BIOLOGY EXTENDED ESSAY

"THE EFFECT OF THE TOOTHBRUSH COVERS AND THE PRESENCE OF TOILET IN THE ENVIRONMENT ON BACTERIAL GROWTH ON TOOTHBRUSHES"

Supervisor**:** Ümit Yaşatürk Midilli

Name of Student: Beril Daloğlu

Candidate Number: 001129-0082

School: TED Ankara College

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ABSTRACT

This extended essay focuses on the effects of using toothbrush covers and the presence of toilets in the preserving environment on the bacterial growth on toothbrushes. Hence, the research question is: “How do the presence of toilet in the environment and the usage of toothbrush covers affect the bacterial growth on toothbrushes?”

To test the hypothesis, which is “both the presence of toilet in the environment and the usage of toothbrush covers increase the bacterial growth on toothbrushes.”, total of 20 toothbrushes were used for a month by 5 different people, 4 toothbrushes for each person. Two of the toothbrushes were covered and the two of them were not. A pair consisting of one toothbrush from each group is put in a room without toilet and the other pair is put in a room with toilet. At the end of a month, the toothbrushes are taken to the laboratory and the bacterial growth on each of them is compared by the analysis of the data obtained at the end of the serial dilution and plate counting method.

It is concluded that the usage of toothbrush covers increase the bacterial growth on the toothbrushes by providing a moist environment to the bacteria, when the presence of toilet in the environment does not affect it. However, because of an unexpected data result caused by the toothpaste remnants on the bristles of some toothbrushes, a different question is raised at the end of this experiment: “Does leaving some toothpaste remnants on the bristles of the toothbrush inhibit the bacterial growth?”

Word Count: 257

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INTRODUCTION

Tooth brushing has been a usual everyday activity to provide oral hygiene. As the toothbrushes become more developed and being used more frequently, the need of keeping them sterile has been necessary. When there is enough water, nutrition, oxygen (for aerobes) and the temperature and pH are appropriate, bacterial growth occurs.[1] As these conditions are provided on a toothbrush, the hygiene tool turns into a home for more than 100 million bacteria.[2] The conformity of the bristles of a toothbrush for bacterial growth makes it essential to take some precautions when storing it. Some of these precautions are replacing the toothbrush with a new one in every 3 - 4 months; not storing them in closed containers so often to prevent moist; and rinsing the toothbrush with water after brushing.[3] Along with these methods to keep the toothbrush clean, there is also an argument as the toothbrushes should not be kept in a room that includes a toilet. When it is considered that most people store their toothbrushes in a bathroom that contains a toilet, it can be said that this suggestion is almost never taken seriously.

The argument about the effects of the toilets on bacterial growth on toothbrushes had taken my attention and made me conduct a research on this subject. Also, when I was researching, I came across some hypothesis regarding the usage of toothbrush covers and I started to think about the relation between the toilets and toothbrush covers. What would change if a toothbrush cover was used to avoid the bacterial growth caused by the environment? Would the cover protect the toothbrush and decrease the bacterial growth or would it increase it because of its longer moist duration? To be able to answer all these questions, I wanted to make an experiment concerning the relation between the environmental conditions and the bacterial growth on toothbrushes. Hence, I decided to write an extended essay based on the research question: "How do the presence of toilet in the environment and the usage of toothbrush covers affect the bacterial growth on toothbrushes?"

[1] "Factors affecting microbial growth"
http://www.foodsafetysite.com/educators/competencies/general/bacteria/bac2.html

[2] http://www.medicinenet.com/truth_about_your_toothbrush_pictures_slideshow/article.htm

[3] http://www.ada.org/en/about-the-ada/ada-positions-policies-and-statements/statement-on-toothbrush-care-cleaning-storage-and-

HYPOTHESIS

There is a wide range of different methods that are developed for the optimum protection of the toothbrushes from bacterial growth. These methods might be either affective or not, differentiating by the conditions of the environment. The effect of the toilets on the bacterial growth on toothbrushes is one of the conditions that are not taken very seriously.

Toilets are the center of bacterial growth in the bathrooms. With flushing, these bacteria are spread out to the environment with droplets according to the "aerosol effect", which has been first studied by the environmental microbiologist, Charles Gerba in University of Arizona.[4] With the bacteria transmitted to the bathroom with the aerosol effect, the toothbrushes that are stored openly are like open fields, waiting for bacterial invasion.

