

BIOLOGY EXTENDED ESSAY

**Investigating the presence of microorganisms in
toothbrush that is kept with a cap and without cap**

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Abstract

Brushing our teeth is important for our oral health. During every tooth brushing, toothbrush interacts with the microorganisms in our mouth. Preservation of toothbrushes is also an important thing for the accumulation of microorganisms in toothbrush. Due to reduce the accumulation of microorganisms in toothbrushes, toothbrush caps have been developed to keep toothbrushes clean and sanitized but their effectiveness is questionable at best.

In this research, the presence of microorganisms in toothbrush with and without cap in the same conditions is investigated. The investigation topic is how the presence of microorganisms in toothbrush changes when it is kept with and without cap in the same conditions.

Toothbrushes are used by family members and they brushed their teeth systematically with a toothbrush that is kept with a cap and another toothbrush that is kept without a cap. In order to get reliable results, toothbrushes are kept in the same physical condition, in the bathroom, same temperature and family members use same type and same brand of toothbrushes and toothpastes.

The experimental results are obtained after the usage of toothbrushes in ten days by the family members. Samples were taken from the toothbrushes in order to plant the samples to the solid growth medium and leave them for incubation. After the incubation period, the solid growth mediums were taken and they are analyzed.

When comparing the solid growth mediums, it is observed that caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized. Therefore, in order to demonstrate the effectiveness of cap it is suggested that toothbrush companies invest on research to find out whether cap is indeed has a real benefit and also to find out whether all toothpastes have a some sort of protection against growth of microorganisms. This research may lead to high economy and savings and avoid environmental pollution.

Word Count: 300

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I. Introduction

More recently toothbrush caps have been developed to keep toothbrushes clean and sanitized but their effectiveness is questionable at best. It is still controversial that caps may reduce contamination of toothbrush. On the other hand, if the effectiveness of toothbrush is inconsiderable, damage to the environment as plastic waste and commercial lost must be discussed. Due to discuss and find out the effectiveness of toothbrush cap, I decided to select this topic.

The toothbrush is an oral hygiene instrument used to clean the teeth and gums that consists of a head of tightly clustered bristles mounted on a handle, which facilitates the cleansing of hard-to-reach areas of the mouth. The toothbrush has soft, end-rounded bristles and be able to remove plaque and usually has been made from synthetic fibers since they were developed.¹

There are several researches related to hygienic protection of a toothbrush, more specifically through a cover to achieve such hygienic effect. The primary purpose of this protection is against airborne contaminants, as well as harmful bacterial and viral agents. “Researchers have established that thousands of microbes can grow on toothbrush bristles and handles. While most of them are harmless members of the mouth's microbial zoo; others can cause cold and flu viruses, the herpes virus that causes cold sores, staphylococcus bacteria responsible for many ear, nose and throat infections, the Candida microbe that causes thrush, and bacteria that can cause periodontal infections.

Usually, people think that, brushing their teeth is enough for their oral health but actually it is not enough. You should rinse your toothbrush thoroughly with water after brushing, but that might not remove everything. During every tooth brushing, toothbrush interacts with the microorganisms in our mouth. We are cleaning our toothbrush after every tooth brushing but its preservation is also an important thing for the accumulation of microorganisms in toothbrush.

¹ <http://en.wikipedia.org/wiki/Toothbrush>

The typical toothbrush is reused for months, never cleaned thoroughly, and usually is stored under warm, moist conditions conducive for bacterial growth. This little piece of plastic, which is the very basis of home daily dental care, repeatedly is plunged into one of the dirtiest parts of the body. More than 300 kinds of microorganisms thrive in the human mouth.²

Toothbrushes are often stored in the bathroom and a bathroom can be a very unclean place. Even if it appears to be clean on the surface airborne contaminants, viral agents, and bacterial agents abound and thrive under such conditions. These same airborne contaminants, bacterial and viral agents are the cause of illness and disease. There is a need to keep toothbrush hygienic and ensure prevention of bacterial and viral contamination.³

More recently toothbrush caps have been developed to keep toothbrushes clean and sanitized but their effectiveness is questionable at best. It is still controversial that caps may reduce contamination of toothbrush. It is also observed that many of the cap inventions have been proposed to reduce contamination of the toothbrush and to prevent bacterial growth on the bristle head due to poor ventilation allowing bacterial growth on the brush head. Bacterial growth is aided by warm moist conditions. Worse yet, some cap inventions don't let ventilation to their covers at all, allowing bacteria growth to flourish.

