"Consumer Report on Common Household Disinfectants!"

Science in the Real World
Microbes In Action

"Consumer Report on Common Household Disinfectants!" is a curriculum unit developed as part of the *Science In The Real World: Microbes In Action Program*. The curriculum units were developed with support from the National Science Foundation, The Coordinating Board for Higher Education, Sigma Chemical Company, Pfizer Foundation and the Foundation for Microbiology.

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At a Glance

Description:
Students will compare common household cleaners for their disinfectant abilities. They will do this by inoculating the cleaners with bacteria. Students will check for growth of the bacteria in the cleaning solution and try to grow the bacteria on a petri dish to back up their observations.

Instructional Objectives:
This lab can easily be used a performance based assessment. At the end of this lab, students will have:

a. worked cooperatively with other students
b. performed a serial dilution
c. observed and recorded data concerning broth cultural characteristics
d. expressed a true understanding for quality controls in industry
e. an understanding about the difference between bacteriocidal and bacteriostatic cleaning agents
f. made streak plates to test and support their descriptive data and observations on the growth of bacteria in their cleaners and broth
g. calculated the percent of solution which is recommended by the manufacturer
h. completed a data table that ranks the cleaners on disinfectant range, scent, cost, and what types of materials the cleaners may be applied
i. prepared a consumer report and graphical analysis using the computer

Time requirements:
This lab will span parts of 3 class periods.

- Day one requires the setup of the standards and the cleaners inoculated with bacteria.
- Day two requires the student groups to record descriptive data on any changes in the tubes as they compare the inoculated tubes with the standards. Students will also streak nutrient agar plates to be incubated for 24 hours.
- Day three requires students to record their results and decide how to rank the cleaners tested.

Curriculum Placement:
This unit can be used to demonstrate science application to test chemicals used by humans and to evaluate whether they are as effective as they claim to be. The lab can be used as an alternative assessment for scientific inquiry or as an introduction to microorganisms and microbiology techniques.

Consumer Report
on Common Household Disinfectants

Introduction and background information:

Your microbiology lab has been hired to perform tests on various cleaning agents to see which of these agents prevent bacterial growth and therefore can truly be called a disinfectant. Your laboratory findings will be published in a famous consumer magazine, which is read by millions of subscribers nationwide. Readers can use this information to know which products are the best disinfectants.

Your lab has developed a protocol (procedure) for testing the effectiveness of various cleaners, and plans to use *Escherischia coli* as the inoculate. (An inoculate is the bacteria we will add to test the disinfectants for bacteriocidal properties.) Your lab chose this bacterium because *E. coli* is a common food and water contaminant. The health department regularly tests for this kind of bacteria when evaluating restaurants for proper hygiene, or testing the safety of our drinking water.

A common source of *E. coli* is any link to animal waste. This type of waste is found on animals, in their feces and colon, and becomes a part of the sewage for animals and humans. *E. coli* is most often transferred to humans from improper slaughtering techniques. That is why it is important that we cook ground beef thoroughly.

E. coli's presence in water may indicate that the water has been contaminated by fecal matter (animal/human waste) that sometimes finds its way into well and ground water supplies used by people. City drinking water is tested at the sewage treatment plants daily. It is important that people drinking water from wells have their water tested often, particularly when there is any doubt about the water purity.

As in any lab testing, one must follow a particular protocol to ensure quality control and repeatable results. Each team in the lab will be responsible for testing one of the cleaning agents by adding a certain percent of the cleaner to nutrient broth. This broth will act as food for the bacteria and will encourage growth, if the chemicals in the cleaner at that particular dilution do not kill the bacteria.

Objective: To determine which household cleaning agents can truly be called disinfectants and to rank them by their effectiveness.

