

# Logbook

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# Timeline

Timetable

12/7/2023 - Information meeting at school.

12/11/2023 - Submit Parental Permission forms.

12/12/2023 - Access google classroom for science fair.  
- Brainstorm ideas for big question.

1/8/2024 - Submit big question and ideas/plans for project.

1/19/2024 - Submit all forms and approvals.  
- Continue background research.  
- Perform experiments and analyze data.

3/1/2024 - Projects due (school).

3/2/2024 - School judging.

3/14/2024 - Projects due (CYSF).

3/15 - 4/10/2024 - Create tri-fold and practice presentation.

4/11/2024 - Set up trifolds (Olympic Oral).

4/12/2024 - CYSF judging.

4/13/2024 - CYSF awards.

# Key

R=replicate (1-3)

D=day of brushing (1-7)

T=Treatment (mouthwash: Listerine, Colgate, Orajel)

C=day of culture (1-7)

(see table on next page)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
7 Choose project + submit big question	8 Background research	9 Background research	10 Background research	11 Background research	12 Background research	13 R1:D1
14 R1:D2	15 R1:D3	16 R1:D4	17 R1:D5	18 R1:D6	19 R1:D7 R1:T	20 R2:D1 R1:C1
21 R2:D2 R1:C2	22 R2:D3 R1:C3	23 R2:D4 R1:C4	24 R2:D5 R1:C5	25 R2:D6 R1:C6	26 R2:D7 R2:T R1:C7 (count)	27 R3:D1 R2:C1
28 R3:D2 R2:C2	29 R3:D3 R2:C3	30 R3:D4 R2:C4	31 R3:D5 R2:C5	1 R3:D6 R2:C6	2 R3:D7 R3:T R2:C7 (count)	3 R3:C1
4 R3:C3	5 R3:C3	6 R3:C4	7 R3:C5	8 R3:C6	9 R3:C7 (count)	10
11	12	13	14	15	16 Interview dentist	17 Conclusions
18	19	20	21	22	23 Applications	24 Update platform
25 Create presentation	26 Create presentation	27 Create presentation	28 Create presentation	29 Create presentation	1 Submit project	2

12/14/2023

## Brainstorming Ideas:

Background

1. Is it possible to make an eco-friendly and portable handfan with magnets?

2. Ways to apply science to the playground:  
Swings - The angles and aerodynamics to get up high, fast.  
Slides - What's the best apparel to go down as quickly as possible?  
Seesaw - How can I lift other people here, but not normally?  
Spinner - What's the most ideal spinning position?

3. Which piece of playground equipment has the most and least germs?

4. Can I create a homemade fertilizer to grow my marigolds?

5. How dirty is my toothbrush and how can I clean it?

12/16/2023

Online sources to get a basic understanding of my proposed projects and to help me decide which one to choose.

1. <https://sciencing.com/make-fan-magnets-7762563.htm>  
<https://www.instructables.com/DIY-Mini-Fan/>

2. <https://www.science.org/content/article/physicists-unlock-secret-childs-swing>

<https://buggyandbuddy.com/playground-science-kids-explain-campo-friction-slide/#:~:text=What's%20Going%20On%3F,they%20go%20down%20the%20slide.>

<https://siobhannixon.wordpress.com/2016/12/18/the-physics-behind-a-seesaw/>

<https://indianapublicmedia.org/lamomentofscience/centripetal-force-and-merry-go-rounds.php>

12/28/2023

(continued)

3. <https://clark.com/health-health-care/playgrounds-are-dirtier-than-bathroom-toilets/>

<https://www.homeadvisor.com/r/playground-germs/>

4. <https://www.homesandgardens.com/garden/unusual-compost-ingredients>

<https://lomi.com/blog/news/homemade-fertilizer-for-plants#:~:text=Quick%20how%20to%3A%20add%20banana,dry%20to%20agitate%20the%20tea.>

1/3/2024  
(continued)

5. <https://www.healthline.com/health/dental-and-oral-health/how-to-clean-toothbrush#electric-toothbrush-head>

<https://www.wikihow.com/Grow-Bacteria-in-a-Petri-Dish>

<https://www.stevespanglerscience.com/lab/experiments/growing-bacteria/>

<https://learning-center.homesciencefools.com/article/bacteria-experiment-guide/>

1/7/2024  
(continued)

Question	Information	Decision
1.	It is possible, but it needs a battery to function.	Not too eco-friendly
2.	Swings: center of mass and pendulum force. Slides: gravity versus friction. Seesaws: levers. Spinners: centripetal force	Very interesting, but more of a research project than an experiment
3.	The dirtiest pieces of playground equipment are the rock walls, baby swings and tunnels (outside).	This has been done, and isn't ideal in winter.
4.	There are many different substances that you can add to fertilizer (weed tea, vacuum leftovers, old wool socks, etc.).	This is very common and requires several months to do.

Question	Information	Decision
5.	Toothbrushes are contaminated with all kinds of microorganisms. The recommended method of decontaminating your toothbrush is to soak it in mouthwash. You can observe the germ growth with an agar growing petri dish.	Interesting and feasible experiment. What's the best way to clean my toothbrush?

