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Gunjan Patel IB Biology HL Yr: 2 The Effects of mouthwash on the human mouth and how it affects the production of bacteria  
Research Question: How does mouthwash affect the growth of bacteria in the human mouth?  
Aim: This internal assessment aims to determine how variations of mouthwash affect the production of bacteria in the human mouth. This is going to be achieved by using sterile cotton swabs to swab the mouths (teeth and gum lining) of five individuals and then placing it in a zig-zag pattern on a petri dish lined with agar. It will be measured by using different types of mouthwashes, such as organic, different brands and different contents in the mouthwash. It will attempt to determine if bacteria in the mouthwash trial will show resistance to the mouthwash during the week by recording the growth daily in a qualitative measure.  
Background: Cosmetic and therapeutic mouthwashes exist for people around the world.

People can buy therapeutic mouthwashes in the store or can be modified via prescription. Meanwhile, cosmetic mouthwash may relieve bad breath for a short period of time and in place of it, will be a refreshing taste but it does not have any active ingredients while therapeutic does. Ingredients in a therapeutic mouthwash may include: cetylpyridinium chloride (which controls plaque and gingivitis growth), fluoride (which is proven to prevent tooth decay) and peroxide (which is used in whitening mouthwashes and toothpaste). Mouthwash is also helpful because it can access places in the mouth where a toothbrush cannot. One of the most common uses of therapeutic mouthwash is to treat halitosis, which means bad breath, which is secondary to the bacterial buildup in the mouth that ultimately causes inflammation and gives off noxious odors. It is also caused from the buildup

by food debris within the mouth that was not removed due to lack of dental hygiene. Many people with halitosis have cavities or gum disease.

However, even in the absence of halitosis, essentially every person has buildups of bacteria in the mouth, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Streptococcus mutans* (main one that causes disease), and *Porphyromonas gingivalis*. Studies have been performed regarding the efficacy of different types of mouthwash and have shown to provide effective control of bacterial growth as well as decreased pain following dental procedures due to the inhibition of the body's natural inflammatory response to bacteria in the mouth. Another popular type of mouthwash is organic mouthwash, as it only contains natural ingredients and the human mouth contains beneficial bacteria and organic mouthwash only removes the hazardous bacteria because it does not contain alcohol and chlorine dioxide. Xylitol, oils, and plant-based extracts are all ingredients in a typical organic mouthwash. While Listerine is the most popular 'name brand' mouthwash, and it contains alcohol which its role is to reduce hydrogen bonds in protein structures, which causes denaturation. Personal engagement: I chose to conduct this experiment because I have always been curious why each mouthwash says that they are number one.

I wanted to find a way to prove which mouthwash is the best at reducing the count of colonies of bacteria and to do this I had four members of my family swab their mouths at the beginning of the week and put it in four agar plates to let it grow throughout the week. Each day after that, I assigned each member of my family a specific mouthwash and at the same time making sure each person ate the same type of food to keep my data accurate. They

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would use the mouthwash once at the end of each day. And at the end of the week, I would swab their mouths and put it on an agar plate to let the bacteria grow. Mouthwash: The mouthwashes that were used and the person who used the mouthwash are listed below:- Listerine Antiseptic Mouthwash containing alcohol (Person #1)- Crest Scope Mouthwash (Person #2)- Crest Pro-health CPC Antiplaque Mouthwash (alcohol-free) (Person #3)- Thera Breath Oral Rinse (Person #4) This mouthwash is clinically tested and uses OXYD-8 to attack only harmful bacteria and eliminate the bad taste in your mouth. Hypothesis: This experiment is to determine how variations of mouthwash affect the growth of bacteria, and it is expected that, out of the list of the used mouthwashes in my experiment, Listerine and Scope brands will have the least number of bacteria grown.

This is due too that they are the only ones that contain alcohol as an active ingredient, which is a known antiseptic that is used worldwide as a disinfectant in multiple places such as a hospital. Furthermore, the “ organic” mouthwashes that are recommended by dentists, contain natural/herbal products. The data on their efficacy are lacking compared to the “ name brand” products. Bacteria that are exposed to mouthwash daily, will develop resistance and will determine if the bacteria show any signs of resistance for one week. I hypothesize, that the Listerine antiseptic mouthwashes will be the most effective in inhibiting the growth of bacteria with the organic “ herbal” brand being the least effective. Variables: Dependent Variable: - The amount of bacteria present - Changes due to mouthwash use - Number of colonies Independent Variable: - Type of mouthwash - Time Controlled Variable: - Temperature where petri dishes are stored

- Light conditions where petri dishes are stored - Size of Petri Dish  
- Type of Agar - Time between both trials - Trial without  
mouthwash Materials:- (10) Pre-poured sterile LB-agar plates- (10) 6-  
inch sterile cotton swabs - four different brands of mouthwash (different  
ingredients) - permanent markers - Rulers - Scotch tape-  
Agar plates - Incubator- Heat lamps Method: 1.

Obtain eight agar plates and sterile swabs from Amazon and get a heat lamp  
in the ready position. 2. Using a sterile cotton swab, swab the inside of  
four individual's mouths and make sure to swab the front teeth along with  
the gums while twirling the swab in a circular motion. Ensure that the  
individuals are eating the same cuisine until the end of the trial 3. After  
swabbing for individuals, remove the lid of the agar plates and carefully twirl  
the cotton swab across the plate in a semi zig-zag pattern, and then label  
each agar plate with the name of the individual (also mouthwash when  
repeating mouthwash) 4. Close the lid of the agar plates and slowly place  
them upside down and move the plates to under the heat lamp. 5.