Materials - per team (2-4)
For Day One
2- 5 ml samples of 10% solution of the cleaner to be tested by your team
1- 4.5 ml tube of sterile water
9- test tubes of 4.5 ml of sterile nutrient broth
2- test tube racks (one for standards and one for incubation)
2- sterile disposable 5 ml pipettes for serial dilution (or 2 sterile calibrated droppers can be substituted for the pipettes and pipette pump)
2- hand pipette pumps
2- permanent markers
Procedure/Day One: One half of your team will be concerned with setting up the proper standards, which will be used for comparison once the inoculated cultures have had a chance to grow. The other half of the team will be concerned with preparing the inoculated cultures. Divide your team into 2 groups and record the names of your group members below who will be responsible for each of these tasks.

Group S Member(s) preparing the standard cultures	**Group A** Member(s) preparing the active inoculated cultures

Group S and Group A will each receive 5 mL of your assigned cleaner, in a 10% solution. Each group will make four 10-fold dilutions of the 10% cleaner solution in nutrient broth. In order to be most accurate, only one person in each group should do all 4 of the dilutions. Put an asterisk (*) by this person's name in the table above. The other members should assist by labeling the tubes and recording necessary information.

Obtain the cleaner your team will be testing and record the name. Write the name of your cleaner below and any information you can extract from the label about what it is made of, the quantity of the cleaner in the container, whether it claims to kill bacteria, and the recommended strength one should use for killing bacteria. If the cost is known, record this information also.

Our Team's Cleaner: _____

Additional information:
_____

_____

_____

Each group will follow the directions below to make tubes 1-6. The labels will be different based on your group (A or S). FOR EXAMPLE: Tubes will be labeled 1A, 2A and so on for one group and the other group will label theirs 1S, 2S and so on.

1. Label the 10% solution provided by the teacher as tube 1A or 1S.

2. Obtain 4 tubes containing 4.5 ml of nutrient broth. Label these tubes 2A-5A or 2S-5S.

3. Locate a sterile pipette. Use this pipette to prepare all four of the 10 -fold serial dilutions of the 10% cleaner provided, as described in the steps marked a-d below. Refer to the diagram on the next page while making your serial dilution.

    a. Pipette 0.5mL of the 10% cleaner solution from tube 1 into tube 2 containing 4.5 ml of nutrient broth. Mix by pipetting up and down two times. Return the cap to tube 1 and place in the rack. Label tube 2 as a 1% solution.

    b. Pipette 0.5mL of the 1.0% cleaner solution from tube 2 into tube 3 containing 4.5 ml of nutrient broth. Mix by pipetting the liquid up and down two times. Return the cap to tube 2 and place in the rack. Label tube 3 as a 0.1% solution.

    c. Pipette 0.5ml of the 0.1% cleaner solution from tube 3 into tube 4 containing 4.5 ml of nutrient broth. Mix by pipetting up and down two times. Return the cap to tube 3 and place in the rack. Label tube 4 as a 0.01% solution.

    d. Pipette 0.5ml of the 0.01% cleaner solution from tube 4 into tube 5 containing 4.5 ml of nutrient broth. Mix by pipetting up and down two times. Return the cap to tube 4 and place in the rack. Label tube 5 as a 0.001% solution and return to the rack.

4. Quality Controls. You are now finished with the 10-fold serial dilution. You will now need to set up some quality controls. Quality controls are used in industry to be certain that the data they collect is not faulty due to a problem with the media or the organisms used in the testing. In this case you are making certain that you are working with viable bacteria and that they will grow in the nutrient broth that you provide them. Therefore if they do not grow, we can infer that the cleaner is acting as the inhibitor.

Group A ONLY:
To make tube 6, obtain a fresh tube of 4.5 ml of nutrient broth. Label this tube (6A: QC)
Using a sterile pipette, add 10 drops (or .5 ml) of a fresh overnight culture of *E.coli* to ALL 6 of prepared tubes, replacing the caps quickly and swirl to mix.

