

THE MOUTHWASH CHALLENGE

Ginny Brown
Churchill HS / Montgomery Co. MD

Abstract

A problem assignment to design a new mouthwash that will combat the growth of bacteria that is frequently blamed for gum disease.

Scenario

You have recently been hired by the research and development department of a large health and beauty aids company. Your first assignment is to design a new mouthwash that will combat the growth of bacteria that is frequently blamed for gum disease.

The Research

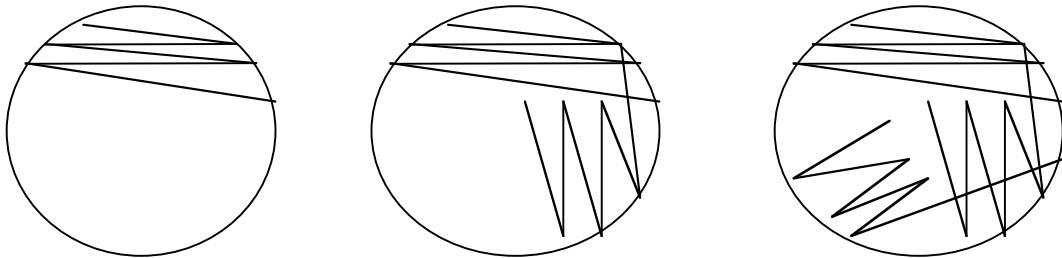
1. The first step is to brainstorm. What different chemicals do you think would be effective killing agents of bacteria and also are safe to put in your mouth? Make a list of them in your data section. Do you think there is a product or group of products currently available that will accomplish the effect you desire? If so, go to your neighborhood pharmacy, grocery store or medicine chest and check out the ingredients. List any ingredients or products that you think may be helpful in your new product on the data page.
2. You are now ready to develop your hypothesis. Make a list of 4 – 6 different chemicals, cleansers or mouthwashes that you think would be effective against tooth bacteria. Let's get started on our experiment!

Materials (per student group)

4-6 petri dishes with nutrient agar
sterile blank disks
inoculating loop
sterile swaps
2-3 tubes of sterile nutrient broth
forceps
sterile water
metric rulers
bunsen burners
samples of 4-6 chemicals, cleaners or mouthwashes (supplied by students)
Also required: Incubator set at 35°C

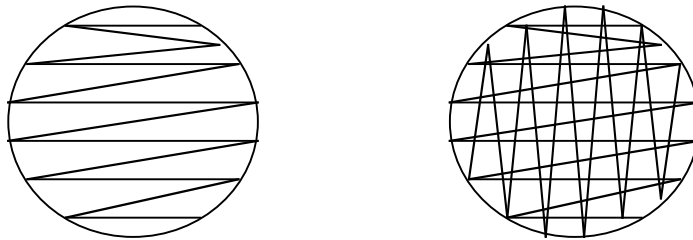
Procedure

1. The first step is to grow a sample of your teeth bacteria. To do this, take a sterile cotton swab and carefully run it back and forth along your front teeth right at the gumline (where the gums meet the teeth). Restrict your sample collection area to the top four front teeth.
2. Next, take a plate of nutrient agar and isolate your bacteria using the following method:
 - a. Rub the swab over the top third of the plate (see diagram on the next page). Dispose of the swab according to the teacher's directions.
 - b. Flame your inoculating loop. Turn plate one quarter turn.
 - c. Follow the diagram below, flaming your loop after each section is complete.



3. Incubate these plates for 24 hours at 35°C.
4. The bacteria that should be growing at this time should look like small, clear pinpoint dots. This is the bacteria that you want to subculture into your broth tube. Be careful not to touch large white colonies. These are yeast and not the cause of gum disease. *Have your plate checked by your teacher before preceeding any further!!*
5. One your plate has been checked, follow the procedure below for setting up your broth culture:
 - a. Flame your inoculating loop and cool.
 - b. Select 5-6 colonies and pick them up with your inoculating loop.
 - c. Remove the top of the broth tube and flame gently.
 - d. Insert the loop with bacteria into the broth tube and swirl gently. Remove the loop and re flame.
 - e. Gently flame top of the broth tube and recap.
6. Incubate your broth culture for 24 hours at 35°C.