Since the use of toothbrush cap is common and concerns public health, I have identified the issue on comparing reproduction rate of microorganisms in toothbrush that is kept with a cap and without cap as the subject of my extended essay and decided to conduct experiments. In this research, the presence of microorganisms in toothbrush with and without cap in the same conditions shall be investigated. The investigation topic is **how the presence of microorganisms in toothbrush changes when it is kept with and without cap in the same conditions.**

² <http://www.dentistry.com/daily-dental-care/dental-hygiene/dental-experts-warn-change-toothbrushes-more-often>

³ <http://www.patentstorm.us/patents/7246400/description.htm>

II. Hypothesis

The toothbrush is an oral hygiene instrument used to clean the teeth and gums that consists of a head of tightly clustered bristles mounted on a handle, which facilitates the cleansing of hard-to-reach areas of the mouth. The toothbrush has soft, end-rounded bristles and be able to remove plaque and usually has been made from synthetic fibers since they were developed.

It is important to keep a toothbrush clean and dry, and in different references it is mentioned that a toothbrush cap helps with this. In order to keep toothbrushes clean and sanitized, different brands of toothbrush caps have been developed but their effectiveness is questionable at best. It is still controversial that caps may reduce contamination of toothbrush.

It is claimed that cap can help protect a toothbrush from coming into contact with germs and bacteria that can enter the mouth when brushing. The subject research is conducted to verify whether toothbrush with or without cap is more vulnerable to disinfection of microorganisms.

Since the oral hygiene was valued by Roman and Greek civilizations there was no any protection of toothbrush. Different brands of toothbrush caps have been developed recently but up to 5 years old, there was no toothbrush caps so people didn't use toothbrush caps and according to the literature, in those times there wasn't any sensational problem of the people around the world about oral health. Toothbrush caps are recently developed in order to keep toothbrush clean.

In light of this information, it was hypothesized that toothbrush caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized. There is no difference in accumulation of microorganisms in toothbrushes that are kept with a cap and without a cap.

During a year, billions of toothbrushes are used in the world. If the effectiveness of the toothbrush caps is inconsiderable, their damage to the nature as plastic waste and commercial lost has importance.

III. Method Development and Planning

The research question is how the presence of microorganisms in toothbrush changes when it is kept with and without cap in the same conditions.

In order to support or reject the proposed hypothesis and answer the given research question the method will be efficient.

The aim of the method must be to investigate the accumulation of microorganisms in toothbrush that is kept with a cap and without a cap. In order to observe the accumulation and production of microorganisms in toothbrush, all the other variables that affect the results must be kept constant. Toothbrushes brand and toothpastes that are used in the experiment must be equal. Toothbrushes must be placed at same physical conditions i.e. keeping in the same bathroom, in the same temperature and also toothbrush must be only used by the same person in order to get efficient results.

First method is that; typical tooth brushing in daily life that is 3 times a day will be divided into two categories as Monday, Wednesday and Friday, tooth brushing with one brush and after brushing, preserve it with a cap and the other weekdays Tuesday, Thursday, Saturday brushing the tooth with the other toothbrush and after brushing, preserve it without a cap with the one that is preserved with a cap at same conditions. The production of microorganisms in the toothbrush lasts 24 hours at least and 48 hours at most. After the typical tooth brushing completed on Saturday night, on Sunday the toothbrushes are taken to the Private Viromed Laboratory and some copies will be taken from the toothbrush in order to observe the accumulation and production of microorganisms in toothbrushes which are kept with a cap and without a cap after brushing. This experiment will be done in 4 weeks in order to get efficient results. After getting the results, the comparison of the accumulation and growth of microorganisms in toothbrushes that are kept with a cap and without a cap can be made.

After the first method, another method can also be used in order to get certain results. In this method; typical tooth brushing in daily life that is 3 times a day will be divided as tooth brushing and preserving it with a cap and without a cap too but in this method, the first 3 days of the week, the person used one of the same toothbrushes and preserve this toothbrush with a

cap . The other 3 days, the person will use the other toothbrush and after brushing, he/she will preserve it without cap at same conditions. After the last brushing, the toothbrushes will be taken to the laboratory and some examples will be taken from the toothbrushes. The accumulation of microorganisms will be observed by the laboratory results. This method will be used after the first method and it will last 4 weeks too. After getting the results, the comparison of the accumulation and growth of microorganisms in toothbrushes that are kept with a cap and without a cap can be made.

The primary reason why I have chosen this method is that these two methods will be helpful for getting certain results because the time duration for toothbrushes at same conditions may affect the accumulation of microorganisms from day to day. In order to get best results, these methods will be used in the experiment.

While selecting the brands of toothbrush and toothpaste to be used in the experiment, the criteria is that the brand must be most available and popular in the market because this experiment is based on the people's oral health so materials must be frequently used by the people in the world.