Leave the agar plates under the heat lamp for 48 hours and after time has  
passed, remove the plates from the lamp and take a picture of the plates to  
document the growth 6. Put the agar plates on a table and near the lamp  
and put it back under the heat lamp for 4 days. Continue to take pictures  
every 24 hours 7. Obtain four different types of mouthwashes, such as  
Listerine antiseptic, Crest non-alcoholic, Crest antiseptic, and organic  
mouthwash, to determine which mouthwash is most effective in killing  
bacteria. 8. After the mouths have been swabbed (step 2), assign a  
mouthwash to each individual (different ingredients, such as organic/non-

alcoholic). And after dinner every day, each individual will need to use the mouthwash they have been assigned<sup>9</sup>. After four days have passed, repeat steps 2-6 but with using mouthwash and also using an incubator to let the plates with agar grow on it.

Data and Analysis: Table 1: The data recorded, describing the growth of bacteria in the trial without mouthwash, in relation to it filling the agar plate

Days Agar plate, person #1 Agar plate, person #2 Agar plate, person #3

Agar plate, person #4 Day 1 (Monday) None (0%) None (0%) None (0%)

None (0%) Day 3 (Wednesday) Light growth (<25%) Light growth (<25%)

Mild growth (25-50%) None (0%) Day 4 (Thursday) Mild Growth (25-49%)

Mild Growth (25-49%) Intermediate growth (50-75%) Light growth

(<25%) Day 5 (Friday) Mild Growth (25-49%) Intermediate growth (50-

75%) Intermediate growth (50-75%) Mild Growth (25-49%)

Person #1, Day 3:

Person #1, Day 5: The

Pictures above demonstrate the growth of bacteria at the end of the first

trial, which was day #5. With person #1 being on the left and person #4

being on the right. On days 1, 3, 4, 5 I recorded the growth of bacteria on the

agar plate and took pictures to show the growth of it on the agar plate.

Calculating how much of the colonies of bacteria, filled up on the plate in

table #1.

We can see that the number of colonies differs in each person due to the meal they ate and the bacteria present in the mouth. Table 2: The data

recorded with the use of mouthwash in the trial, describing the growth of

bacteria in the trial without mouthwash, in relation to it filling the agar plate

Days Agar plate, person #1 Agar plate, person #2 Agar plate, person #3

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Agar plate, person #4 Day 1 (Monday) None (0%) None (0%) None (0%)  
None (0%) Day 3 (Wednesday) Discreet growth (<5%) Light Growth (<20)  
Medium growth (25-40%) Light Growth (<20) Day 4 (Thursday) Light growth  
(<20%) Medium growth (25-40%) Medium growth (25-40%) Light Growth  
(<20) Day 5 (Friday) Medium growth (25-40%) Medium growth (25-40%)  
Intermediate growth (50-75%) Medium growth (25-40%) These  
pictures were taken on day #3, and starts from person #1 on the left and  
ends on person #4 on the right. We can see that the three mouthwashes  
that contain alcohol (1, 2, 4) reduced the growth of bacteria the most, with  
person #3 who used nonalcoholic mouthwash, bacteria growth was  
noticeable and covered at least 50% of the agar plate. These pictures were  
taken on day #5, and starts from person #1 on the left and ends on person  
#4 on the right. We can see that at the end of the trial, bacteria growth  
slowed down even more after being left in an incubator to grow for four days.  
Except person #3, who used Crest nonalcoholic mouthwash. We can  
determine from this test that Listerine was the best at reducing the growth  
of bacteria, with Crest nonalcoholic mouthwash being the worst.

Conclusion: The hypothesis stated in the beginning does support the  
conclusions that were proposed from my data, The Listerine mouthwash was  
the most effective in killing the bacteria due to its key ingredient alcohol,  
while the Crest nonalcoholic mouthwash was the least effective due it not  
having alcohol. The graphs showed the number of colonies of bacteria  
present in the agar plate, and it is evident that after day 3, the colonies  
stopped growing or reduced after being left in the incubator during the trial.  
The graph also shows that person #3 who did not use alcohol, the colonies of

bacteria on that agar plate had a high number of colonies of bacteria growing, and that the mouthwash offered minimal resistance. One of the limitations of this experiment was that there were not multiple trials and because of that, I was unable in calculating the standard deviation of the colonies of bacteria. Another limitation, is that I did not have access to an incubator or more people to make my trial more accurate. I would have to check on the agar plates at an appropriate time and sometimes, the room would be locked for testing. My experiment was conducted at two different places, one was at home, so I could swab my family members mouth and another was checking the incubator at school, which was only at certain times.

Also, some of my family members ate different foods which could've potentially affected my data, such as during work or at the gym. The agar plates that were used during the trial were purchased from Amazon and were set up to my specifications and included sterile swabs. My first order, the agar plates were damaged and had to request another order be shipped.

This delayed my experiment and reduced the number of trials that could be done to one week. In order to combat these errors if i were to redo the trial again, the way the trials and experiments were conducted would need to be standardized to make the results more reliable. Such as have all the materials and equipment set up before starting the experiment. Overall, the experiment was organized but the delays and low number of trials that occurred could've been eliminated if all material was present before starting and had access to an incubator or a heat lamp in my house.