Group S ONLY:
To make tube 6, obtain a fresh tube of 4.5 ml of water. Label this tube (6S: QC)

5. Record the color, clarity, and look for any sediment or particles visible in all of the tubes as compared to water (clear and colorless, this is tube 6S). Use the descriptions below when recording your observations in your chart.

   A. color--describe the color of the liquid.

   B. clarity--describe the clarity. Clear broth is called transparent. Cloudy broth is called turbid.

   C. particles--describe any particles that may be floating in the broth by swirling the tube. Sometimes they settle at the bottom of the tube that can be seen when tube is swirled. Use the word fine for small particles, which sometimes can add to the turbidity of the liquid and use the word flocculent for the larger particles that are sometimes visible.

Name _____

Date_____

Team Data Table

Tube and Contents	Initial Description of **Standards**- Day 1	Observed Changes in **Standards**- Day 2	Initial Description of **Inoculants**- Day 1	Observed Changes in **Inoculants**- Day 2	Conclusions- Disinfected (D) or Did Not Disinfect (ND)
1: 10%					
2: 1%					
3: 0.1%					
4: 0.01%					
5: 0.001%					
6: QC					

Members of the team who prepared the standards (Group S) will place their tubes in the refrigerator until the next class period. Be sure that your rack is well marked with your initials, class period, and date.

Members of the team who prepared the inoculants (Group A) will place their tubes in an incubator set at 37 degrees Celsius for 24-48 hours. *E.coli* grows best at 37 degrees Celsius therefore we call this the optimum temperature for growth. Be sure that your rack is well marked with your initials, class period, and date.

Questions: Questions 1-4 can be answered after day 1.

1. What is the purpose of this lab?

2. Why do we make a set of standards?

3. Why are these standards not the control?

4. Which tubes are the control tubes in this experiment? Explain how each of these tubes will be used as a control.

5. Why do you think that the set of standards will be kept in the refrigerator?

Materials For Day Two - per team (2-4)
2 permanent markers
1 sterile calibrated dropper for adding the inoculum
1 5 ml broth culture of overnight E. coli
1 sterile nutrient agar petri plate
1 wire inoculating loop with bunsen burner or 6 sterile disposable loops or 6 sterile toothpicks wrapped and autoclaved in foil

Procedure/Day Two: (All team members)

1. Remove your test tubes from the incubator or refrigerator.

2. As a team, record any visible difference in the standards by swirling each tube and observing. Leave data table blank if there is no change.

3. Obtain the inoculants from the incubator. Observe tube 6A first, so you can see what *E. coli* looks like after growing in broth.

4. Look at the inoculants at each concentration for any evidence of bacterial growth by comparing the inoculant to the standard at each concentration. Be sure to record data on the color of the cleaner, clarity, and any visible particles that can be seen after swirling.

5. Based on your observations label on the group data table whether your team concludes that the cleaner at the different concentrations disinfects or does not disinfect. If your team is uncertain about any be sure to note this with your data as to why you are uncertain.

More Background:

Disinfectants are thought by most people to mean that they kill the bacteria. Those that do kill the bacteria are called **bacteriocidal**. However, sometimes disinfectants do not kill the bacteria, but they can disinfect by keeping the bacteria from reproducing or growing to harmful levels. This type of disinfectant is referred to as **bacteriostatic**. To determine which type of cleaner you have, you can grow some of the liquid from each tube on a nutrient agar petri plate.

If the cleaner has killed the bacteria, it will not grow on the nutrient agar.

Place bacteria on nutrient agar------> no growth = bacteriocidal

The bacteria may have stopped growing therefore your data includes no signs of growth but the bacteria was not killed in the presence of cleaner will be able to grow on nutrient agar. A situation where bacterial growth is retarded from growth to harmful levels is called bacteriostatic.