For ease of follow up of experimental conditions for family members took part in the experiment. Firstly, eight toothbrushes of the same brand, model and make were purchased from the market. The selected brand for testing was Banat which had specifications quoted "nylon 6.12 bristles with specifically rounded tips prevent the tooth and gums for injuring; the rubber covered handle prevents slipping and provides an easy and effective brushing". It was also stated on the package of the toothbrush that "Your toothbrush remains hygienic with its protective cap". In order to preserve the same testing conditions, the testers use the toothpaste brand called Colgate Total 12.

All of them contain caps. Then, four caps were removed. Each tester i.e. family members, was provided with two toothbrushes with one having a cap on it. The toothbrush with cap was marked through a sticker at its bottom end. Each tester was instructed to use the toothbrush with cap in the morning and the one without cap in the evening. Each tester kept his/her toothbrushes in a plastic cup. Testers i.e. family members, kept their toothbrushes on the counter of washing basin in the bathroom. Testers began tooth brushing as in the morning of 13th of September 2012 Thursday until 23th of September 2012 Sunday evening. Before

toothbrushes were analyzed in the laboratory, they were marked in pairs as 1C, 2C, 3C, 4C and numbered. Each number represents the testers and the C accounted for recognition of the toothbrush with cap. The eight toothbrushes tested shall be examined in the Viomed laboratory on 24th September 2012 to observe the accumulation of microorganisms in the toothbrushes that were kept with cap and without cap. Laboratory conditions provide taking samples from the toothbrushes by serum physiologic to plant it to the solid growth medium and plantation process with the laboratory equipments such as loop, serum physiologic, sterile Stuart transport swab and solid growth media.

In this investigation, in order to observe the presence of microorganisms in the toothbrushes, solid growth media is used as it is important for reproduction of microorganisms and is stated in the literature;

“In the laboratory, bacteria are usually grown using solid or liquid media. Solid growth media such as agar plates are used to isolate pure cultures of a bacterial strain. However, liquid growth media are used when measurement of growth or large volumes of cells are required. Growth in stirred liquid media occurs as an even cell suspension, making the cultures easy to divide and transfer, although isolating single bacteria from liquid media is difficult. The use of selective media (media with specific nutrients added or deficient or with antibiotics added) can help identify specific organisms.”⁴

“Solid media contains agar, which is a compound that goes into water solution at temperatures approaching boiling, and then, once in solution, solidifies the medium at room (<40°C) temperature.

Subsequent exposure to high temperature (i.e., boiling) will melt the medium. Exposure to relatively low temperatures (i.e., >40°C), however, will not melt the medium, thus allowing incubation of solid medium at various temperatures (compare to gelatin which liquefies at 37 °C)

Once boiled, agar-containing medium will stay liquid at 45 °C. This allows solid medium to be poured into various vessels at temperatures that will not kill most cells (nor melt vessels), followed by a solidification of the medium.

Colonies represent piles of cells descended, assuming pure culture technique and sufficiently few colonies on a single plate, from a single parent cell, all growing on or in a solid medium.

The four phases of bacterial growth can be observed within a single colony, with the edges displaying lag and log phases and the interior can display stationary and then decline phases.”⁵

In order to get accurate results; all variables that affect the reproduction of microorganisms are kept constant such as pH, moisture, nutrient content, oxygen and light. All samples preserved in same conditions as in equal solid growth media so that the amount of oxygen, the light in the media, pH level, moisture in the environment and nutrient content is same. For example all samples were left for incubation for reproduction in incubator at 37 °C.

⁴ <http://en.wikipedia.org/wiki/Bacteria>

⁵ <http://www.mansfield.ohio-state.edu/~sabedon/black06.htm>

- Eight toothbrushes labeled as “1” and “1 C” (Appendix 1- Method Development and Planning figure 6)

IV. Method

Material List

- 8 toothbrushes 4 with caps and 4 without caps (figure 6)
- Loop with 3 millimeter diameter
- Serum physiologic (%0.9 Isotonic NaCl) (Appendix 2- Material List figure 9)
- Sterile Stuart Transport Swab (Appendix 2-Material List figure 8)
- 8 Round glass discs (Appendix 2 –Material List figure 7)
- Glove
- Incubator (Appendix 2-Material List figure 10)

In line with the above plan, the samples were analyzed in the laboratory. The laboratory test report was received on 27th of September.

Method

1. Place the toothbrushes in tubes with sterilized serum physiologic (Appendix 3- Method figure 12)
2. Place tubes in vortex (Appendix 3- Method figure 13)
3. Start vortex to stir tubes (Appendix 3- Method figure 13)
4. Stop vortex and take out tubes
5. Take out toothbrushes from the tubes
6. Through to help of loop with 3 millimeter diameter take out samples from each tube
7. Place samples from the tube into solid plantation (culture) growth medium i.e. round glass tablets (Appendix 3- Method figure 15)
8. Leave 8 round glass tablets in 37 °C incubator for incubation between 24 to 48 hours.
9. Take out the samples and open the lids and observe if there are changes on the surface.
10. If there is change, record them.

