

TED ANKARA COLLEGE FOUNDATION HIGH
SCHOOL

Comparison of the bactericidal effects of most-known, medical-level mouth antiseptics, Baticon, Tantum Verde, Andorex, Listerine and lemon on *Streptococcus mutan*.

Biology Extended
Essay

Supervisor: Sevim SARAL
Name of candidate: Emir Taha Çiftci
Candidate number: 1129- 0101
Word count:

Abstract

The aim of this extended essay is to investigate the antibacterial properties of the most-known mouth products, Baticon, Tantum Verde, Andorex, Listerine and lemon (the natural antiseptic) on *Streptococcus mutan* in lab conditions. So that this extended essay includes the comparison of these products on an *Oral Bacteria* of a healthy mouth.

My research question was: "Is there a significant mean difference among mouth antiseptics Baticon, Tantum Verde, Andorex, Listerine and lemon in terms of their antibacterial effects on *Streptococcus mutan* in laboratory conditions?"

My hypothesis was: "There would be a significant mean difference in terms of antibacterial activity between Baticon, Tantum Verde, Andorex, Listerine and lemon. The most-known mouth antiseptic Tantum Verde will be the most effective antibacterial product against *Streptococcus mutan*, followed by the other well-known product Baticon. The third most effective product will be the commercial mouthwash, Listerine. Andorex will take the 4th place and finally the natural antiseptic lemon."

In order to test the hypothesis and to answer the research question, the Kirby-Bauer antibacterial testing method was used. *Streptococcus mutan* population was cultured on Mueller-Hinton agar plate in laboratory conditions. The products (Baticon, Tantum Verde, Andorex, Listerine and lemon) which are all liquid, were dropped onto the agar plate. The plate is incubated at the incubation temperature of bacteriological incubator. At the end, the diameters of the exclusion zones were measured and compared to each other. This measurement gave the necessary data about the antibacterial effect of the products.

Results show that, the area of which Andorex dropped had the largest diameter of the exclusive zone. The second largest diameter was formed by the natural antiseptic, Lemon. The diameter of Listerine, the most-known antibacterial mouthwash, took the 3rd place, followed by Baticon. Finally, Tantum Verde had the smallest diameter.

Moreover, according to the results of ANOVA showed that there was a significant mean difference between Baticon, Tantum Verde, Andorex, Listerine and lemon in terms of their antibacterial effects on *Streptococcus mutan* in laboratory conditions with an arrangement as from most effective to the least effective one as following: Andorex, lemon, Listerine, Baticon, Tantum Verde.

Word Count : 348

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INTRODUCTION

When I was at my grandparents house, I realized a lemon in their bathroom. When I asked them why the lemon is there for, they said they squeeze lemon juice and gargle after they brush their teeth in order to maintain oral hygiene. I was fairly surprised when I first heard that they use lemon for a mouthwash. I thought they use it since they are old and probably don't know about the new mouthwash products but after I did a research, I figured that lemon is one of the most effective antibacterial agent. However, I still had a question in my head: Compared to other well-known antiseptic mouthwashes, to what extent is lemon juice a good oral antibacterial?

To start with, It should be clear that the mouth is full of bacteria like many areas of the body. Although most of them are harmless, some of them can cause serious dental problems or oral infections. With a good oral health care, such as brushing the teeth and using antibacterial mouthwashes, these bacteria can be kept under control.

Only brushing the teeth may not be sufficient for good oral hygiene since some antibacterial chemicals do not exist in toothpaste. Most of the people use mouthwashes or mouth rinse to enhance oral hygiene. Mouthwash is a liquid solution that is swished around inside the mouth to cover the teeth, gums and tongue, and then is spit out and is not diluted. There are some commercial mouthwashes such as Listerine®, Colgate® and Scope® which are available in almost every drugstore and supermarket and the most of the population prefers to use these prepared mouthwashes instead of the natural antibacterial mouthwashes like Lemon Juice. In addition, there are some people that use the antiseptic gargles and sprays which aren't known as much as the commercial mouthwashes. For instance, Tantum Verde®, Baticon® and Andorex® have antibacterial effect on the oral bacteria. However, which kind of products would be the most effective in oral bacteria?