There is also such belief that the toothbrushes can be protected from bacteria spread from outside by covers. However, some sources suggest that toothbrush covers increase the bacterial growth rate on the contrary, because they obstruct the drying process of the toothbrush which provides moist environment that is ideal for the bacterial growth.[5]

Accordingly, it can be hypothesized that the bacterial growth on the toothbrushes is affected by the presence of toilet in the bathroom and the usage of covers. It is expected that the bacteria on the toilet closet will transmit on the bristles of the toothbrush and increase the bacterial growth. Also, because of the moist environment created by the cover the bacterial growth will, again, increase.

[4] http://serendip.brynmawr.edu/exchange/node/1839
[5] http://www.popsugar.com/fitness/How-Keep-Your-Toothbrush-Germ-Free-23459538

METHOD DEVELOPMENT AND PLANNING

In this experiment, first, toothbrushes that are used for a certain time interval is needed. As the research question includes both the effect of presence of toilet in the environment and the usage of a toothbrush cover on the bacterial growth rate, 4 toothbrushes are needed for a trial: 1 without and 1 with a cover that are kept in a room with toilet; 1 without and 1 with a cover that are kept in a room without toilet. These 4 toothbrushes are used by a person for a month consequently. For example, when the toothbrush without cover that is kept in a room with toilet is used in the morning, the one with cover and kept in a room with toilet is used in the afternoon and the next day the toothbrushes that are kept in a room without toilet is used in the same order. These steps are repeated as a loop for a month which is the least possible time period to allow bacterial growth.

While storing the toothbrushes, it should be considered that all the other conditions, except the usage of a cover and the presence of a toilet in the room, should be kept constant to get the ideal results. At first I thought that the person that uses all of the toothbrushes should be kept constant. However this point created some problems. As the toothbrushes are used to cleanse the oral cavity, they hold oral flora which means "bacteria and other organisms that normally inhabit the oral cavity"[6] and it is also known that "more than 300 types of bacteria are found living in the mouth"[7]. Hence, these facts constitute the problem that number and type of bacteria that is transmitted to the toothbrushes from mouth will differ depending on the person who uses the toothbrushes. This problem affects the outcomes of the experiment as the data that is obtained at the end of the experiment could and most probably would be different if the person who uses the toothbrushes had been different. Because of this fact, the results will be more accurate when the person using the toothbrushes changes in every trial and the conclusion is made according to the mean value of the data taken as a result of these trials. Other variables that should be kept constant during the experiment are the distance between the toothbrushes and the toilet, and the time left between the usages of the toothbrushes. For example, when a toothbrush is used in the morning it will be used again in the morning 2 days later so there will be 48 hours left between two usages and this time interval must be the same for all other toothbrushes. The reason for 48 hours of time gap is that there are only 5 people living in the house that the experiment was conducted and as 4 toothbrushes in total must be used by a person; the minimum time that could be left between usages of a particular toothbrush was 48 hours with a person brushing their teeth twice a day. One other point that must be considered is that all the toothbrushes must be a regular, manual toothbrush without any special features that might affect the bacterial growth rate.

After a month of using the toothbrushes that are stored in different conditions, they are taken to the laboratory for bacterial analysis. At first it seemed appropriate to directly cultivate the bacteria on the toothbrushes on blood agar plates with cotton tip swabs and the experiment was conducted that way. However, after 24 hours of autoclaving, there was no

[6] http://medical-dictionary.thefreedictionary.com/oral+flora
[7] Clint J. Hays "Bacteria In The Oral Flora: A Study Of Antimicrobial Agents In Toothpastes On Toothbrushes"

bacteria culture on the plates. That result did not satisfy me because it seemed unlikely that the toothbrushes which have been used for a month are completely bacteria-free. Hence, I have done research on the outcomes of this experiment and finally found an answer: When the toothbrushes are being used, toothpaste is also used with them which includes antibacterial ingredients that may inhibit the transmission of the bacteria on the agar plates with using cotton tip swabs so, the culture on the toothbrushes cannot be directly plated on the agar plate for bacterial counting because in that way, bacterial growth on the plate will most probably not occur. As a result of this, I decided to use a different method that will allow the bacterial growth. At first the toothbrushes must be kept in "Brain Heart Infusion Broth" for 24 hours, which provides the optimum environment for cultivation of dental pathogens. Also using broth provides direct contact between the bristles of the toothbrush and the medium which will allow the cultivation of the bacteria inhibited because of the toothpaste[8]. After that, using the serial dilution method will be appropriate for this experiment. Serial dilution method is used when "the number of bacteria in a culture must be determined but the entire culture cannot be directly inoculated onto the agar plate, because even 100 microliters of bacterial culture may contain billions of bacteria that would overcrowd the agar plate and a serial dilution means diluting the culture several times by the same dilution factor"[9]. In this experiment, 100 µl of culture is taken from the sample and added to 900 µl of sterile water then, 100 µl of culture is taken from this new diluted sample and added to another 900 µl of sterile water. These steps are repeated 10 times which will equal to 10 diluted cultures. Later on, 100 µl of all diluted cultures will be taken and be plated to blood agar bases. While plating the dilution order of the cultures must not be mixed. In the end, there will be 10 plated blood agar plates with increasing dilution order. After 24 hours of incubation in 37°C incubator, the bacteria on the plate can be counted. The plate that will be used for counting should be chosen according to the availability of the density of bacteria colonies for counting on that plate. If the density is too high then it will not be possible to count the colonies because they will be too close that cannot be distinguished by human eye. Hence, it will be more appropriate to choose a plate that includes more diluted bacteria culture. Then the original concentration of bacteria will be calculated by multiplying the final number of colonies on the plate by the dilution factor[10] (this will be shown in detail in the method part).