See Appendix 3- Method

V. Results

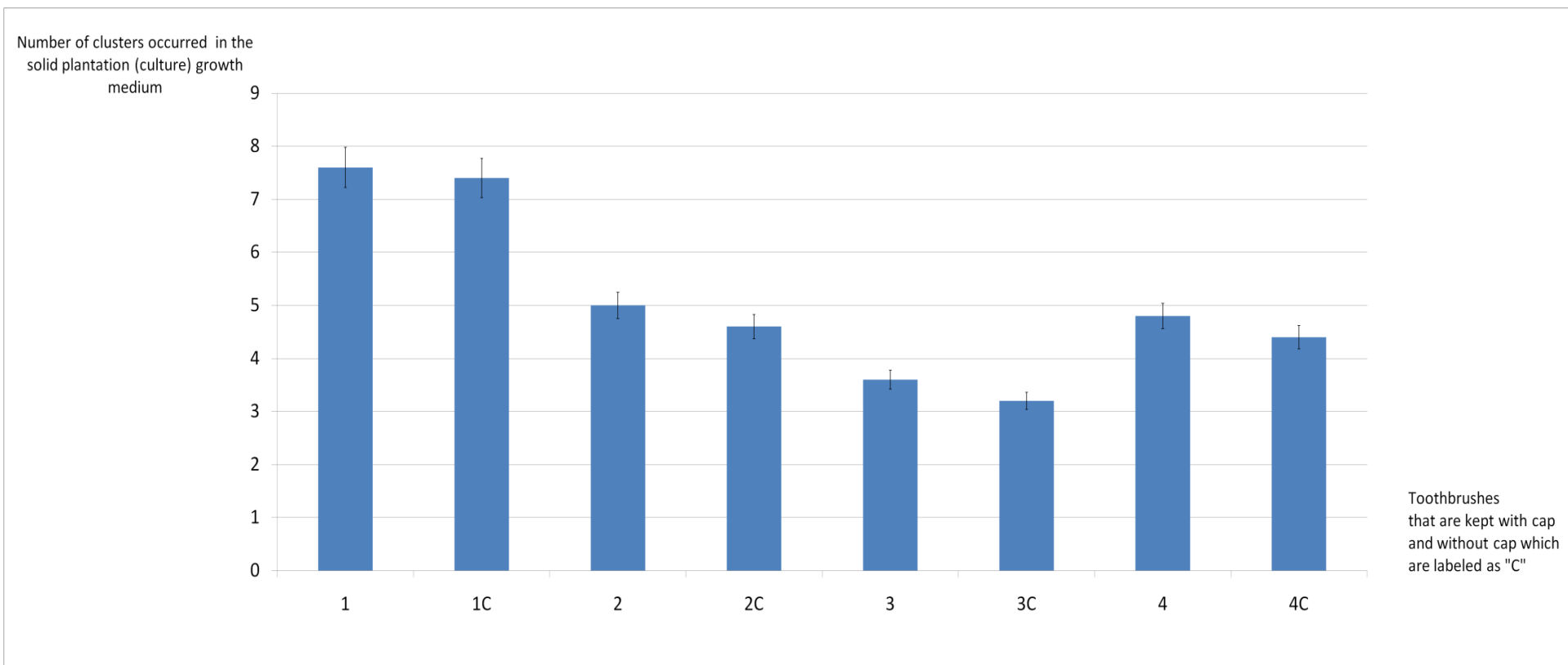
In order to get efficient results; all variables that affect the reproduction of microorganisms are kept constant such as pH, moisture, nutrient content, oxygen and light. All samples preserved in same conditions as in equal solid growth media so that the amount of oxygen, the light in the media, pH level, moisture in the environment and nutrient content is same. For example all samples were left for incubation for reproduction in incubator at 37 C.

As indicated in the test report obtained from the laboratory, the observation as regard to growth of microorganisms in toothbrushes that is kept with a cap and without cap. Numbers represents the family members, and “C” represents the toothbrushes that are kept with a cap.