The lemon is a green tree which has a yellow fruit, called Lemon. Oral microbiologist claimed that the lemon's antibacterial properties is not even comparable to other plants because Lemons contain the substances such as calcium, magnesium, vitamin C and citric acid which can break down the cell membranes of the oral bacteria. In the past experiments, lemon juice is found to be active against oral bacteria. Therefore, it has been revealed that it may be used in oral care materials. Simply, the low pH of juice makes it antibacterial and the more concentrated it is, the lower the pH. Thus, some

bacteria can't stand against it since the cell membranes of the bacteria is not protective enough for the low pH level in lemon juice. The lemon is used for oral medication in some countries such as India, in traditional medicines Siddha Medicine and Ayurveda. ¹

Tantum Verde is drug of antibacterial activity, which can be used as a spray or mouthwash. Many people carry Tantum Verde with them most of the time because it is fairly easy to use the spray instead of the mouthwash. In an experiment on the antibacterial effect of Tantum Verde, results showed that Tantum Verde has an actual bactericidal effect on oral pathogens. ²

Another product called Andorex has a lot of similarities with Tantum Verde. Andorex is mouth-rinsing solution that has an antiseptic property which reduces the amount of oral bacteria. Unlike Tantum Verde, Andorex is not a well-known Oral hygiene product. Moreover, It prevents the dental problems in the mouth with its strong antibacterial content called chlorhexidine gluconate. Andorex can be used as a spray and gargle after brushing the teeth. ³

A fairly popular antiseptic gargle among the European countries is Baticon. Baticon is used after brushing the teeth by a great amount of people. In the past few years, Baticon was used only for injuries in order to kill the bacteria. However, it is currently used by more people as a gargle after brushing the teeth to enhance oral hygiene. The Baticon is usually diluted when its used as a gargle but in the experiment, Baticon will not be diluted because of any reaction that could occur after mixing. In addition, there is a risk that Baticon could lose its antibacterial effect when it is diluted.

The last product I picked for this experiment is Listerine which is a brand of modern antiseptic mouthwash product with the slogan "Kills germs that cause bad breath." The reason why Listerine is going to be used in this experiment is that the Listerine has a great reputation and it is the most popular mouthwash in the oral health care sector. Unlike the other antiseptic products, Listerine is available in every drugstore and has a better taste than the others. A study was published in 2012 proved that performing a 30 second rinse with Listerine prevents gum decay and oral infections. ⁴

The topic of this research is comparing of the antibacterial effects of Listerine, lemon, Tantum Verde, Andorex and Baticon on *Streptococcus mutans*.

During my research, I have seen many experiments and studies were done to reveal if Listerine was better than the other brands. Also, I have seen a study of the antibacterial effects of the lemon. However, I was not able to find any research done on comparison of the modern mouthwashes (Listerine), antiseptic gargles and sprays (Tantum Verde, Andorex, Batiodin) and a natural antiseptic lemon. On the other hand, the antibacterial effect of lemon which interested me the most, led me to choose this topic. In this research, my aim is to make a distinction between the five oral antiseptic products by measuring the diameters of exclusion zones in *Streptococcus mutans* culture on Mueller-Hinton Agar plates.

I have used "*Streptococcus mutan*" instead of Staphylococcus aureus, Streptococcus pyogenes, Helicobacter pylori, Candida albicans, Actinomyces viscosus, Porphyromonas gingivalis, Prevotella intermedia, and Actinobacillus actinomycetemcomitans because almost every oral hygiene company claims that they prevent the dental cavities and tooth decay. The "*Streptococcus mutan*" colonize and maintain a dominant presence in the oral cavity and causes the tooth decay. Therefore, comparing the agents on "*Streptococcus mutan*" by its antibacterial effect would be the most logical way. ⁵

The biological terms I used in my extended essay are “Antibacterial” and “Antiseptic.” Antibacterial simply means the destroying or inhibiting the growth of bacteria and the antiseptics are agents or solutions that kills microorganisms that causes illnesses. Bactericidal antiseptic kills the bacteria and the bacteriostatic antiseptic weakens the bacteria. ⁶

HYPOTHESIS

Lemon is primarily used for lemonade. The sour taste of lemon juice took part in cooking and baking, too. Moreover, lemon juice is found to be active against oral bacteria. However, most people buy artificial antiseptic products instead of using lemon juice. The reason why people do not prefer to use a natural antiseptic is that they don't rely on its antibacterial property as much. Using antiseptic drugs is known as quicker, easier and a “clear” way in order to get rid of the oral bacteria. However, the level of lemons antibacterial property is still a doubt in my mind.