\*Selected project



# Background Research

1/8/2024

## Ways to clean your toothbrush:

Method	Pros	Cons
Boiling Water (30 sec.)	- Gets rid of some harmful bacteria.	- Can damage the toothbrush. - Can burn your skin.
UV light (5 min.)	- Most effective germ killer.	- Very expensive. - Can also damage brush. - Weekly
Hydrogen Peroxide (15 min.)	- Great at germ killing. - Easily accessible.	- $H_2O_2$ = Poison. - Needs to be done daily.
Mouthwash (15 min.)	- Disinfects bacteria. - Cheap and simple. - Widely available.	- May occasionally wear down the bristles. - Weekly

## \* Selected Method

### Sources:

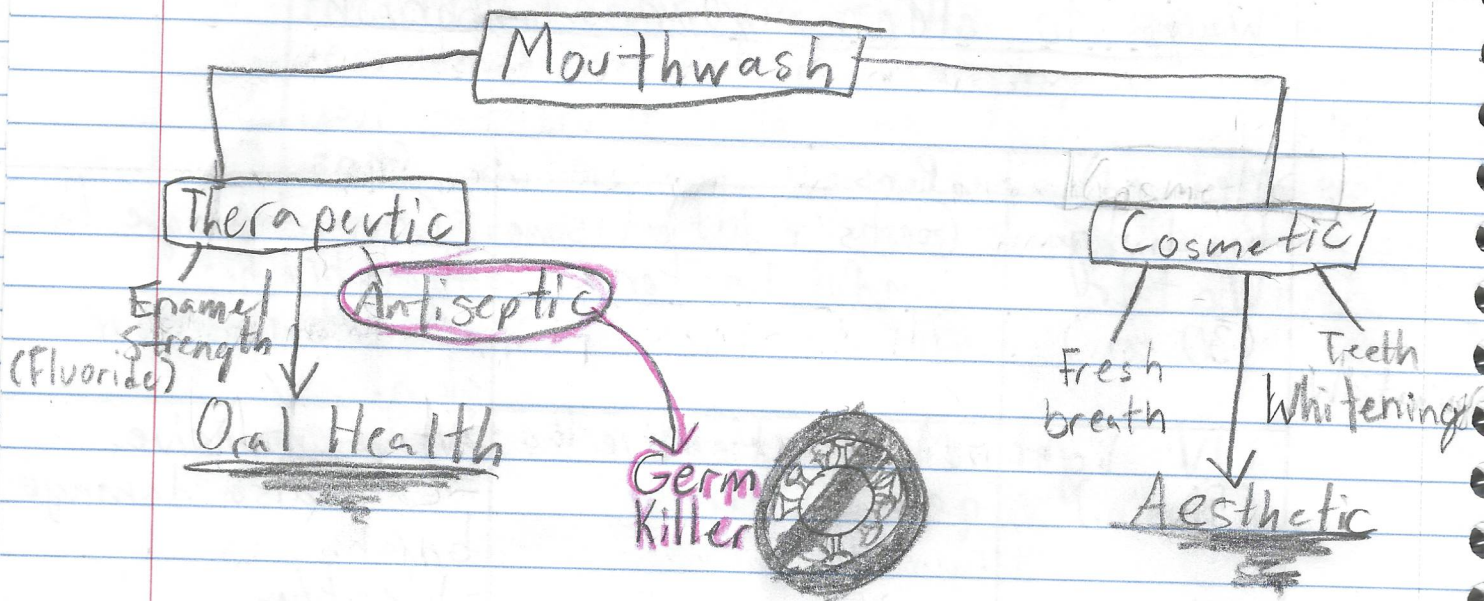
"How to clean your toothbrush." WebMD.com, April 8, 2022. Online.

"How to disinfect your toothbrush and keep it clean." Healthline.com. June 25, 2020. Online.

Which type of mouthwash should I use?

1/9/2024

## Types of mouthwash:



\*I should use an antiseptic mouthwash to disinfect my toothbrush.

\*I realized that my mouthwash and toothpaste only contain Sodium Fluoride, so although they are therapeutic, they only strengthen my teeth's enamel, but don't kill germs.

↓  
I need to change my mouthwash!

Antiseptic - a substance that prevents the growth of disease-causing microorganisms.

## Components of mouthwash:

- ✓ - Cetylpyridinium Chloride (CPC): It is added to mouthwash to reduce bad breath.
- ✓ - Chlorhexidine: It can be used to help control plaque and gingivitis.
- ✓ - Essential oils: Controls plaque and gingivitis.
- Fluoride: Helps prevent tooth decay.
- Sodium fluoride: Prevents tooth decay and decreases tooth sensitivity.
- ✓ - Eucalyptus: prevents plaque and gingivitis.
- ✓ - Hydrogen Peroxide ( $H_2O_2$ ): Used for teeth whitening and penetration of the soft tissues of teeth.

## Sources:

"Types of mouthwash and their uses." Listerine-me.com. February 2023. Online.

"Antiseptic vs. Antibacterial mouthwash: which should you use?" Byte.com. January 3, 2023. Online.

11/01/2024

## Functions of antiseptic components in mouthwash:

Antiseptic	Mechanism of Action	Spectrum
Povidone-Iodine (PVP-I)	Inhibits microbial protein synthesis (creation).	Gram positive, Gram negative, bacteria spores, fungi, protozoa and some viruses.
Chlorohexidine (CHX)	Lower concentration is bacteriostatic (it affects the cell membrane to stop bacterial reproduction). Higher concentration is bactericidal (cell death by cytolysis).	Gram positive, Gram negative, fungi and some viruses.
Triclosan (TRC)	Inhibition of several enzymes.	Gram positive, Gram negative, bacteria and fungi.
Benzethonium chloride (BTC)	Disrupts membrane and causes bacterial cell death.	Bacteria, fungi and viruses.
Cetylpyriminium Chloride (CPL)	Membrane disruption that leads to bacterial cell death.	Gram positive bacteria and yeast.
Essential oils (Menthol, Thymol, Eucalyptol)	Disrupts protein function in bacteria and cell membranes in fungi.	Bacteria, fungi, viruses and yeast.
Hydrogen Peroxide ( $H_2O_2$ )	Disrupts membranes, DNA and other cell components.	Bacteria, viruses, yeast and bacteria spores.