Toothbrush	Reproduction of microorganisms (positive (+), negative (-))
1	+
1C	+
2	+
2C	+
3	+
3C	+
4	+
4C	+

Number of clusters occurred vs Toothbrushes Table								
Number of clusters occurred Toothbrushes								
	1	1C	2	2C	3	3C	4	4C
Trial 1	8.00	8.00	5.00	4.00	3.00	2.00	5.00	4.00
Trial 2	7.00	8.00	4.00	4.00	3.00	3.00	5.00	3.00
Trial 3	7.00	7.00	5.00	4.00	4.00	3.00	5.00	5.00
Trial 4	8.00	7.00	6.00	5.00	4.00	4.00	4.00	5.00
Trial 5	8.00	7.00	5.00	6.00	4.00	4.00	5.00	5.00
MEAN	7.60	7.40	5.00	4.60	3.60	3.20	4.80	4.40
MODE	8.00	7.00	5.00	4.00	4.00	3.00	5.00	5.00
MEDIAN	8.00	7.00	5.00	4.00	4.00	3.00	5.00	5.00
RANGE	1.00	1.00	2.00	2.00	1.00	2.00	1.00	2.00
VARIANCE	0.30	0.30	0.50	0.80	0.30	0.70	0.20	0.80
SD	0.55	0.55	0.71	0.89	0.55	0.84	0.45	0.89
SE	0.24	0.24	0.32	0.40	0.24	0.37	0.20	0.40
t	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26
%95CI(SE X T _(0,05,df))	0.55	0.55	0.72	0.90	0.55	0.85	0.45	0.90
%95CI(EXCEL)	0.34	0.34	0.44	0.55	0.34	0.52	0.28	0.55

Number of clusters occurred vs Toothbrushes Graph



	Average number of clusters occurred						
	Toothbrushes with cap	Toothbrushes without cap					
1C	8	8	1	t-Test: Paired Two Sample for Means			
	8	7					
	7	7					
	7	8					
	7	8					
2C	4	5	2			8	8
	4	4		Mean	4.736842105	5.105263158	
	4	5		Variance	2.649122807	2.210526316	
	5	6		Observations	19	19	
3C	6	5	3				
	2	3		Pearson Correlation	0.861518352		
	3	3		Hypothesized Mean Difference	0		
	3	4		df	18		
	4	4		t Stat	-1.933206698		
4C	4	4	4				
	4	4		P(T<=t) one-tail	0.034548786		
	5	5		t Critical one-tail	1.734063592		
	3	5		P(T<=t) two-tail	0.069097571		
	5	5		t Critical two-tail	2.100922037		

VI. Data Analysis

According to the data obtained, the average growth of microorganisms as clusters in the solid plantation growth surface is approximately equal to the each variable which is keeping toothbrush with a cap and without a cap, caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized and based on the experimental results, it is verified.

Obtained data verifies the hypothesis that caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized. In first family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 7.4 and the toothbrush that is kept without cap the average number of clusters occurred is 7.6 on the growth surface (culture). Also, in second family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 4.6 and the toothbrush that is kept without cap it is 5 on the growth surface (culture). In third family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 3.2 and the toothbrush that is kept without cap it is 3.6. The number of clusters which are occurred on the solid plantation growth surface represents the production of microorganisms. Test results showed that the average number of clusters in the solid plantation surface is approximately same to each other. This shows that caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized.

VII. Evaluation

In this research, the presence of microorganisms in toothbrush with and without cap in the same conditions investigated. The research question is how the presence of microorganisms in toothbrush changes when it is kept with and without cap in the same conditions. The hypothesis is that toothbrush caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized.

Test results showed verifies the hypothesis that caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized. In first family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 7.4 and the toothbrush that is kept without cap is 7.6 on the growth surface (culture). Also, in second family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 4.6 and the toothbrush that is kept without cap is 5 on the growth surface (culture). In third family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 3.2 and the toothbrush that is kept without cap is 3.6 on the growth surface (culture).

Based on the results, it can be stated that it is controversial that caps may reduce contamination of toothbrush. Toothbrush caps have been developed to keep toothbrushes clean and sanitized but based on the experimental results; they don't have an impact on reducing reproduction rate of microorganisms in toothbrushes.

The toothbrushes are kept in same physical condition, in the bathroom and same temperature. Microorganisms will have reproduced as the environment is very much suitable for contamination because toothbrushes are in contact with the air. Also in order to take them to the laboratory to get experimental observations and results, they are packed with separate refrigerator bags and each family member's toothbrush that are with cap and without cap are packed in one bag so they may contact with each other. Before taking them to the laboratory, the main prediction is that they are already produce microorganisms because the refrigerator bags contain millions of bacteria and microorganism so that it affects the reproduction of microorganisms in toothbrush.

According to the t test paired two sample of means, p one tail is 0.034548786 so that it is smaller than 0.05. Due to this result, hypothesis 1 that is toothbrush caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized is accepted and hypothesis 0 that is caps have effectiveness in keeping toothbrushes clean and sanitized is rejected.

VIII. Conclusion

In this experiment, the effectiveness of toothbrush caps is questioned. Toothbrush caps have been developed to keep toothbrushes clean and sanitized but their effectiveness is questionable at best. On the other hand, if the effectiveness of toothbrush is inconsiderable, damage to the environment as plastic waste and commercial lost must be discussed.