The spray or gargle type oral antiseptics such as Baticon, Tantum Verde and Andorex are known for their strong antiseptic effect on bacteria. Although they have a plenty of similarities, these 3 products vary from one to another. Each product has its special content. On the other hand, Tantum Verde is one of the most-known oral antiseptic products. Therefore, in my hypothesis, Tantum Verde is going to be the most effective on oral bacteria because of its popularity and good reputation.

Baticon is another well-known antiseptic product. In addition, it has been a good oral antiseptic gargle, recently. When its compared to Tantum Verde, it is not as popular but the reviews of this product show that It is started to become the most effective one in the market.

Andorex is a product which is not known as much as the other products due to its unavailability in the drugstores. Nevertheless, It can be found in some pharmacies. . For instance, Andorex has some extra content which makes it stronger and more effective on oral bacteria.

The most popular mouthwash Listerine is currently been used by a lot of people. It is very easy to be found, even in the supermarkets. There is a great amount of good review of Listerine. Therefore, these reviews and the products popularity leads me to think that Listerine will be the second most efficient product on oral bacteria.

All the reviews, the content information and the products popularity, had an impact on the formation of my hypothesis as "There would be a significant mean difference in terms of antibacterial activity between Baticon, Tantum Verde, Andorex, Listerine and lemon. The most-known mouth antiseptic Tantum Verde will be the most effective antibacterial product against *Streptococcus mutan*, followed by the other well-known product Baticon. The third most effective product will be the commercial mouthwash, Listerine. Andorex will take the 4th place and finally the natural antiseptic lemon."

DEVELOPMENT & PLANNING

I have done a research on the experimental technique in testing the bactericidal function of liquid antiseptic oral health care products. I have decided to use the Kirby-Bauer disk diffusion susceptibility testing method. In the information I gathered, I learned that the Kirby-Bauer test which is also called the disc diffusion, is first used in the 1950s. Kirby-Bauer test is still used in some laboratories. This test is basically used to determine the resistance or sensitivity of bacteria to specific chemicals. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism. ⁷

The main reason why I have chosen this method to use is that I am going to be able to have quantitative data for my results of the experiment. This testing method is not complicated because it enables you to see the antibiotic effect of the products on oral bacteria with simple materials. Kirby-Bauer testing method is also known of its reliability and high-accuracy.

After the bacterium is swabbed on the plates called Agar, the liquid antibiotic products will be applied. After the certain amount of time, there will be no growth in the intermediate area of the disc. This non-growth area is called zone of inhibition or zone of exclusion. After the zone size are measured, I will be able to determine the level of antibiotic property of each product. I am going to take notes during the experiment to draw a chart in order to give a result of sensitive, resistant or intermediate.

After I had an idea of the method that I am going to use in my experiment, I

called my uncle, Doğan Çiftci who is a General Surgeon in the hospital called "Can Hastanesi." He told me it would be convenient to do the experiment with the Kirby-Bauer testing method. In addition, he told me that they have a fully-equipped laboratory which I can use. Eventually, I contacted a laboratory assistant, Halit Sakarya. He told me that they can help me with my experiment so we arranged a date and did the experiment, successfully.

By the end of the experiment, the measurements of the diameters of the inhibition zones will be compared. The one with a greater diameter is going to be the most effective against the *Streptococcus mutan*. So that my independent variable of the experiment is the antiseptic products I am going to use on agar plates and my dependent variable is going to be the diameter of the zones of exclusion.

The oral bacteria that I planned to use is *Streptococcus mutan* because it is easy to investigate and because of the reasons I listed in the Introduction Section. I have used liquid solutions in my experiment in order to drop the same amount of mouth-wash on the Agar plate. I didn't want to use any solid oral antiseptic because it would make it harder to measure.