Place bacteria on nutrient agar------> growth = bacteriostatic

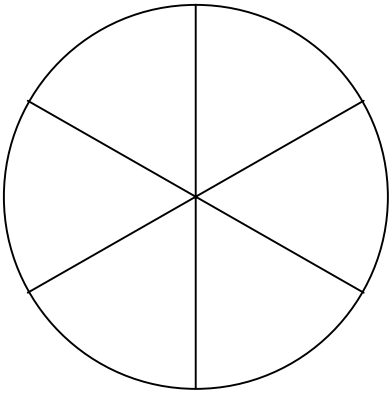
6. Based on your observations of the inoculated tubes after 24-48 hours of incubation at 37^{o} C, write a hypothesis about what levels you believe that you would find active bacterial growth.

Hypothesis:

_____

_____

7. Mark the bottom of your petri dish containing nutrient agar as you see it in the diagram on the page below. Label each wedge as C for control, 10 %, 1%, 0.1%, 0.01 and 0.001%. Be sure to label your plate with your initials, class period and date on the perimeter of the plate. Using sterile technique, streak a loop full of liquid from each of the tubes inoculated with E. coli (tubes 1A-6A). If you do not have wire or 6 sterile inoculation loops, you can use 6 clean/sterile cotton applicators....one for each tube. One streak in the center of the wedge is all that is needed to determine growth after 24 hours of incubation.



****Caution**: With cotton applicators, it is easy to add too much liquid on the wedge and the liquid may run into other test areas. Also be careful to streak the concentrations in the correct wedge of your dish based on your labels. When you have finished, place the plate upside down in the incubator set at 37 degrees Celsius. You will check your results the following class period before making your final report to your supervisor.

Day two questions:

6. Based on your observations, at what %(s) if any, does your cleaner prevent growth of E.coli?

7. Why did you streak tube 6A?

Procedure/Day 3:

Using the diagram from the previous page, draw any noted growth on each of the sections after 24-48 hours of incubation at 37^{o} C. You now have enough data to decide if the cleaner was bacteriocidal or bacteriostatic at the prepared concentrations.

Day three questions:

8. Based on your observations of the streak plate after incubation, at what %(s), if any, does your cleaner prevent the growth of E. coli on nutrient agar?

9. Does this coincide with your data from the team observations in the tubes? Explain your findings

10. Is your cleaner bacteriocidal or bacteriostatic at certain dilutions? Explain your answer.

11. Use the directions on the disinfectant container that you have tested to calculate the percent solution that the manufacturer recommends. Show your work. (Example: full strength = 100%)

12. Based on your data, do the manufacturers recommend a percent that is effective in disinfecting the bacteria? Explain your answer.

13. Are there any other tests that you would like to try?

Answer questions 14-20 using data on all of the cleaners tested in your class.

14. Which cleaners are disinfectants?

15. Which cleaners are bacteriocidal?

16. Which cleaners are bacteriostatic?

17. Are different cleaners effective at the same concentrations? Explain your answer.

18. Compile the data your lab (class data) has collected about each cleaner into an easy to read, well-organized computerized spreadsheet. Include the ranking of the disinfectants tested for their scent, the cost, works on hard surfaces, works on fabrics, and any concerns. Be sure to include an explanation to the reader, which indicates how you rated them. For example, you may decide that the cost factor is most important. In other words, "which disinfectant gives you the most for your money?" Another group may decide that scent or whether they ruin fabric is most important therefore they will support their ranking based on these properties. Your team must choose your parameters and give explanations of your reasons for their ranking in your report to inform the consumer . (See a sample of what needs to be done on the following page.)

Which Disinfectant is the Best?

Rank	Disinfectant	Scent	Cost	Works On	Range of Disinfecting	Other Information
Best:	Sample A	floral	$.10/gal	hard/fabrics	.1% & up	**may want to spot test prior to using on fabrics
Worst	Sample B	pine	$.25/gal	hard/fabrics	10% & up	**once diluted to recommended level it will not control bacterial growth.

Sample A is the best disinfectant because it disinfects *E. Coli* at the manufacturer's recommended dilution. It also has a pleasant scent, is economical, and works well on most surfaces and fabrics.