It is still controversial that caps may reduce contamination of toothbrush and in light of this idea, an experimental environment is prepared in order to investigate the reproduction rate of microorganisms in toothbrushes that is kept with a cap and without cap.

In order to get reliable results the dependent variables are kept constant. Family members use same type and same brand of toothbrushes and toothpastes. Also the preservation of toothbrushes is important in reproduction of microorganisms because microorganisms are directly contact with the air. In order to get efficient results, toothbrushes are kept in the same physical condition, in the bathroom and same temperature.

The experimental results are obtained after the usage of toothbrushes in ten days by the family members. They brushed their teeth systematically with a toothbrush that is kept with a cap and another toothbrush that is kept without a cap. Samples were taken from the toothbrushes in order to plant the samples to the solid growth medium i.e. round glass tablets and leave them for incubation at 37 °C in incubator. After 24-48 hour period of incubation, the solid growth mediums were taken and they are analyzed.

In order to identify the reproduction rate of microorganisms, another type of researches; based on reproduction rate of microorganisms in solid growth mediums are observed in the laboratory.

When comparing the solid growth mediums, it is observed that caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized. In first family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 7.4 and the toothbrush that is kept without cap is 7.6 on the growth surface (culture). Also, in second family members' toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 4.6 and the toothbrush that is kept without cap is 5 on the growth surface (culture). In third family members'

toothbrush that is kept with a cap in the solid plantation growth, the average number of clusters occurred is 3.2 and the toothbrush that is kept without cap is 3.6 on the growth surface (culture). According to the anova results; p value is 1.18742E-11 so as it is smaller than 0.05, it verifies the hypothesis that is caps have inconsiderable effectiveness in keeping toothbrushes clean and sanitized.

Considering that there are quite a number of manufacturers producing toothbrush with a cap. Generally, manufacturers make plastic caps. There is a high amount of plastic material used for cap itself. The production of cap material through plastic cost a lot of money and it doesn't degrade in nature. Certainly it causes environmental pollution. They are polymers, very long chain molecules that consist of subunits (monomers) linked together by chemical bonds. The monomers of petrochemical plastics are inorganic materials (such as styrene) and are not biodegradable so that they have an impact on environmental pollution.

Therefore, in order to demonstrate the effectiveness of cap it is suggested that toothbrush companies invest on research to find out whether cap is indeed has a real benefit and also to find out whether all toothpastes have a some sort of protection against growth of microorganisms. This research may lead to high economy and savings and avoid environmental pollution.

