

AAI Teacher Research Fellowship Curriculum Development
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OVERVIEW:

Title: Standing on Guard for You

The science concepts covered in this laboratory activity will allow students to differentiate between specific and nonspecific immunity and examine chemicals involved in non-specific resistance. Students will also observe the bactericidal properties of normal body fluids and learn how to handle safely fluids containing microorganisms.

Using aseptic techniques, a spectrophotometry and preparing dilutions. The placement of this laboratory activity could be in several areas (i.e. immune system, microbial life, and diseases). The body's ability to stand off diseases can be specific or non-specific. This investigation will show students how our nonspecific immunity protects us all the time. There are many lines of defense our body has to keep us well. Millions of infectious viruses, bacteria, and other organisms are in the air you breathe, on every object we touch, and even on our skin. Why don't these pathogens make us sick all the time? Students will have a greater understanding of the many ways in which our bodies fight off disease and build on their knowledge of their immune system.

SCIENCE BACKGROUND:

Our body's resistance can be divided into two types: specific or adaptive immunity and non-specific or innate immunity. Specific immunity is antigen-specific and exposure results in an immunological memory. Non-specific is an immediate response and is not dependent on an antigen and will not result in immunological memory. Tears and saliva are elements of the non-specific (innate) immune system. Both contain lysozyme a protein that is a bactericidin and exhibits an antimicrobial activity. This protein breaks down the bacterial cell wall (peptidoglycan). Lysozyme activity can be detected by the lysis of bacterial cells using a spectrophotometer.

LEARNING OBJECTIVES:

A required laboratory report including data table, graphing and a conclusion drawn by students upon assessment of data collected. Basic laboratory skills include spectrophotometry, handling microorganisms, preparing dilutions, pipetting, and graphing.

TIME REQUIREMENTS:

This laboratory activity can be done in two block periods or five one-hour class periods. One block period is needed to review procedure and demonstrate the equipment used and discuss collection of tears and saliva and how to handle potential hazardous body fluids and microorganisms. A discussion of the role of lysozyme discovered by Flemming in 1929 would be a good introduction to this lab activity as well.

ADVANCE PREPARATION:

Prepare nutrient agar plates or these can be purchased already prepared for culturing *Micrococcus lysodeikticus*, *Micrococcus luteus* or for bacterium students choose to culture from a selected source. The number of plates will vary for the number of students. One plate per student will be needed. A lysozyme buffer will need to be prepared and kept in the refrigerator. This can be purchased as well from any biological supply such as Flinn or Carolina. Follow the directions for preparing the buffer and culture the bacterium for at least 48 hours at 37°C in incubator.

MATERIALS AND EQUIPMENT:

This lab can be done for any number of students. Each student will need one culture plate with bacterium colonies, a petri dish (for the collection of tears or saliva), 4.5 lysozyme buffer for dilution of tears or saliva, additional 5 mls of lysozyme buffer for the suspension of the bacterium colonies, one 1ml pipette, 5 ml pipette, Spectrophotometer tubes(2), Spectrophotometer, timing device, and graph paper. Cost will vary according to how many students this lab is being prepared for and if students are going to work in groups of two.

A dependable incubator for cultures suitable for most school laboratories can be purchased for approximately \$300. A 48 hr culture is the optimal bacterial culture to use in this activity. Cultures can be purchased and teacher can plate on prepared nutrient agar for 48 hours prior to use. Bacterial cultures will cost about \$10 and the prepared nutrient agar plates will vary according to number used but will cost about \$20 for 10 plates. Lysozyme buffer can also be found in any chemical and biological catalog for about \$30 for one gram, this will make two liters of buffer using package directions and adjusting it to a pH of 6.0. Storage for this buffer is at 25°C. One gram of lysozyme buffer will make enough buffers for about 200 students. A Spectrophotometer designed for high school science labs will cost approximately \$800 and can be as high as \$1500. Most of this expensive equipment cost could be off set by using an equipment loan program from a local university. Disposal of all of the biohazard materials used in this laboratory activity can be minimal by soaking everything in a 10% household bleach solution overnight.