## Brand decision:

- Equate: Eucalyptol, 0.092%, Menthol, 0.042%, Thymol, 0.060%.
- Listerine: Eucalyptol, 0.092%, Menthol, 0.042%, Thymol, 0.060%.
- Colgate: Cetylpyridinium Chloride, 0.075%.
- Orajel: Hydrogen Peroxide, 1.5%.

↓ Note: I read this information on the product label.

## I choose:

- Listerine (same as Equate, but more popular).
- Colgate.
- Orajel.

## Sources:

Mc. Donnell G, Russell AD. Antiseptics and disinfectants: activity, action and resistance. Clin Microbial Rev. 2001 Jan;14(1):227.

Garrido, L.; Lyra, P.; Rodriguez, J.; Viana, J.; Menendez J. S.; Barroso, H. Revisiting Oral Antiseptics, Microorganism Targets and Effectiveness. J. Pers. Med. 2023, 13, 1332.

1/11/2024

## How to grow bacteria at home:

1. Count how many petri dishes you will need. For each one, add 1.2 grams of agar and 60 ml of water to a beaker. Stir until dissolved.
2. Microwave for 3 minutes. Carefully fill each petri dish with agar until halfway full.
3. Dip a sterile cotton swab in warm water and swab it onto a surface. Then, trace in a gentle zigzag line onto the dishes. Clean surface and repeat.
4. Place in a dark box at room temperature for a week. Check every few days to avoid overgrowth.
5. Record results. Then, place in a sealed plastic bag to dispose.

## Sources:

"How to grow bacteria in a petri dish." [Wikihow.com](https://www.wikihow.com/Grow-Bacteria-in-a-Petri-Dish). June 28, 2023. Online.

"Growing bacteria." [Stevesspanglerscience.com](https://www.stevespanglerscience.com). 2023. Online.

"How to grow bacteria and more." [Homesciencebooks.com](https://www.homesciencebooks.com). 2023. Online.

1/12/2024

## Contaminants in the mouth, toothbrush and bathroom:

### Mouth microflora:

- Bacteria (Gram positive and negative cocci and rods)
- Fungi
- Viruses
- Archaea
- Protozoa.

### Toothbrush microbiome:

- Streptococcus
  - Staphylococcus
  - Lactobacilli
  - Pseudomonas
  - Klebsiella
  - E. (scherichia). coli
  - Candida → Fungi
- Bacteria

### Bathroom microcontaminants:

- E. coli
- Salmonella
- Staphylococcus Aureus

### Sources:

Deo PN, Desmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019 Jan-Apr; 23(1):122-128. doi:

Continued:

Naik R, Ahmed Mujib BR, Telagi N, Anil BS, Spoorthi BR. Contaminated toothbrushes - potential threat to oral and general health. J family Med Prim Care. 2015 Jul-Sep; 4(3):444-8. doi:

"Germs in your bathroom: everything you need to know." Bathroomcity.co.uk. 2024. Online.



1/13/2023

## How does agar work?:

Nutrient agar is made of <sup>0.5%</sup> peptone, <sup>0.3%</sup> beef extract and <sup>0.5%</sup> agar. The beef extract contains nitrogen compounds, salts, vitamins and carbohydrates. Peptones also offer natural nitrogen, specifically via long-chain peptides and amino acids. Finally, it has 0.5% sodium chloride and distilled water, which adjust salt levels and maximize replication.

## Sources:

"Nutrient agar: composition, preparation and uses." [Microbiologyinfo.com](https://microbiologyinfo.com). 2024. Online

"Nutrient agar - non-selective solid media for microbiology." [Biotrend.com](https://biotrend.com). 2020. Online.

# Additional Research

1/21/2024

Why is it called a "Petri" dish?:

The petri dish is named after Julius Richard Petri, a German bacteriologist.

What microorganisms can grow in agar?:

Bacteria, fungi and protozoa can be cultured in agar, however viruses and archaea are nearly impossible to grow.

Sources:

Mahajan M. Etymologia: Petri dish. Emerg Infect Dis. 2021 Jan; 27(1): 261. doi: 10.3201/eid2701.ET2701.

2/9/2024

What is the difference between biological and technical replicates?

A biological replicate is when culture of a live (or dead) organism occurs in several different samples with the same variables. However, a technical replicate is when identical conditions are provided, but it's the same sample measured multiple times.

Sources:

Bell G. Replicates and repeats. BMC Biol. 2016 Apr 7;14:28. doi: 10.1186/s12915-016-0254-5.

What is the most common antiseptic mouthwash?

Listerine is the best seller mouthwash, with Brajel ranking 12 and Colgate ranking 60.

Sources:

[https://www.amazon.ca/Best-Sellers-mouthwash/zgbs/best-sellers/ref\\_aud\\_r\\_16371172011](https://www.amazon.ca/Best-Sellers-mouthwash/zgbs/best-sellers/ref_aud_r_16371172011)

2/16/2024

## Dentist interview:

Q1: Does a dirty toothbrush add more bacteria than it cleans?