After calculating number of bacteria in every sample, the mean value of the data will be taken for every different toothbrush storing condition and the results will be compared to come to a conclusion on which conditions are healthier in terms of bacterial growth.

[8] http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1135&org=133
[9] J. Kirk Brown' s text, Ch3 "Quantifying Bacteria: Serial Dilution and Plate Count"
[10] J. Kirk Brown' s text, Ch3 "Quantifying Bacteria: Serial Dilution and Plate Count"

MATERIALS

- 10 manual Colgate® toothbrushes
- 10 manual Colgate® toothbrushes with covers
- Autoclave
- Incubator
- 20 Disposable cell culture flasks (250 ml)
- 200 Eppendorf tubes (1.5 ml)
- 200 petri dishes
- Blood agar base
- Brain heart infusion broth
- 200 Bacteriological disposable loops
- 1000 µl Volume pipette
- 1000 µl pipette tips
- Eppendorf tube rack for 1.5 ml
- Distilled water

METHOD

Procedure:

I. Pre-Laboratory Steps
- 2 Colgate® manual toothbrushes and 2 Colgate® manual toothbrushes with covers are bought.
- 1 toothbrush with cover and 1 without cover is put in a room with toilet and the other pair is put in a room without toilet.
- The toothbrushes are used for a month consecutively, twice a day. (A toothbrush is used once in 48-hours)
- At the end of a month, the toothbrushes are put in separate plastic bags and taken to the laboratory.

II. Laboratory Steps[11]
- Laboratory apron and gloves are worn during all of the steps below to avoid any contact with the bacteria and prevent any external contamination on the materials.
- The toothbrushes are put in the flasks (the bristles should be at the bottom, in the broth) and the opening of the flasks are covered with sterile napkins, hold still by plasters.

- The flasks are put in 37°C incubator.
- The flasks are observed and taken out of the incubator when the broth is completely cloudy. The time passed until the broth becomes cloudy is noted down.
- Serial dilution method is conducted (see Appendix):
  i. 10 eppendorf tubes are taken (1.5 ml each) and they are filled with 900 µl distilled water with using pipette.

[11] See Appendix 1 for the preparation method of Brain Heart Infusion broth and Blood Agar base

ii. 100 µl of broth that had been taken out of the incubator is taken with pipette and put into the first eppendorf tube.
iii. The tube is shaken and 100 µl of the mixture in this tube is taken and put into the other tube. This tube is also shaken and another 100 µl of the new mixture is taken and put into the next tube. These steps are carried out until the last tube is filled with 100 µl of the mixture taken from the previous tube.
iv. 100 µl of the mixtures prepared in the eppendorf tubes are taken with pipette and spread on the blood agar plates prepared before.
v. The mixture is distributed to the whole surface of the agar plate with bacteriological disposable loop.

vi. The agar plates that had been plated are put in 37°C incubator for 24-hours.
vii. After 24-hours, the plate which is the most appropriate for counting is taken and the bacteria colonies are counted.
viii. The number of bacteria in the original culture is calculated by multiplying the number of colonies counted by the dilution factor. (described below)
ix. These steps are repeated for the other 3 cell culture flasks.

Calculating Concentration of Bacteria (CFU/ml)[12]:

$$\left(\frac{Number\ of\ colonies\ forming\ units\ grown}{volume\ of\ diluted\ LB\ broth\ with\ bacteria\ plated}\right)\ x\ (Dilution\ factor)\ X\ (Conversion\ factor)$$

[12] J. Kirk Brown' s text, Ch3 "Quantifying Bacteria: Serial Dilution and Plate Count"

RESULTS

TABLE 1: The number of colonies (CFU/ml) counted on the blood agar plates that were plated by diluted broth at the end of the serial dilution method. "$\pm \sqrt{n}$" is the uncertainity value of the number of colonies, in which *n* is the number of colonies calculated in each trial.