According to my researches, agar plate is convenient to make an experiment on the bacteria. On the plates, the oral bacteria called *Streptococcus mutan* was grown. Basically, the agar plate contains acid 17.5 grams of hydrolysate of casein, 2 grams of beef extract, 1.5 grams of starch and 17 grams of agar. These contents speed up the bacteria growth on the plate. The incubator Stuart SI19 is used because overcomes this problem with forced air circulation. Also, external contamination is avoided by wearing gloves in the laboratory. ⁸

After the solutions dropped on the agar plate, It has kept in the incubator. The incubators conditions were the same for all Agar plates which had different antiseptic solutions on them in order to avoid the errors that could happen during the experiment.

At the end of the experiment, I suppose that the Tantum Verde will have the largest diameter. The second largest will be Baticon. The third most effective product which will have the third largest diameter will be Listerine. Andorex will take the 4th place and finally lemon will have the smallest diameter.

MATERIALS

- 100 ml Listerine® Antibacterial Mouthwash (2 Drops will be used)
- 100 ml Baticon® (2 Drops will be used)
- 100 ml Tantum Verde® (2 Drops will be used)
- 100 ml Andorex® (2 Drops will be used)
- 100 ml Regular lemon juice (2 Drops will be used)
- 5 Agar Plates
- *Streptococcus mutan*
- Distilled water
- The Stuart SI19 Incubator
- Millimetric ruler (uncertainty: $\pm 0.5\text{mm}$)



METHOD

A) The preparation of the nutritional agar

- 1) (See Appendix)

B) The measurement of antibacterial agents

- 1) 2 drops of each antibacterial agent is collected in the measurement needle The needles are changed for every product.

C) Applying the antibacterial agents on the agar plates

- 1) 2 drops are applied at the center of the each Agar Plate.
- 2) 5 antibacterial agents are applied on different agar plates.
- 3) The 5 agar plates are kept in the incubator set to 36.5°C for 24 hours.

D) Recording of the results

- 1) On each agar plate, the diameter of the exclusion zones are measured and recorded.

This procedure is repeated for 5 times in order to reduce the uncertainty of the experiment.



RESULTS

TYPE OF THE ANTIBACTERIAL PRODUCT	ZONES OF EXCLUSION DIAMETERS (± 1 mm)				
Trials	1	2	3	4	5
TANTUM VERDE	13	11	17	12	12
ANDOREX	34	33	36	32	33
LEMON JUICE	32	30	31	28	29
LISTERINE	27	25	28	23	24
BATICON	23	21	23	20	22

Table 1: The diameters of exclusion zones recorded after 24 hours of incubation of *Staphylococcus mutan* grown on agar plates.

DATA ANALYSIS

(Formulas used to calculate the values in the tables and graph)

The Mean ⁹

$$\bar{X} = \frac{\sum X}{N}$$

\bar{X} is the symbol for the mean.

Σ is the symbol for summation.

X is the symbol for the value recorded.

N is the symbol for the number of trials.

Standard Deviation ¹⁰

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{N}}$$

σ is the symbol for standard deviation.

X is the symbol for the value recorded.

N is the symbol for the number of trials.

\bar{X} is the symbol for the mean.

Σ is the symbol for summation.

Standard Error ¹¹

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

S is the sample standard deviation

n is the size (number of observations) of the sample.