A1: Probably not, but it's highly recommended to change your brush every 3-6 months so that it still works.

Q2: Does a different toothbrush bristle design impact the amount of germs cleaned?

A2: There are no studies showing evidence of the impact, so just pick the one that suits your liking.

Q3: What is your preferred method of toothbrush cleaning?

A3: Changing the brush often is the key to a clean mouth, but a UV light works well. However, in terms of mouthwash, a prescription mouthwash with CPC is the king of killing germs.

## Sources:

- Dr. Jiwa. Family braces beacon hill. February 16, 2024.

2/20/2024

## Are all germs bad?

Not all germs are bad. In fact, the vast majority of them actually benefit us. Some things that good germs do include helping us digest our food, break down natural waste and curdle milk to make cheese.

However, there are some harmful germs that can make us sick. E. coli cause diarrhea, streptococcus causes strep throat and the coronavirus results in Covid. While most bacteria have a bad reputation, bad bacteria only make up a small portion of bacteria in general. You can wash your hands all you want, but bacteria will still remain, and since most bacteria are helpful, it isn't such a bad thing.

## Sources:

- Cline-Ransom, Lesa. "Germs: fact and fiction, friends and foes" October 2022.

2/21/2024

How do germs grow and how fast do they grow?:

There are two ways for bacteria to grow. They can multiply or they can divide. When a bacterium multiplies, it first penetrates the wall of a human cell. It then injects its RNA (ribonucleic acid) into the cell's DNA (deoxyribonucleic acid), which causes the cell to instantly produce hundreds of identical virus copies. The cell will later burst to release the bacteria.

The other way a bacterium can reproduce is to divide. To do so, the bacterium goes through a process called binary fission, or dividing itself. The bacterium makes a copy of its DNA, splits a wall between itself, splits into two layers and then bacteria separate. This usually takes about 20 minutes.

Sources:

-Nye, Bill. Zoehfeld, Kathleen. "Great big book of tiny germs". April 2006.

2/22/2024

What is standard deviation and how do you reduce it?  
Each value from the colonies counted

$$\sigma = \sqrt{\frac{\sum (C_i - \mu)^2}{N}}$$

↑ Standard deviation  
↑ The amount of trials

The standard deviation is the gap between the most spread-out values (which are sometimes close) from the mean. To reduce this, simply increase the variable "N", so you divide by a greater amount.

Sources:

- "Standard deviation": En.wikipedia.com, February 8, 2024.  
Online.

2/24/2024


## What are the main aspects of bacteria? :

### All bacteria have/are:





- Prokaryotes (nucleus-less organisms)
- Cell membrane
- Single-celled organisms
- DNA
- Cytoplasm

\* Most bacteria are healthy in your body, but only if they don't spread to other bones, organs and tissues.

### Some bacteria have:

- Pili (special "hairs")  Actually proteins!
- Flagellum (tail)

### Bacteria can be shaped like:

- Balls (such as cocci) 
- Rods (such as bacilli) 
- Corkscrew-like (such as spirilla) 
- Square (such as walsbyi) 

### Sources:

- Taylor, Marianne. "Discovering the microscopic world." 2023.
- Moold, Steve. "The bacteria book." November, 2018.



1/7/2024

Big question:

What's the best brand of mouthwash to clean my toothbrush?

Big Quest

1/12/2024

Hypothesis:

I predict that 15 min<sup>+</sup> of Orajel mouthwash will clean my toothbrush the best, because it has the highest percentage of antiseptic components.

1/11/2024

Materials:

- Mouthwash: Colgate, Listerine and Orajel
- Toothbrushes (12)
- Timer
- Medicine cups
- Toothpaste
- Petri dishes
- Agar growth medium
- Swabs
- Distilled water
- Box
- Thermometer
- Heated blanket