		Number of Colonies (CFU/ml)	
		Environment with toilet ($\pm\sqrt{n}$)	**Environment without toilet ($\pm\sqrt{n}$)**
Toothbrush with cover	Person 1	1.8×10^{10}	3.0×10^{10}
	Person 2	1.5×10^{9}	1.5×10^{9}
	Person 3	2.7×10^{10}	2.7×10^{10}
	Person 4	2.0×10^{10}	2.0×10^{10}
	Person 5	0	1.5×10^{10}
Toothbrush without cover	Person 1	1.0×10^{10}	1.3×10^{10}
	Person 2	1.5×10^{8}	1.5×10^{8}
	Person 3	5.0×10^{9}	2.0×10^{9}
	Person 4	1.2×10^{10}	1.6×10^{9}
	Person 5	0	4.0×10^{8}

TABLE 2: Time taken until the broth becomes completely cloudy which indicates the bacterial growth for each group.

Groups	**Time taken until the broth becomes cloudy (hours) (± 1)**
Toothbrushes with cover stored in an environment with toilet	12
Toothbrushes without cover stored in an environment with toilet	24
Toothbrushes with cover stored in an environment without toilet	12
Toothbrushes without cover stored in an environment without toilet	24

TABLE 3: The qualitative observations made during the experiment

Smell	Color
No smell	Brain Heart Infusion broth is dark yellow, clear and transparent before bacterial growth
No smell	After the bacterial growth Brain Heart Infusion broth is still dark yellow but blurry and non-transparent
No smell	Blood agar plates are in the color of blood; red before the bacterial growth
No smell	Blood agar plates are still red but covered with millions of white tatches after the bacterial growth occurs

DATA ANALYSIS

TABLE 4: Mean, Standard deviation and Standard error values of the number of colonies counted on the agar plates that has been plated with the diluted broth.[13]

Groups	Count	Sum	Mean	Standard Deviation	Standard Error
Toothbrushes with cover stored in an environment with toilet	5	6.65×10^{10}	1.33×10^{10}	1.195×10^{10}	5.342×10^{9}
Toothbrushes without cover stored in an environment with toilet	5	2.72×10^{10}	5.43×10^{9}	5513574158	2465745323
Toothbrushes with cover stored in an environment without toilet	5	9.35×10^{10}	1.87×10^{10}	1.1267×10^{10}	5038849075
Toothbrushes without cover stored in an environment without toilet	5	1.72×10^{10}	3.43×10^{9}	5406431355	2417829605

TABLE 5: Analysis of Variance (ANOVA): Two factor with replication statistical calculation for all groups. Usage of cover, presence of toilet and the relation between them are represented by "Sample", "Column" and "Interaction" respectively. This table will be used for making the analysis of the effectiveness of the independent variables. If the P-value is smaller than alpha value, 0.05, then it can be said that that group makes a significant difference on the results so H_0 (null hypothesis) is rejected when H_1 (alternative hypothesis) is accepted.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.69325E+20	1	6.69E+20	8.130788784	0.011546186	4.493998418
Columns	1.445E+19	1	1.45E+19	0.175535033	0.680809276	4.493998418
Interaction	6.845E+19	1	6.84E+19	0.831513701	0.375370159	4.493998418

[13] See Appendices for the calculation of mean, Standard error and deviation.

TABLE 6: Confidence interval for each group. Confidence interval shows the amount of error allowed in the data. The upper and lower boundaries represent the interval that the mean value can be found in, when the errors in an experiment is considered.

Groups	Confidence Level (95.0%)	Mean	Upper	Lower
Toothbrushes with cover stored in an environment with toilet	14832558694	13300000000	28132558694	-1532558694
Toothbrushes without cover stored in an environment with toilet	6846006534	5430000000	12276006534	-1416006534
Toothbrushes with cover stored in an environment without toilet	13990087849	18700000000	32690087849	4709912151
Toothbrushes without cover stored in an environment without toilet	6712971172	3430000000	10142971172	-3282971172

EVALUATION

The aim of this investigation was to observe the effects of toothbrush covers and the presence of toilet in the environment on the bacterial growth on toothbrushes. Toothbrushes with and without covers were used by 5 different people and kept in environments with and without toilet. At the end of a month the toothbrushes were taken to the laboratory and bacterial counting was made as a result of serial dilution method (see page 4).

According to the results and the data analysis, it can be said that half of my hypothesis which was "usage of toothbrush cover will increase the bacterial growth on a toothbrush" is proved to be correct however, the second half which was "presence of toilet in the environment will increase the bacterial growth on a toothbrush" is proved to be wrong.