IX. Appendix

Appendix 1-Method Development and Planning

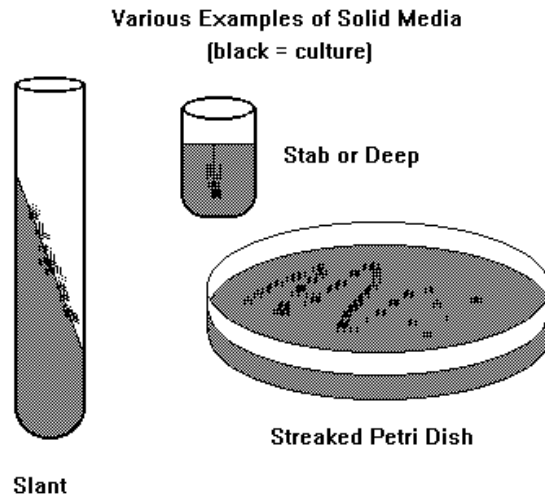


Figure 1

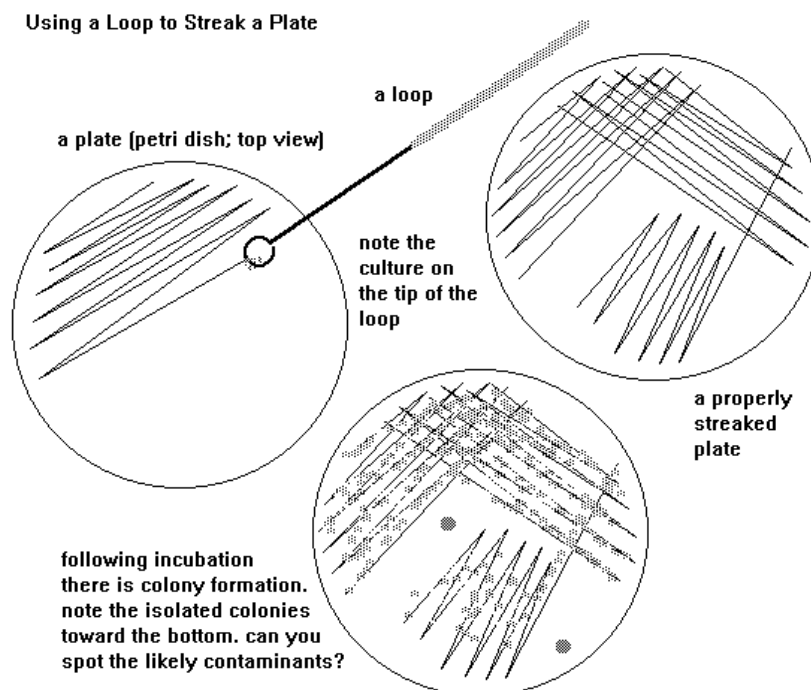


Figure 2



Figure 3



Figure 4



Figure 5



Figure 6

Appendix 2-Material List



Figure 7



Figure 8

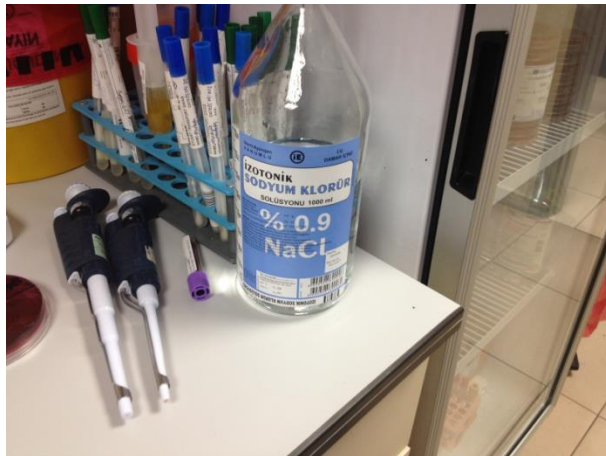


Figure 9



Figure 10

Appendix 3-Method



Figure 11



Figure 12



Figure 13



Figure 14



Figure 15

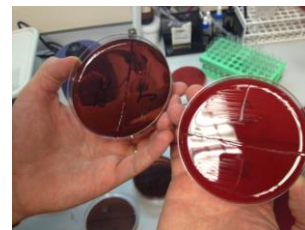
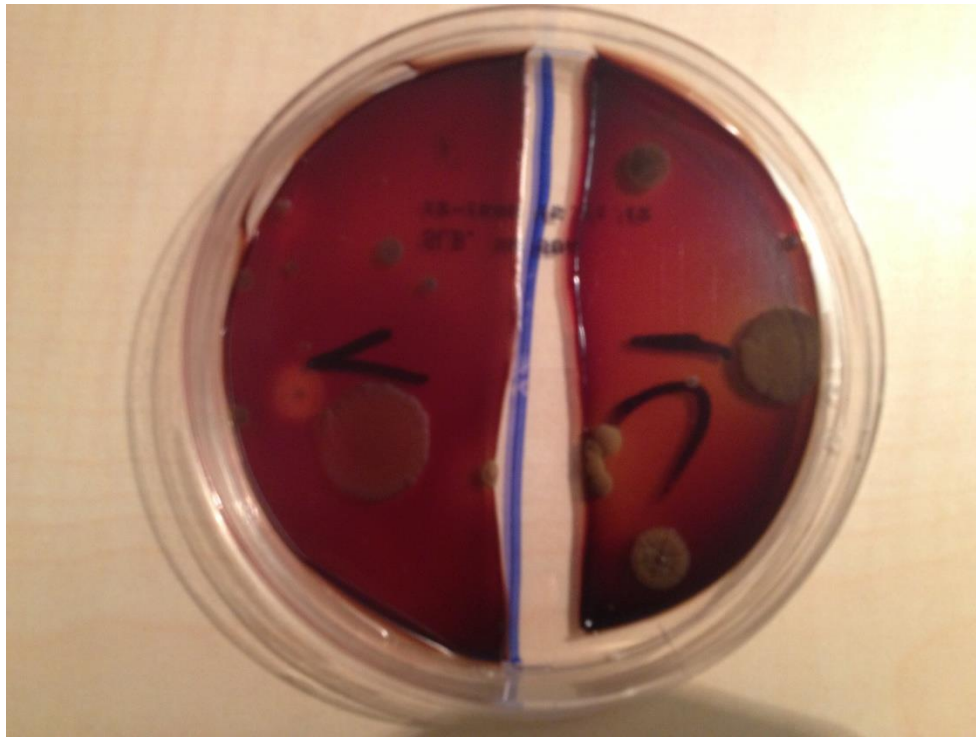