Material

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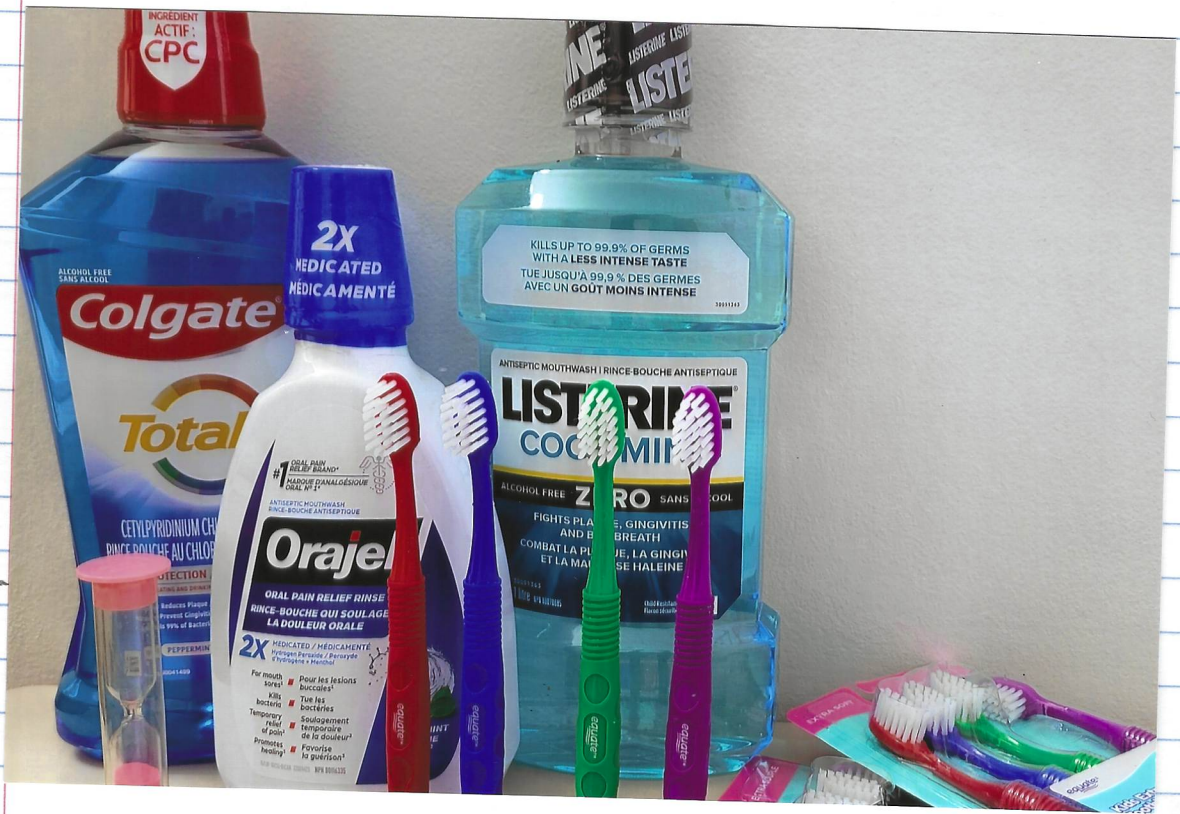
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1/21/2024

Project costs:

- Toothbrushes:  $\$4.78 (3) = \$14.34$
- Toothpaste:  $\$2.98$
- Listerine:  $\$4.88$
- Orajel:  $\$14.49$
- Colgate:  $\$7.27$
- Agar set:  $\$37.95$
- Distilled water:  $\$1.87$
- Total:  $\$83.78$

1/12/2024

### The Mouthwash Type procedure:

1. First, I will brush my teeth with 4 brushes at the same time every day, with the same amount of toothpaste on each one.

2. After each session, I'll rinse them with warm, running water for 30 seconds, letting them air dry in an upright position.

3. After 1 week, I'll use a different treatment on each brush:

- Brush 1: nothing (negative control) (red)

**RED**

- Brush 2: soaked in Orajel mouthwash for 15 minutes

**PURPLE**

- Brush 3: soaked in Listerine mouthwash for 15 minutes

**GREEN**

- Brush 4: soaked in Colgate mouthwash for 15 minutes.

**BLUE**

4. After letting each brush dry overnight, I will swab all 4 brushes, and culture the bacteria in a petri dish containing agar growth medium. The plates will be kept in the researched conditions for 5 days.

5. I will take pictures and count the colonies to determine the effects of each treatment. I'll repeat this experiment 3 times to ensure my results' accuracy and reliability.

\* Note: I will also swab a brush fresh from the pack (new brush). **ORANGE**

## Agar growth:

1. In a 16oz measuring cup, add 10 grams of nutrient agar and 750ml of distilled water. Mix until dissolved.
2. Heat and stir in the microwave for 7 minutes.
3. Carefully pour into petri dishes until halfway full. Replace lids.
4. Let the agar cool for about 1-3 hours.

## Sources:

"Science experiment for kids grow your own bacteria". Seancevolutionlaboratories.com, 2022.

\*Note: To dispose, simply place dishes in a ziploc bag, close airtight and throw it away in the garbage.

