

“Is Bleach a Good Disinfectant?”

Science in the Real World: Microbes In Action

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At a Glance

Description:

This lab demonstrates the effectiveness of bleach as a disinfectant.

Time Requirement:

The activity will take one fifty minute class period and 15- 20 minutes the next day for observations and analysis.

Curriculum Placement:

This would fit well into a unit on bacteria or on hygiene.

Equipment

Test tube racks
Screw capped culture tubes
Permanent markers
Sterile transfer pipette
Sterile cotton- tipped applicators
Petri dishes
Sterilizer/ autoclave
Incubator

Materials

E. coli broth culture
Nutrient agar plates

Is Bleach a Good Disinfectant?

Introduction: Bleach is a solution of sodium hypochlorite and water that is very effective in killing microorganisms. It can enter microbial cells where it reacts with many cellular components, destroying them and killing the cell. The bleach you buy in the grocery store is a 5.25% solution, although a higher concentration (“Concentrated Bleach”) is also available. Bleach is an effective and economical disinfectant that is commonly used in drinking water and in swimming pools. This experiment will determine the concentration of bleach that effectively kills the common bacterium *E. coli*. Note that this common strain of *E. coli* is a normal inhabitant of the human intestinal tract. It is not the pathogenic strain called *E. coli* O157:H7, which causes food poisoning.

Objective: To determine the concentration of household bleach that effectively kills *E. coli*.

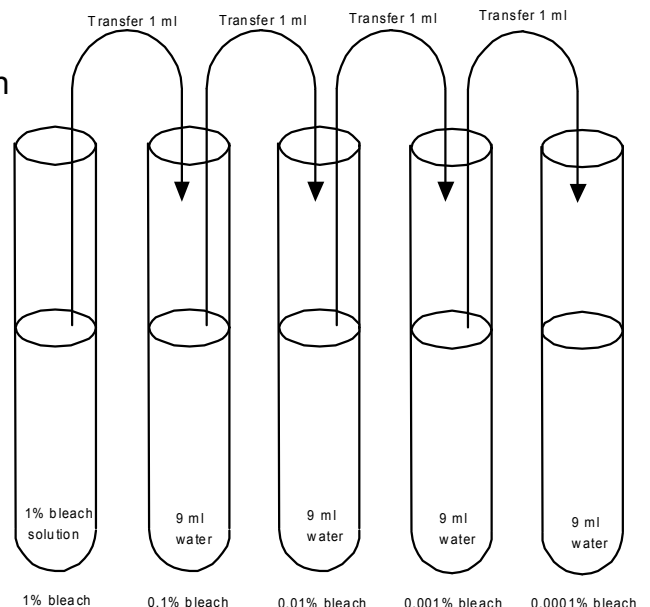
Safety note: Bleach can ruin clothing so students should be careful to keep the bleach away from their clothes.

Materials - per group

- 5 screw cap test tubes containing 9.0 ml of sterile water
- 5 nutrient agar plates
- 2 ml *E. coli* culture
- 5 sterile cotton swabs
- 5 1.0- ml pipettes
- 1 tube containing 5 ml of 1% bleach
- 10% bleach for use as disinfectant

Procedure - Day 1

1. Disinfect your work area by cleaning it with the 10% bleach solution
2. Make 4 10-fold serial dilutions of the 1% bleach solution provided, as described in the steps below.
 - a) Place 1.0 ml of the 1% bleach solution into a tube containing 9.0 ml sterile water. Shake well. Label this tube 0.1% bleach. Set the 1% bleach tube aside.



- b) Place 1.0 ml of the 0.1% bleach into a tube containing 9.0 ml sterile water. Shake well. Label this tube 0.01% bleach.
 - c) Place 1.0 ml of the 0.01% bleach into a tube containing 9.0 ml sterile water. Shake well. Label this tube 0.001% bleach.
 - d) Place 1.0 ml of the 0.001% bleach into a tube containing 9.0 ml sterile water. Shake well. Label this tube 0.0001% bleach.
3. The last tube of water is the control. **There is no bleach in this tube.**
 4. Add 0.1 ml of *E. coli* to the 0.1%, 0.01%, 0.001%, 0.0001% and control tubes. Shake well.
 5. **Leave all the tubes at room temperature for 30 minutes.**
 6. Label the bottom of each nutrient agar plate with the concentration of bleach, your group number or initials and the date. One plate is for the control.
 7. Shake each tube. Place a fresh sterile swab in each tube containing the diluted bleach to soak the swab with the liquid.
 8. Place the swab on the surface of the appropriately labeled nutrient agar plate and gently spread the liquid all over the surface of the plate.
 9. Spread the samples on each of the remaining plates, using a fresh swab for each. You should use one nutrient agar plate and one new swab for each tube, including the control.
 10. Incubate all plates at 37°C for 24 hours.
 11. Disinfect your area with the 10% bleach solution again.