First of all, after the toothbrushes were put in the flasks filled with broth, the time taken for the broth to become completely cloudy were 50% less in the flasks with toothbrushes with cover. However, the presence of toilet in the environment clearly did not affect the bacterial growth rate because there was not any time difference between the flasks with toothbrushes that had been stored in an environment with and without toilet (see Table 2).

Another point that made me come to the conclusion described above was the mean values of the number of colonies that were obtained as a result of the serial dilution method. The mean values for the environment with toilet are 1.33×10^{10} for covered, 5.43×10^{9} for uncovered and for the environment without toilet are 1.87×10^{10} for covered, 3.43×10^{9} for uncovered. Hence, it can be seen that when a comparison is made concerning only the usage of cover, regardless of the presence of toilet, it is seen that there are significant differences between the numbers of colonies. However, when a comparison is made concerning the presence of toilet, regardless of the usage of covers, it cannot be said that the values differ significantly (see Table 4). This result is also supported with two factor with replication ANOVA (Analysis of Variance). The p-value of the sample, which is the usage of cover is 0.011546186; of the columns, which is the presence of toilet in the environment is 0.680809276; and of the interaction, which is the relation between these two variables is 0.375370159 (see Table 5). As it is seen, only the p-value of the sample is less than 0.05, which means H_1 (alternative hypothesis) that is concerning only the usage of cover is accepted; but for the presence of toilet and its relation with usage of covers, H_0 (null hypothesis) is accepted.

In an experiment done on the effectiveness of using chlorhexidine and Listerine on the contamination of toothbrushes, also the effectiveness of the usage of toothbrush covers was observed by the Department of Community Dentistry in Jaipur Dental College, India. The result of that experiment supports the results obtained by this investigation as in that experiment it was concluded that "use of a cap leads to growth of opportunistic microorganisms like Pseudomonas aeruginosa, which may cause infection in the oral cavity."[14] Although I could not find any previous experimental results concerning the effect of

[14] http://europepmc.org/abstract/med/17508674

toilets or their relation with the usage of covers on the bacterial growth on toothbrushes, there was an experiment mentioned in the Australian Dental Journal, concerning the effect of usual storage methods on the bacterial growth on toothbrushes. However, it was concluded that there was not any kind of relation found between these different usual storage methods of 10 different people and the microbial load found in the laboratory. This result might support the result in this experiment concerning the effect of the presence of toilets on the contamination on toothbrushes.[15]

In order to see the validity of the mean values, confidence interval, standard deviation and standard error were calculated. All these statistics are seen to be quite high: confidence interval varying from -1532558694 lower, 28132558694 upper to 4709912151 lower, 32690087849 upper; standard deviation varying from 5406431355 to 1.195×10^{10}; standard error varying from 2417829605 to 5.342×10^{9} (see Table 4 and 6). The highness of these values may seem to be the result of some systematic and random error however; it must be considered that all the samples in this experiment belong to a different individual. In each trial of this experiment, a different person used the toothbrushes. This had to be done to get a more general and valid average values, because with only one person using all of the toothbrushes, the results would be specific only to that particular individual as a result of the oral flora (see page 5). However, making different individuals use the toothbrushes has caused significant differences among the numbers of bacteria that has grown on the toothbrushes in different trials. This result suggests that the individual oral flora is a factor that cannot be disregarded while making a research on bacterial growth on toothbrushes. The reason for the wide error bars on Graph 1 is again the oral flora. If there had been more trials in this experiment and the variety of people had been larger, these error bars would be shorter but the difference between the columns would most probably be the same nonetheless because my hypothesis depends on the difference between the average values, not the amount of bacterial growth separately.

Increasing the number of trials would increase the variety of people that using the toothbrushes and as the sample space increases, the error decreases and the results gets closer to the theoretical. However, increasing the number of trials alone does not remove all the errors. In Table 1, it is seen that there was no bacterial growth on two of the toothbrushes that person 5 had used. While making the experiment, I have realized that these toothbrushes had toothpaste remnants left on the bristles of them. Hence, it can be said that, as a result of this, the antibacterial properties of the toothpaste inhibits the bacterial growth on the toothbrushes. To get more valid results in this experiment, the toothbrushes should be cleaned carefully after usage. Also, another experiment that concerns the effect of toothpaste on the bacterial growth can be done to verify this theory.

Moreover, the toothbrushes that have been used in this experiment were manual toothbrushes, without any additional properties. However, there are lots of different types of toothbrushes generated and some have anti-bacterial properties. Hence, in daily life the results may vary depending on the properties of the toothbrushes that are being used.