The mouthwash type procedure



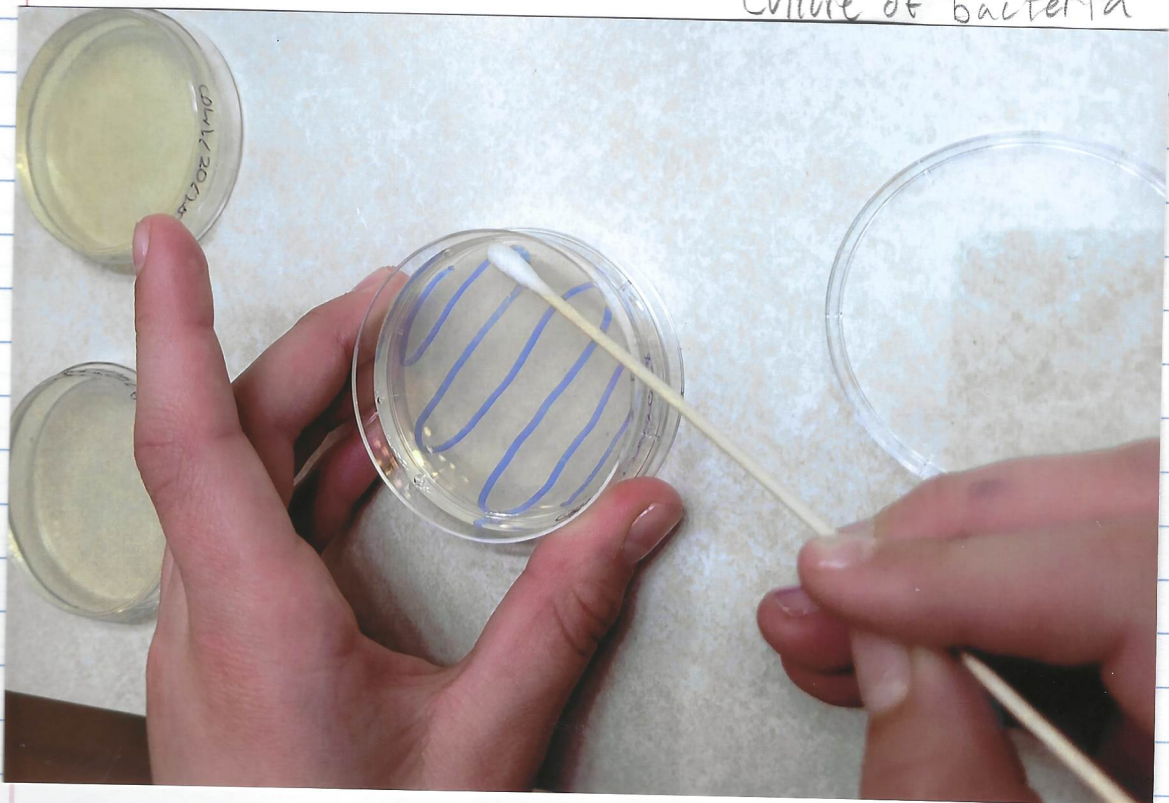
Brushing my teeth



Mouthwash cleanser



Swabbing the brushes

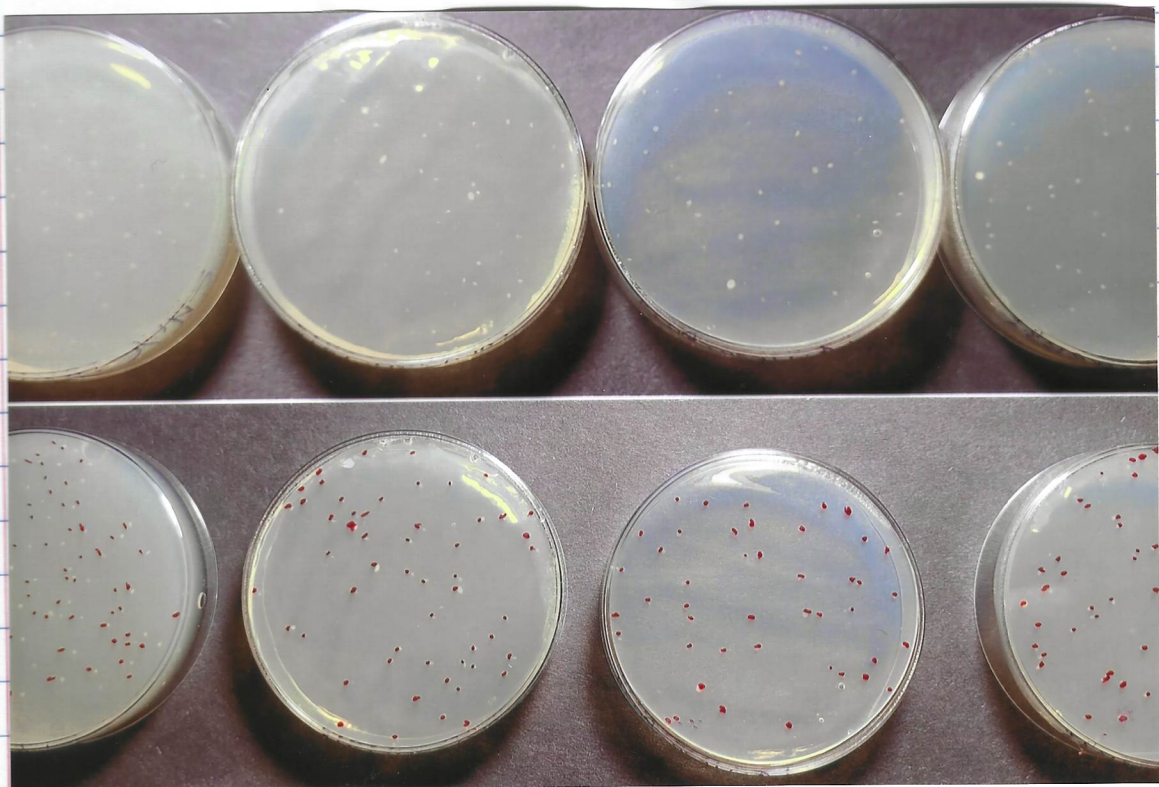


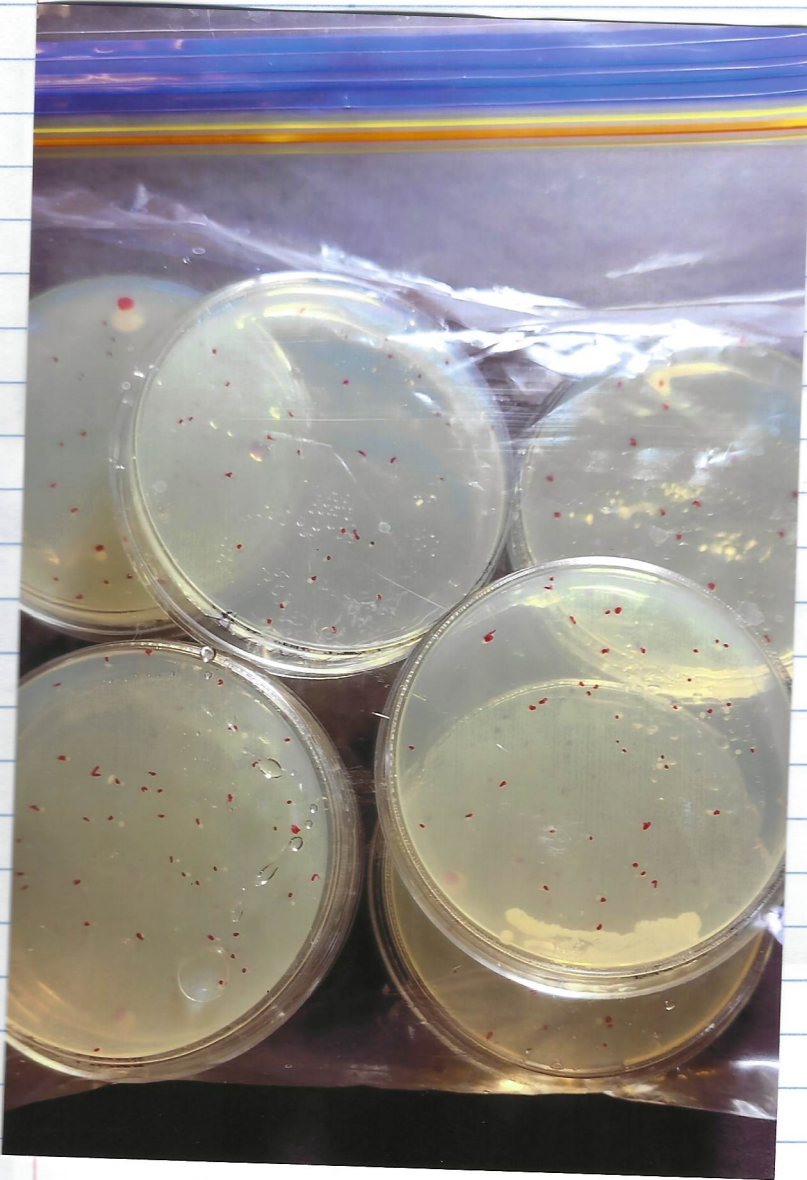
Culture of bacteria





Counting Colonies





Agar disposal

1/10/2024

### Manipulated variable:

My manipulated variable will be the treatment of each brush: type of mouthwash.

### Responding variable:

The number of bacterial colonies.

### Controlled variables:

- Type of toothbrush: I will buy 12 of the same brand and model of toothbrushes at the same store.
- Duration of cleaning: I will set a 15-minute timer and leave the toothbrushes in the solution until the timer goes off.
- Amount of mouthwash: I will equally measure 15ml of mouthwash every time.
- Brushing: I will use 4 brushes (negative control + 3 treatments) at the same time.
- Duration of brushing: I will always set a 2-minute timer every day, right before brushing my teeth.
- Toothpaste: I will use the same quantity and type of toothpaste for every brush.

= Before treatment: after brushing, I will rinse with warm, running water and leave to dry in an upright position.

- Bacterial growth: all petri dishes will be swabbed with the same type of cotton swab and stored in the same dark box, swabbed in the same pattern. All plates will be stored in the same location for the same amount of time of 5 days.

1/26/2024

Colony count:

New brush: 59

Negative control: 87

Orajel: 42

Listerine: 43

Colgate: 51

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2/2/2024

Colony count:

New bush: 31

Negative control: 48

Orajel: 48

Colgate: 41

Listerine: 46

219/2024

Colony count:

New brush: 41

Negative control: 58

Orajel: 20

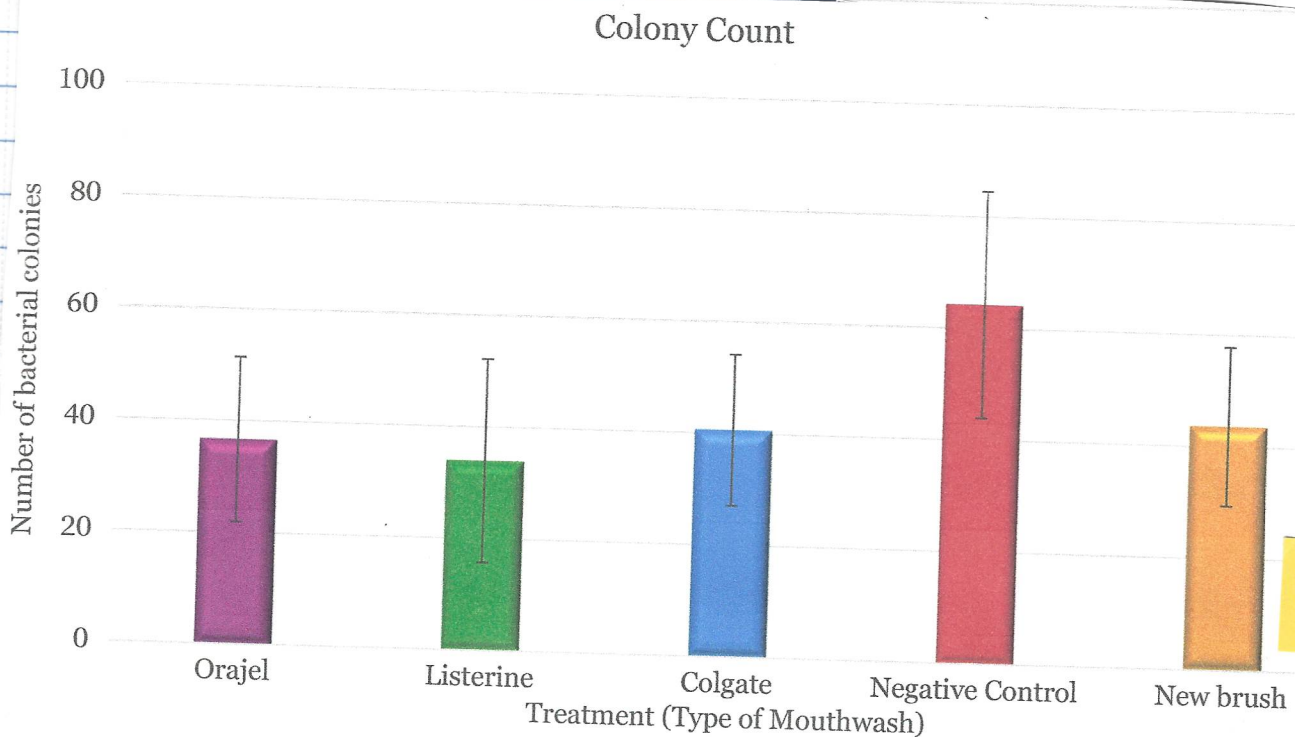
Colgate: 27

Listerine: 13

2/9/2024

## Number of colonies per treatment: The Mouthwash Type Test

Replicate	Orajel	Listerine	Colgate	Negative Control	New brush
1	42	43	54	87	59
2	48	46	41	48	31
3	20	13	27	58	41
Mean	37	34	41	64	44
SD	15	18	14	20	14



- Orajel wasn't the best mouthwash.
  - All the mouthwashes were about the same in killing germs, compared to the negative control.
  - The mouthwashes were about the same as a new brush.
  - A new brush isn't 100% clean, as many believe.
  - The standard deviation was very large, which could be improved by having more replicates.
- HYPOTHESIS REJECTED!**

Results

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## Possible sources of error:

- There were only three replicates.
- Not all colonies were the same size.
- Environmental conditions.
- "Population" sample: 1.
- Temperature: wasn't an incubator.
- Germ transfer.

2/17/2024

One of the most easily accessible methods of toothbrush cleaning is to soak it in mouthwash. However, not all types of mouthwash work for this job, as only antiseptic mouthwashes are intended to eliminate germs. For instance, Listerine, Colgate and Orajel are all antiseptic mouthwashes but which one will clean my toothbrush the best?

To assess which mouthwash works best to clean my brush, I brushed my teeth with 4 brushes at the same time for 1 week. Then, I soaked each brush in a different brand of antiseptic mouthwash (and a negative control). I evaluated the effectiveness of the brand of mouthwash tested by swabbing the toothbrush and culturing the corresponding bacteria in agar plates.

After doing the experiment, I can conclude that my hypothesis was incorrect. My hypothesis was that, "cleaning my toothbrush with Orajel mouthwash for 15 minutes will work best, because it has the highest percentage of antiseptic components." However, my replicates prove that 2// of the mouthwashes tested had a similar effect on diminishing bacterial growth compared to the negative control. In fact, my study shows that cleaning the toothbrush with any tested mouthwash was about as successful as a new brush (which isn't as clean as you might think!).

Although the Orajel mouthwash had the highest percentage of antiseptic components (1.5%), compared to Listerine (0.194%) or Colgate (0.075%), each mouthwash had

Conclusion:

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different active ingredients. For instance, Orajel contains hydrogen peroxide, whereas Listerine has a mixture of essential oils, such as eucalyptol, thymol and menthol, and Colgate has cetylpyridinium chloride. Ergo, it is possible that the effectiveness of these compounds may vary. In other words, a lower amount of one chemical may have the same biological effect as a higher or lower amount of another. As my teacher always says, "more is not better, better is better." This may explain, in part, why Orajel didn't show the best results.

Another thing I noticed was a large standard deviation in my test replicates. This may have also contributed to the lack of difference between the mouthwashes. Having more replicates could minimize the error bars, and allow for more accurate data. Additionally, the lack of a proper incubator for this experiment, to keep the bacteria at a constant and optimal temperature value, may have also influenced the variability of the experiment.

Finally, some mouthwashes may have different activities depending on the time. This is why I would like to do an experiment next year, focusing on the time the mouthwash needs to function best (even though this means I have to go through the pain of brushing my teeth with four brushes again!).

In summary, any of the antiseptic mouthwashes I tested - Orajel, Listerine and Colgate - were effective at reducing toothbrush bacteria, compared to an untreated brush. They worked to keep my brush as

good as new.

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2/23/2024

During this project, I observed that a new brush isn't really as clean as you think. However, it's cleaner than the brush you use every day, ergo it is important to find a way to clean the brush you use. One way to achieve this is to sanitize your toothbrush with antiseptic mouthwash. This will restore it to the level of, as good as new.

This experiment made it clear that using any of the antiseptic mouthwashes - Orajel, Listerine or Colgate - reduced bacterial growth on the toothbrush. Even though brushing and flossing regularly is a great start to oral hygiene, cleaning your toothbrush is also a healthy habit to keep. "Everything in the human life cycle is related to the mouth: fertility, childbirth, sleep, mental health, success in school, finding a mate, getting a job, systemic disease and aging." ("If your mouth could talk." Dr. Kami Hoss, April 2022.) In conclusion, this experiment supports cleaning a toothbrush with antiseptic mouthwash as part of a better oral hygiene routine.

Applica