition of growth, agglutination, etc.)*
14. From your observations, what did you conclude? *(i.e. Gram positive/negative, Acid-fast positive/negative, motile/non-motile, presence/absence of catalase, presence/absence of endospores, presence/absence of Protein A, identity of bacteria based on metabolic activity)*
15. Using your conclusion from the third and the flow chart, circle all possible bacteria that could be in your sample.
    1. *M. smegmatis*
    2. *B. subtillis*
    3. *Ps. aeruginosa*
    4. *S. aureus*
    5. *S. epidermidis*
    6. *E. coli*
    7. *S. marcescens*
    8. *K. pneumonia*
16. What is the fourth test you performed?
    1. Gram stain
    2. Acid-fast stain
    3. SIM motility assay
    4. Catalase test
    5. UV Sensitivity
    6. Staphyloside test
    7. Enteropluri Tube
17. What did you observe in the test? *(i.e. color of cells, shape of cells, bubbles, inhibition of growth, agglutination, etc.)*
18. From your observations, what did you conclude? *(i.e. Gram positive/negative, Acid-fast positive/negative, motile/non-motile, presence/absence of catalase, presence/absence of endospores, presence/absence of Protein A, identity of bacteria based on metabolic activity)*
19. Using your conclusions and the flow chart, identify the bacteria in your “unknown” sample.
    1. *M. smegmatis*
    2. *B. subtillis*
    3. *Ps. aeruginosa*
    4. *S. aureus*
    5. *S. epidermidis*
    6. *E. coli*
    7. *S. marcescens*
    8. *K. pneumonia*