WHAT IS EXPECTED FROM STUDENTS:

Students will write a formal laboratory report showing data collected, results graphed and a discussion of a conclusion about their data. A rubric and scoring guide should be given to students.

ANTICIPATED RESULTS:

Most students will collect data showing the decreasing absorbance of the solutions (using the Spectrophotometer) as the bacterial cells lyse. If student compare saliva activity to their tears they will find that tears have more lysozyme activity. There are few sources of error in this activity. Possible errors will include for the typical high school student pipetting correctly the dilutions of the solutions. This error can improve by prior practice with water. Another error might be timing as the absorbance of the solutions are recorded and zeroing the Spectrophotometer to read the absorbance.

CLASSROOM DISCUSSION:

“Stop and Think” questions:

- *Why are we not constantly sick?
- *Why does you eyes and mouth not have infections all the time?
- *Why would your salvia or tears need to kill bacteria?
- *Are bacteria always in your mouth or eyes?
- *What is in your mouth and eyes to keep you from being sick?
- *What could be learned from our nonspecific immunity?

ASSESSMENT:**LABORATORY REPORT GUIDELINES:**

Name _____

*Writing is an integral part of scientific discovery and learning. The purpose of scientific writing is two-fold. One purpose is to have a clear record of what you did. Another is to help you clarify and consolidate in your own mind what you did and what can be concluded from your experiment.

*Keeping good lab notes is a critical part of doing experiments. In your laboratory spiral begin each lab experiment or lab activity with the general instructions. These instructions may be given in oral instructions, written materials or on the overhead for you to copy.

*If a formal laboratory report is required, the teacher will specify the due date. Formal laboratory reports have no minimum length but should be complete and no longer than 3 pages.

*Failure to turn in laboratory report on the due date will result in partial credit.

Laboratory Format:

TITLE:

This should be clear and concise. It should also be relevant to the experiment.

INTRODUCTION/BACKGROUND INFORMATION/HYPOTHESIS:

This is an explanation in your own words of the background to the experiment

and why you are doing it or what question you are attempting to answer (hypothesis). Two or three paragraphs should be adequate. **Do Not Copy** the instructions or the information you have already been given word for word. This should be in **Your Own Words!!!**

MATERIALS AND METHODS:

A complete list of **ALL** the materials needed to conduct this experiment with a **step-by-step** procedure of how the experiment was carried out. These steps must be numbered, sequential and complete.

DATA:

This is the result you obtain in your experiment including both actual data and **ANY** calculations, graphs or data tables you will need to show your results in a meaningful way.

CONCLUSION:

You will need to evaluate your data and explain what the data shows or indicates. This is where you show you understood what you did and what your results mean.

Laboratory report scoring guide:

Name _____

Category and points:

Introduction/Background Information/Hypothesis:

5= The purpose of the lab or the question to be answered during the lab is clearly stated with appropriate scientific information about the concept.

3= The purpose of the question to be answered is identified but the information about scientific concept is not clear or not stated.

1= No information is given about scientific concept and the question to be answered is not identified.

Materials/Methods:

5= All materials and setup used in the experiment are clearly and accurately described with numbered step-by-step instructions to complete the experiment.

3= Almost all the materials and setup used in the experiment are clearly and accurately described. The step-by-step instructions are almost all listed and accurate.

1= Many of the materials and setup used in the experiment are not listed or inaccurately described. The instructions are not step-by-step and are not complete.

Data:

5= Appropriate calculations, data table, graphs and drawings are shown and the results are correct and labeled appropriately.

3= Data table, calculation, graphs or drawings are correct but labeled incorrectly.

1= No data table, calculations, graphs or drawings are not shown.

Conclusion:

5= Statement includes whether the data supported the hypothesis and what was learned from this experiment.

3= Statement about what was learned from this experiment but does not include a statement about the data collected.