Figure 16

Appendix 4- Presence of Microorganisms in Solid Growth Mediums



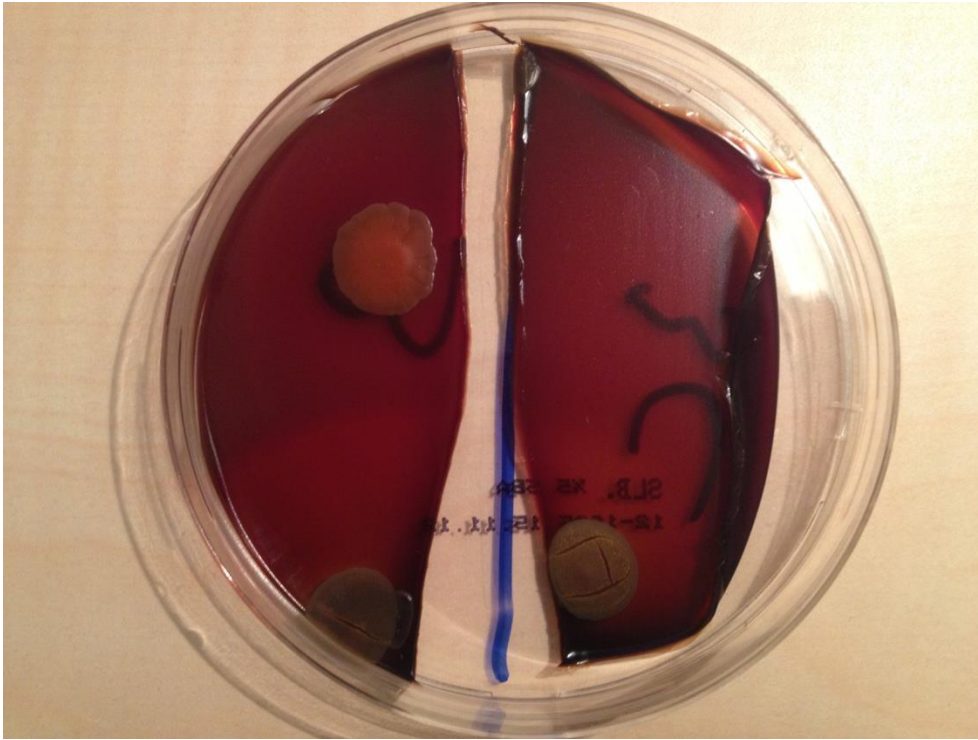
1

1C



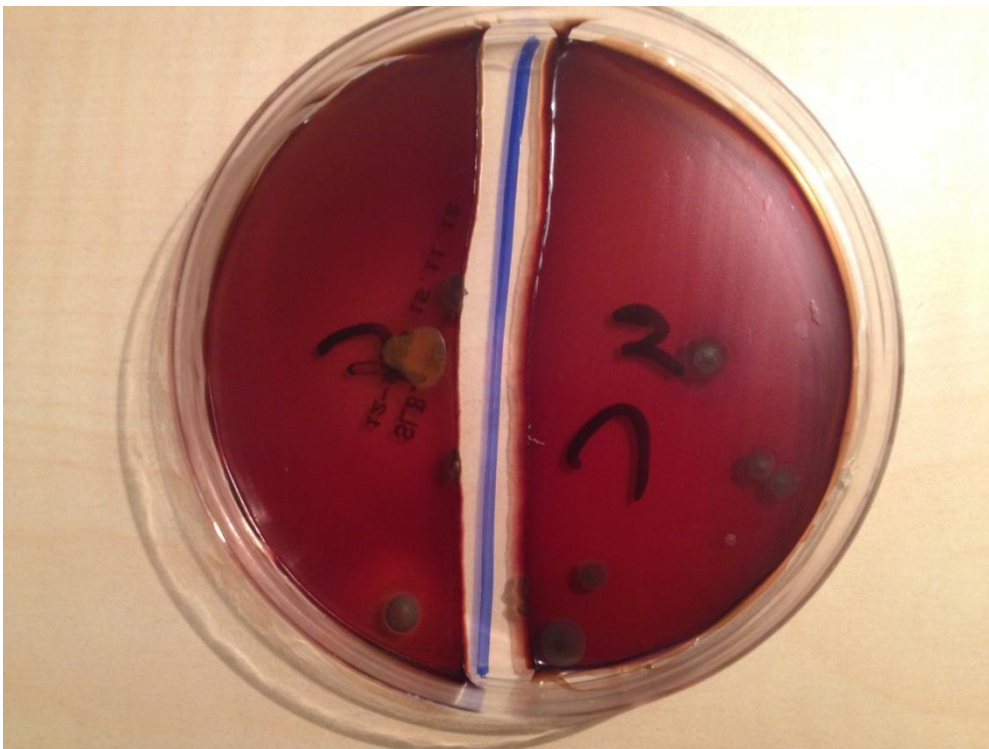
2

2C



3

3C



4

4C

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- <http://en.wikipedia.org/wiki/Toothbrush>
- <http://www.dentistry.com/daily-dental-care/dental-hygiene/dental-experts-warn-change-toothbrushes-more-often>
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