Your typed final consumer report must include the following:

1. Title of Article for the consumer report magazine.

2. Graphic Analysis Presentation done on the computer:

3. Discussion of findings including what influenced your decisions on ranking the cleaners.

Teacher Guide

This unit is designed to provide a group of students with a hands-on experience of how microbiology might be used in testing commonly used products found in any household. Given that the students have some basic skills in aseptic technique, one can use this as a performance based assessment. The spreadsheet of data collected and the written lab report can be graded as separate entities or as one large unit grade. The lab spans three class periods. This works well with the block schedule.
The incubation time for the liquids may be up to 48 hours. For the petri dishes, however, you may want to check after 24 hours to see if growth has occurred. If growth has occurred, you may store dishes in refrigerator until needed. Store dishes upside down in the refrigerator until the day of the class.

Instructional Objectives

At the end of this unit of activities the student should be able to:

1. demonstrate the methods of scientific inquiry by:

    a. stating a problem.
    b. writing a hypothesis.
    c. performing an experiment according to given directions.
    d. working in cooperative groups.
    e. gathering data
    f. analyzing multiple sets if data in order to draw conclusions.
    g. writing a report based on their findings.
    h. showing an understanding of the difference between a control and a set of standards.
    i. showing an understanding for the difference between a bacteriocidal cleaner and a bacteriostatic cleaner in different dilutions.

2. demonstrate the following laboratory skills:

    a. Use a pipette to measure volume.
    b. Perform a serial dilution.
    c. Create a spreadsheet/table of data
    d. Use aseptic techniques to inoculate a sterile tube.
    e. Use aseptic technique to streak a petri dish

Materials - per team (2-4)
For Day One
2- 5 ml samples of the cleaner (10% solution) to be tested
1- 4.5 ml tube of sterile water
9- test tubes of 4.5 ml of sterile nutrient broth
2- test tube racks (one for standards and one used for incubation)
2- sterile disposable 5 ml pipettes for serial dilution (or 2 sterile calibrated droppers can be substituted for the pipettes and pipette pump)
2- hand pipette pumps
2- permanent markers
Incubator set at 37 degrees Celsius

Materials For Day Two - per team (2-4)
2- permanent markers
1- sterile calibrated dropper for adding the inoculum
1- 5 ml broth culture of overnight E. coli
1- sterile nutrient agar petri plate
1- wire inoculating loop with bunsen burner or 6 sterile disposable loops or 6 sterile toothpicks wrapped and autoclaved in foil.
Incubator set at 37 degrees Celsius

Materials for preparation of the broths and agars:
Large flasks or beakers to prepare the broths and agars. While sterilizing, never fill your containers more that one-half full to prevent boil-over.
Metric Balance
Weigh boats
Spatula
Sterilizer or autoclave

Timeline

Broths or Plates may be done the week before the lab.

Preparation of the Nutrient Broths: (9- 4.5 ml tubes per student team of 2-4)
Weigh 8.0 grams of nutrient broth powder. Add to 1.0 liter of distilled or deionized water in a 2.0-liter flask or beaker and mix well until all the powder is dissolved. Dispense into tubes or flasks using a graduated cylinder or a pipette. Sterilize at 121^{o} C for 20-25 minutes. If you are using a pressure cooker, heat to 15-20 lbs of pressure and maintain this for 20 minutes.

Preparation of the Nutrient Agar Plates: (1 plate per student team of 2-4)
Weigh 23.0 grams of nutrient agar powder. (If you have nutrient broth and plain agar, you may weigh 8.0 grams of nutrient broth powder and add 15.0 grams of plain agar for the same results.) Add to 1.0 liter of distilled or deionized water in a 2.0-liter flask or beaker. Mix well by heating the liquid until powder and agar dissolves. Dispense into smaller container to sterilize as describes above in the nutrient broth directions.
After sterilization, cool to 50 degrees Celsius. Swirl to mix agar with the nutrients. Using aseptic technique by barely lifting the lid enough to pour 20-25 mL per plate (approximately one-fourth full) and quickly return the lid. Once solidified, return to plastic sleeves and store in the refrigerator until ready to use.