7. You are finally ready to set up your test. Record keeping most critical from this point on. Make sure to label your dishes distinctly with permanent marker!! Follow the procedure below:
- On the bottom of the petri dishes (the side with the agar in it), divide the dish into quarters.
 - Mark each quarter with an identifying number or letter that corresponds to one of the chemicals that you are going to test.
 - Take a sterile swab. Using the same technique as before, uncap your broth tube and dip the swab in. Recap the broth after removing the swab and flaming the side of the tube.
 - Swab the entire plate. Turn the plate one quarter turn and swab the entire plate again. (Refer to the figure below.)



- Dispose of the swab according to the teacher's directions.
 - Flame your forceps. Pick up the sterile disk and briefly soak it in one of the chemicals you are testing. Place it in the center of the appropriate quarter of your plate.
 - Repeat step f for each of your chemicals and for sterile water.
 - Place a blank disk in any remaining sections.
8. Incubate your plates for 24 hours at 35°C.
9. You are looking for clear spaces around your disk where no bacterial growth has taken place. These will look like little circles around your disk. These are called *zones of inhibition*. Your next step is to measure the diameter of these zones. The diameter is directly proportional to the effectiveness of the chemical that your are testing. Record all results in the table in your data section.
10. Dispose of all bacterial cultures according to the teacher's directions. Failure to do so could result in serious consequences!

Data

Brainstorming

Chemicals that could kill bacteria:

Ingredients that you would like to test against bacteria:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Data Table

Chemical	Zone of Inhibition (cm)
1.	
2.	
3.	
4.	
5.	
6.	
Sterile Water	
Blank Sterile Disk	

Analysis/Conclusions:

1. What chemical worked the best? _____
2. What was the purpose of the blank disk and the disk with the sterile water?

3. In formulating your mouthwash, what chemicals, cleansers and/or ingredients would you want to use? What would you want to leave out? Why?

4. What are some sources of error in this test protocol?

5. What other studies would you want to do before submitting your final mouthwash recipe to your superiors?

Teacher Notes

This lab is intended for introductory biology/honors biology students. The main objective is to familiarize students with prokaryotic organisms and expose them to some laboratory techniques used in studying them.

Disposal of Materials

Swabs should be placed in a beaker with enough bleach in the bottom to cover the cotton tip. The swabs should soak in the bleach a minimum of 30 minutes. The can be disposed of in the regular trash.

Plates should have bleach poured over the top of the agar and soaked overnight. This will kill the bacterial and liquefy the agar, which can then be poured through a screen down the sink. Culture tubes should be soaked in bleach overnight, then washed in hot, soapy water the next day.

Sterile Technique

An important aspect of this lab is the demonstration of sterile technique. Most students find the coordination difficult. They find it helpful to see how to split the tasks between members of a pair. Cooperation of members should be emphasized as success depends on it!

Handwashing is vitally important and should be stressed both before starting lab as well as when the lab is completed each day!

Sources of Error

Tooth bacterial can be very finicky. It is recommended that both students in a pair set up an initial culture in hopes that one will grow. It may also be helpful to double inoculate the broth tubes to ensure growth.

Additional sources of error the students may encounter include:

- a. Diffusion rates in agar can vary for different chemicals. This rate can affect the zone size.
- b. Mistakes in sterile technique can have a variety of appearances. The blank disk and disk with sterile water serves as controls to check the student's technique
- c. When attempting the bacterial lawn, make sure that the plate is completely swabbed. If gaps are left between the streaks, it could lead to errors in zone measurement.

Other Tests Students May Want to Perform

- a. Taste Tests – no one wants to use a mouthwash that doesn't taste or smell good!
- b. Safety Tests – no one wants to use a mouthwash that could be toxic to them!
- c. Replication of Results – this will ensure precision and accuracy of results!