[15] http://onlinelibrary.wiley.com/doi/10.1111/j.1834-7819.1998.tb06101.x/epdf

Another point that should be considered if the experiment were to be repeated is the time left between the usages of the toothbrushes. In this experiment, a particular toothbrush was used once in 48-hours to let every toothbrush be used as much as possible and equally. Because there are only 5 people living in the house that the toothbrushes were kept and there were 4 toothbrushes for every person to use, the shortest time gap between brushings were 48-hours when a person brushed their teeth twice a day. However, this time might be too long because especially for the toothbrushes without covers, the bristles of the toothbrush might stay dry for too long, causing the bacteria on them to die. Also in daily life, most people brush their teeth at least once a day which makes 48-hours time interval too long when compared to a real life scenario. In addition, the toothbrushes can be used for more than a month to have more accurate results.

During the experiment, brain heart infusion broth and blood agar were used. However, the results can be supported with using different type of medium as different mediums allow the cultivation of different type of organisms. Also, in this experiment the toothbrushes were only tested for bacterial growth, but there can be some other organisms that contaminate the toothbrush like viruses and fungi. For more extended results, these organisms that contaminate the toothbrushes can be researched and different experiments concerning them can be made.

CONCLUSION

As a result of this experiment, it is obtained that while the usage of cover affects the bacterial growth on toothbrushes; the presence of toilet in the environment does not. The bacteria just like any other living organisms, look for an environment that will provide them the optimum conditions for survival. The moist is one of these conditions that bacteria need to survive and reproduce. The covers that are used to protect the toothbrushes from the bacteria coming from toilets actually make the reverse effect. Usage of covers provide a moist environment in a longer duration when compared to the toothbrushes without covers and because of this, moist-loving bacteria grow on the toothbrushes with cover with much higher rate. Some beliefs suggested that the covers protect the toothbrush from bacteria coming from the toilet however, not only this belief is proved to be wrong but also it is concluded that the toilets does not affect the bacterial growth at all.

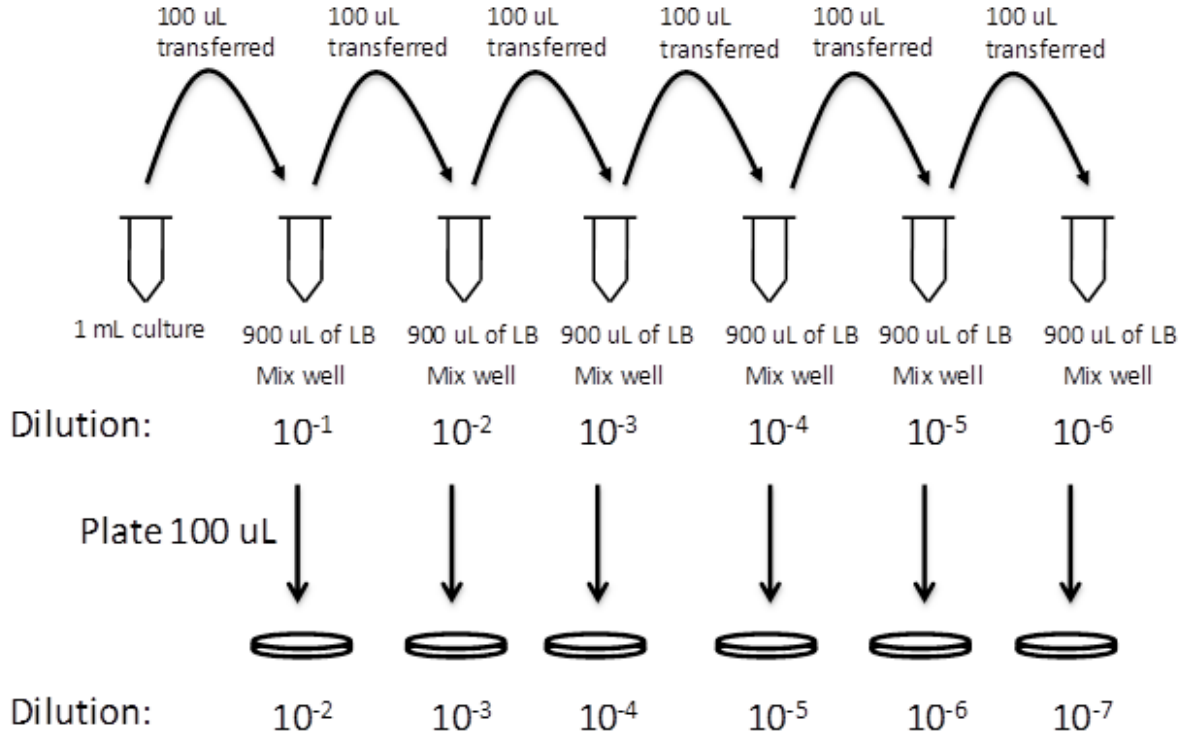
How to protect the toothbrushes from bacterial growth has been a topic that includes many different and contrasting theories. In this experiment while answering some questions that have been discussion subjects, other questions are raised: “Does leaving some toothpaste remnants on the bristles of the toothbrush inhibit the bacterial growth? If it does, does the result of this method vary between different toothpaste brands?”