The day before the lab or the morning of the lab:

Preparation of the 10% solutions of the cleaners you will provide your student groups: Pipette 10 mL of cleaner into a flask or beaker. Add 90 mL of water. Mix well by pipetting the solution up and down several times. Pipette 5 ml samples into test tubes for the each group to test this cleaner.

Sources of Supplies

Carolina Biological Supply Co.
2700 York Road
Burlington, NC 27215
(800) 334-5551

Escherichia coli	RG-15-5067	1 plate
Dehydrated Nutrient Agar	RG-78-5301	100 g
Dehydrated Nutrient Broth	RG-78-5360	100g
Disposable Calibrated Pipettes	RG-73-6096	Pack of 50
Petri Plates	RG-74-1250	Pack of 20

You may purchase your cleaners at the grocery store or have the students bring in bottles of cleaner used in their homes.

Answer Key to Questions in the Lab:

Day One Questions 1-5:

1. *The purpose of the lab is to test the cleaner for disinfectant properties and to decide whether it is bacteriocidal or bacteriostatic at the made dilutions.*

2. *Standards allow us to compare the tube with the inoculated organism to a tube that lacks the organism and view the cleaner as the cleaner was at the time of the original dilution.*

3. *Standards were not incubated and do not have bacteria added to them.*

4. *The tube set up for quality control is the control tube. (Tube 6 A) Nutrient broth and bacteria only .*

5. *The refrigerator does not allow for contaminated growth of bacteria is a short period of time. Also there will be less evaporation of the capped tubes is they are kept cool.*

Day two

1. *Answers will vary.*

2. *6A is the control tube that shows growth of bacteria is broth only. Answers will vary based on their recorded data from here.*

Teacher Hints and Trouble Shooting

Cooperative learning is certainly involved in this activity for each team of 2-4 students must depend on the others results. The team will divide into 2 groups having 1-2 students in each group. One group is responsible for making the standards and the other groups is responsible for making the cleaner dilutions inoculated with bacteria. You may want to emphasize this to the class before they begin. Also stress the importance of following a protocol precisely to obtain reliable results.

It is recommended that student inoculated broths should be incubated for 48 hours. Making their decisions about growth is tricky at 24 hours, without a trained eye.

If you have wire loops for streaking the plates this is your best bet. However you can sterilize toothpicks in foil.

To cut down on your costs, have the students bring in the household cleaners. Let them know that they must bring in the bottle that contains all the pertinent information.

It is nice to have some that work well and some that do not work well. I would include Lysol brand disinfectant cleaner and bleach as those that work well at some dilutions. Spic and Span and Mr. Clean have not worker well at these dilutions. Also some of the disinfectant sprays are already diluted and may not work very well after the serial dilution.

Allow extra time for the final written report, as the students are required to make a table showing how they analyzed the class data and what parameters they chose to use. Each group should be a little different. Some may choose how well they disinfect while others may be more interested in how it makes their house smell.

If you have the time, a 3-5 minute presentation by the team could be done.

Dear Parents,

Your child is about to embark on a real world science challenge to discover which household disinfectants really work on bacteria. This concept of how bacteria can be killed or controlled by disinfectants added to cleaners is an important application of how knowledge microbiology is useful to us in our everyday world.

The students will be asked to work cooperatively with other teams to determine the effectiveness of cleaners that they have brought from home. You may want to find out their results as they will rank their effectiveness. Who know, you may want to switch your cleaners after their findings are known.

Ask you child about the concepts of this unit. As always, if you feel that you have any questions, please feel free to contact me or make an appointment to come in and see us in action!