1= No statement made about the data collected and no statement about what was learned from this experiment.

Appearance/Organization:

5=Lab report is neat and handwritten, using all headings. Material is visually organized.

3=Lab report is neatly written but has no headings and not organized.

1=Lab report is sloppy, with many cross-outs and has no apparent organization.

Spelling/Punctuation/Grammar:

5=Report has one or fewer errors in spelling, punctuation and grammar.

3=Report has two to four errors in spelling, punctuation and grammar.

1=Reports has more than four errors in spelling, punctuation and grammar.

STUDENT SECTION:

RATIONAL:

Microorganisms are constantly invading us and if we had no resistance to these microorganisms, we would constantly be ill and would even die. We have body defenses that prevent this from happening. Some of these defenses remove microorganisms and if they do get in fight them while they remain inside. Our ability to do this is called” resistance “and it is a part of our immune system. Nonspecific resistance refers to our defenses that protects us against pathogens, regardless of what kind they are. This is the first line of our defenses. We will look at one of this first line of defenses, which is a chemical defense. Lysozyme is a chemical enzyme capable of breaking down the cell wall of bacteria. This chemical defense enzyme can be found in our perspiration, tears, saliva, nasal secretions, and tissue fluids. It has antimicrobial activity. In the laboratory activity you will investigate this antimicrobial activity.

MATERIALS:

Per Student:

9.5 ml of lysozyme buffer

nutrient agar plate of 48hr.old bacterium culture

bent glass rod (in the shape of an “L”)

10 ml glass test tube

0.5 ml tears or saliva

Petri dish

1-ml pipette (2)

5-ml pipette (2)

Spectrophotometer tube (2) one to zero instruments and one for

test

Goggles, aprons are suggested

PROCEDURE:

1-Students collect tears or saliva in Petri dish. If collecting tears using a cut onion may help produce tears.

2-Prepare a 1:10 solution of tears or saliva by adding 0.5 specimen to 4.5 ml lysozyme buffer. This buffer will help maintain the optimum pH for the enzyme to work.

- 3-Prepare the bacterial suspension by scrapping bacterium from agar plate with glass rod into test tube and adding 5 mls of lysozyme buffer to tube. Stir with glass rod until mixed.
- 4-Mix equal parts of specimen preparation with bacterial suspension in a spectrophotometer tube (2-2.5 ml can be used depending on the size of the Spectrophotometer tube).
- 5-Set the wavelength of the Spectrophotometer to 540nm on absorbance using a spectrophotometer tube with 5 ml. of lysozyme buffer only to zero instrument (this is called the blank).
- 6-Remove the blank tube and replace with the tube with equal parts of bacterial suspension and specimen preparation.
- 7-Read the absorbance at 30seconds, 60 seconds, 120 seconds, 180 seconds, 240 seconds, 5 minutes, and 10 minutes.
- 8-Record time and absorbance on data table.
- 9-Plot enzyme activity on the graph paper. Absorbance should be on the Y-axis and the time on the X-axis. If you have done two specimens (tears and saliva) use one line for tears and another line for saliva.
- 10-PLACE THE PETRI DISH, DILUTION TUBE AND SPECTROPHOMETER TUBE IN THE CONTAINER WITH 10% BLEACH SOLUTION.

DATA COLLECTED:

Students should construct a data table showing time and absorbance of each body fluid tested. A data table could at this point include absorbance from other members of the class. The class could be also divided into those using tears and those using salvia and even males and females.

DISCUSSION/ANALYSIS:

- *Why are tears and saliva potential biohazards?
- *Was lysozyme enzyme present in your tears or saliva?
- *How could you tell if they were present?
- *If you compared tears to saliva, what did you find?
- *What effect did the lysozyme enzyme specimen preparation have on the bacterial suspension?
- *Do you think plants have this same lysozyme enzyme activity?
- *How might you determine if plants contain this enzyme and has the same antimicrobial activity?