APPENDICES

APPENDIX 1: Preparation of Brain Heart Infusion Broth and Blood agar base

- Laboratory apron and gloves are worn during all of the steps below to avoid any external contamination on the materials.
- Brain Heart Infusion Broth is prepared:
  i. 37 g of Brain Heart Infusion powder is added to 1 liter of distilled water and mixed.
  ii. The mixture is sterilized by autoclaving at 120°C for 15 minutes.
  iii. It is distributed into 4 disposable 250 ml cell culture flasks
- Blood agar base is prepared:
  i. 37 g of powder is added to 1 liter of deionised water and after 10 minutes it is swirled to mix.
  ii. The mixture is sterilized by autoclaving at 120°C for 15 minutes.
  iii. After 15 minutes, it is cooled to 47°C in refrigerator.
  iv. It is poured into 40 petri dishes. The thickness should be approximately 5-8 mm.
  v. The plates are kept in room temperature for solidification.
  vi. They are kept in refrigerator until used.

APPENDIX 2: Figure 1: Visual example of the serial dilution method[16]. (In this experiment the dilution is made 10 times, not 6 as in this figure.)



[16] http://2014.igem.org/Team:CSU_Fort_Collins/Notebook/KillSwitch/Sep

APPENDIX 3: The mean, standard deviation and standard error values are calculated by the formulas below:

- Formula of Mean[17]:

$$\overline{x} = \frac{\sum x}{N}$$

N: Number of trials (count)

x : Number of colonies in the chosen trial

Example calculation for the toothbrushes with cover preserved in an environment with toilet:

$$\frac{1.8\ x\ 10^{10} + 1.5\ x\ 10^{9} + 2.7\ x\ 10^{10} + 2.0\ x\ 10^{10} + 0.0}{5} = 1.33 \text{ x } 10^{10}$$

- Formula of Standard Deviation[18]:

$$s = \sqrt{\frac{\sum (x - \overline{x})^2}{N - 1}}$$

x: Number of colonies in trial i
$\overline{x}$: Mean value
N: Number of trials (count)

Example calculation for the toothbrushes with cover preserved in an environment with toilet:

$$\sqrt{\frac{(1.8\ x\ 10^{10} - 1.33\ x\ 10^{10})^2 + (1.5\ x\ 10^{9} - 1.33\ x\ 10^{10})^2 + (2.7\ x\ 10^{10} - 1.33\ x\ 10^{10})^2 + (2.0\ x\ 10^{10} - 1.33\ x\ 10^{10})^2 + (0.0 - 1.33\ x\ 10^{10})^2}{4}}$$

$= 1.195 \text{ x } 10^{10}$

- Formula of Standard Error[19]:

$$\mathrm{SE}_{\bar{x}} = \frac{s}{\sqrt{n}}$$

s: Standard deviation value
n: Number of trials (count)
$\overline{x}$: Mean value

Example calculation for the toothbrushes with cover preserved in an environment with toilet:

$$\frac{1.195\ x\ 10^{10}}{\sqrt{5}} = 5.342 \text{ x } 10^{9}$$

[17] http://standard-deviation.appspot.com/
[18] http://standard-deviation.appspot.com/
[19] https://en.wikipedia.org/wiki/standard_error

APPENDIX 4:

T.C.
HİTİT ÜNİVERSİTESİ DEKANLIĞI
Temel Tıp Bilimleri Bölüm Başkanlığı
Tıbbi Mikrobiyoloji Anabilim Dalı

Sayı : 30134845/ 29/07/2015
Konu : Beril Daloğlu

To whom it may concern;

I agree that Beril Daloğlu, May 2016 session International Baccalaureate program student with candidate number 001129-0082, has conducted her biology extended essay experiment on her own, with using the materials of our laboratory.