Name _____

Date _____

Data and Analysis

Analysis- Day 1

1. Predict which concentration will be the most effective in killing the bacteria?
2. Which concentration will show the most bacterial growth?

Procedure - Day 2

Count the colonies and record the results in the table below. Some plates will have too many colonies to count (TNTC - too numerous to count). Some plates may have many colonies, but you can estimate the number by counting one quarter of the plate and multiplying that number by 4.

Data Table

Concentration of bleach	Number of <i>E. coli</i> colonies
0.1%	
0.01%	
0.001%	
0.0001%	
0% (Control)	

Analysis- Day 2

1. How do your results differ from your predictions from yesterday?
2. In the lab, we use a 10% bleach solution to clean up our areas. Is this a reasonable concentration, too high, too low? Why do you think that concentration was chosen?

Teacher’s Guide

Instructional Objectives

At the end of this activity, the students should be able to:

1. Demonstrate the following laboratory skills:
 - a. Comparing and contrasting
 - b. Following directions
 - c. Performing serial dilutions

2. Demonstrate the methods of scientific inquiry by:
 - a. Performing an experiment according to given directions
 - b. Gathering and organizing data
 - c. Analyzing data
 - d. Applying understanding of a specific principle to a more general purpose

Sources of Supplies

Carolina Biological Supply
2700 York Road
Burlington, NC 27215
(800) 227-1150
www.carolina.com

<u>Description</u>	<u>Stock Number</u>	<u>Quantity</u>
Sterile Transfer Pipette	RG-21-4551	100
Screw-cap culture tubes (16 x 125 mm)	RG-73-1505	60
<i>Escherichia coli</i> culture	RG-15-5065	1 tube
Sterile Cotton-tipped Applicators	RG-70-3032	200

Preparation

In advance:

1. Obtain a culture of *Escherichia coli* (Carolina Biological, Ward, Presque Isle)
Note that this common strain of *E. coli* is a normal inhabitant of the human intestinal tract. It is not the pathogenic strain called *E. coli* O157:H7, which causes food poisoning.
2. Prepare nutrient broth according to instructions on package and sterilize about 25 ml in several 125-ml flasks.
3. Sterilize 99 ml water in a bottle or flask. This will be used later for the 1% bleach solution.
4. Prepare nutrient agar plates according to instructions on package. Pour plates of about 25 ml/plate. You will need 5 plates per student group.
5. Prepare screw cap test tubes containing 9 ml water and sterilize the tubes. You will need 5 tubes per group of students.
6. Sterilize 2 empty screw cap test tube per group of students. One tube is for the *E. coli* culture and one tube is for the 1% bleach solution.

The day before class:

Inoculate nutrient broth flasks with *E. coli*. You will need about 2 ml per student group. Incubate the culture at 37°C overnight.

The day of class:

1. Pipette the *E. coli* culture into sterile screw cap tubes. You will need about 2 ml per tube and one tube per student group.
2. Prepare the 1% bleach solution. Dilute 1 ml of concentrated bleach from a fresh bottle into 99 ml of water in a flask or bottle. Mix well and pipette about 5 ml into sterile screw cap tubes (1 per student group).

Note: dilute bleach solutions rapidly lose their effectiveness, so the dilute solution should not be made far in advance.

Data and Analysis- Answer Key

Analysis- Day 1

1. Predict which concentration will be the most effective in killing the bacteria?

Answers will vary, students are likely to predict that 0.1% will be the most effective.

2. Which concentration will show the most bacterial growth?

Answers will vary, students should be able to predict that the control will be the first to show growth.

Procedure - Day 2

Count the colonies and record the results in the table below. Some plates will have too many colonies to count (TNTC - too numerous to count). Some plates may have many colonies, but you can estimate the number by counting one quarter of the plate and multiplying that number by 4.

Data Table- (Example Data)

Concentration of bleach	Number of <i>E. coli</i> colonies
0.1%	0
0.01%	6
0.001%	TNTC
0.0001%	TNTC
0% Control	TNTC

Analysis- Day 2

1. How do your results differ from your predictions from yesterday?

Answers will vary.

2. In the lab, we use a 10% bleach solution to clean up our areas. Is this a reasonable concentration, too high, too low? Why do you think that concentration was chosen?

This concentration is very high. It is used because more dilute bleach concentrations quickly lose their effectiveness.

Teacher's Hints and Troubleshooting

- Make sure to warn students about the effect of bleach on their clothes. Even leaning against a counter that has been previously bleached will ruin clothing.
- Have students use new pipettes and new swabs for each concentration to avoid cross-contaminating tubes.
- *E. coli* requires an incubation at 37°C. It will grow at room temperature, it will just take longer.
- The thirty-minute disinfection time period is essential. It gives the bleach a chance to kill the bacteria.