Prof. Dr. H. Ayşegül TAYLAN ÖZKAN
Temel Tıp Bilimleri Bölüm Başkanı

Hitit Üniversitesi Rektörlüğü, Tıp Fakültesi Dekanlığı,Bahçelievler Mah. Çamlık Cad.
No:2 19030 Çorum Tel : 0364 222 11 00-01 Faks: 0364 222 11 02
e-Posta: tipfak@hitit.edu.tr İnternet Adresi: www.tip.hitit.edu.tr

Bilgi için: İmren ALTIN
Hemşire
Dahili Tel:5004

BIBLIOGRAPHY

- Bilgehan, Hakkı. "Chapter 7." *Klinik Mikrobiyolojik Tanı*. N.p.: n.p., 1995. 131-44. Print.
- Brown, J. Kirk. "Quantifying Bacteria." *Serial Dilution and Plate Counts* (n.d.): n. pag. Web. <https://www.tracy.k12.ca.us/sites/emckay/Shared%20Documents/biotech/Quantifying%20Bacteria%20lab%20results%20and%20conclusion.pdf>.
- Hays, Clint J. "Bacteria In the Oral Flora: A Study of Antimicrobial Agents in Toothpastes on Toothbrıshes." *May 2006, Volume 1 Bacteria in the Oral Flora: A Study of Antimicrobial Agents in Toothpastes on Toothbrushes* 1 (2006): 167-74. *Saint Martin's University Biology Journal*. May 2006. Web.
- "Describe the Conditions Favorable to the Growth of Bacteria in Food." *Food Safety Education*. Department of Food, Nutrition and Packaging Science, Clemson University, n.d. Web. 06 Feb. 2016. <http://www.foodsafetysite.com/educators/competencies/general/bacteria/bac2.html>.
- "Dental Health Pictures Slideshow: The Ugly Truth About Your Toothbrush - MedicineNet." *MedicineNet*. N.p., n.d. Web. 06 Feb. 2016. <http://www.medicinenet.com/truth_about_your_toothbrush_pictures_slideshow/article.htm>.
- "Toothbrush Care: Cleaning, Storing and Replacement." *Toothbrush Care: Cleaning, Storing and Replacement*. American Dental Association, Nov. 2011. Web. 06 Feb. 2016. <http://www.ada.org/en/about-the-ada/ada-positions-policies-and-statements/statement-on-toothbrush-care-cleaning-storage-and->.

- Tan, Sarah. "Think before You Flush or Brush." *Think before You Flush or Brush*. Serendip Studio, 15 Jan. 2008. Web. 06 Feb. 2016. <http://serendip.brynmawr.edu/exchange/node/1839>.
- Sugar, Jenny. "What Not to Do With Your Toothbrush." *RSS*. N.p., 7 June 2012. Web. 06 Feb. 2016. <http://www.popsugar.com/fitness/How-Keep-Your-Toothbrush-Germ-Free-23459538>.
- "Oral Flora." *TheFreeDictionary.com*. The Free Dictionary by Farlex, n.d. Web. 06 Feb. 2016. <http://medical-dictionary.thefreedictionary.com/oral+flora>.
- "CM1135, Brain Heart Infusion Broth | Oxoid - Product Detail." *CM1135, Brain Heart Infusion Broth | Oxoid - Product Detail*. N.p., n.d. Web. 06 Feb. 2016. <http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1135&org=133> .
- "Standard Deviation." *Simple Example of Calculating*. N.p., n.d. Web. 06 Feb. 2016. <http://standard-deviation.appspot.com/>.
- "Standard Error." *Wikipedia*. Wikimedia Foundation, n.d. Web. 06 Feb. 2016. <https://en.wikipedia.org/wiki/standard_error>.
- "Bacterial Contamination and Decontamination of Toothbrushes After Use." *Bacterial Contamination and Decontamination of Toothbrushes After Use.* New York State Dental Journal, 2007. Web. 06 Feb. 2016. <http://europepmc.org/abstract/med/17508674>.
- Taji, S. S. "The Microbial Contamination of Toothbrushes." Australian Dental Journal, 1998. Web. 06 Feb. 2016. <http://onlinelibrary.wiley.com/doi/10.1111/j.1834-7819.1998.tb06101.x/epdf>.
- Karibasappa, G. N. "Assessment of Microbial Contamination of Toothbrush Head." *National Center for Biotechnology Information*. U.S. National Library of Medicine,

Jan.-Feb. 2011. Web. 06 Feb. 2016.

<http://www.ncbi.nlm.nih.gov/pubmed/21525668>.

- "Team:CSU Fort Collins/Notebook/KillSwitch/Sep." *Team:CSU Fort Collins/Notebook/KillSwitch/Sep*. N.p., n.d. Web. 06 Feb. 2016.

<http://2014.igem.org/Team:CSU_Fort_Collins/Notebook/KillSwitch/Sep>.