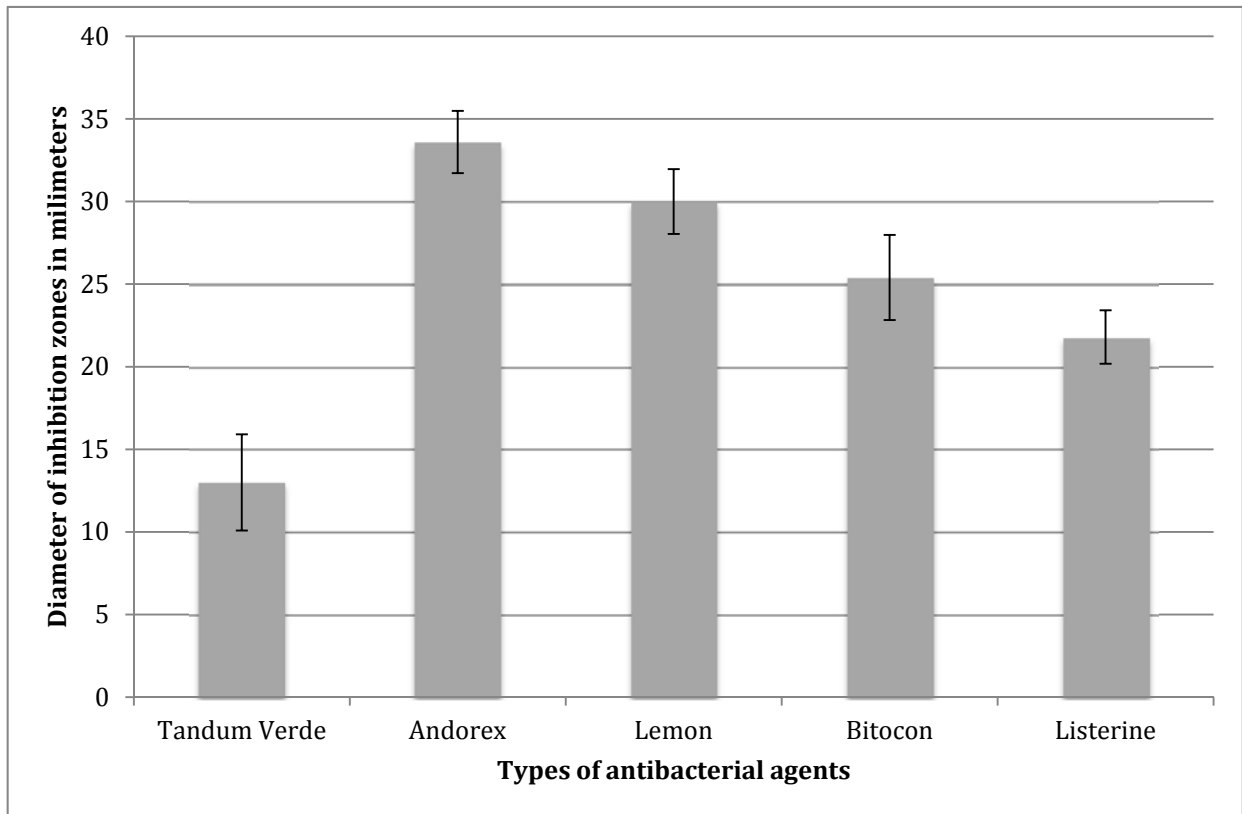
	COUNT	SUM	AVERAGE	VARIANCE	STANDART DEVIATION
TANTUM VERDE	5	65	13	5.5	2.34
ANDOREX	5	168	33.6	2.3	1.52
LEMON	5	150	30	2.5	1.58
BATICON	5	127	² 5.4	4.3	2.07
LISTERINE	5	109	21.8	1.7	1.30

Table 2: The mean values, standard deviations and standard errors of the diameters of exclusion zones on Agar plates, which have different antibacterial agents on them.

Groups ----- Calculation	Tantum V.	Andorex	Lemon	Biticon	Listerine
Mean	13	33.6	30	25.4	21.8
Standard Error	1.048808848	0.678232998	0.707106781	0.927361849	0.583095189
Standard Deviation	2.34520788	1.516575089	1.58113883	2.073644135	1.303840481
Count	5	5	5	5	5
Confidence	2.911960193	1.883076689	1.963243161	2.574769268	1.618931785

Table 3: Descriptive Statistics for each antibacterial agent.

Microsoft Office Excel 2011 and Texas Instruments TI-84 is used for the calculations.



Graph 1: The comparison of the inhibition zones obtained from Listerine, Andorex, Lemon, Tandum Verde and Baticon. The error bars indicate standard error for each group.

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p-level</i>	<i>F crit</i>
Between Groups	1,265.36	4	316.34	97.03681	8.38996E-13	2.86608
Within Groups	65.2	20	3.26			
Total	1,330.56	24				

Table 4: ANOVA calculation for all groups.

H_1 : There is a significant mean difference between the antibacterial effects of agents on the oral bacteria.

H_0 : There is not a significant mean difference between the antibacterial effects of agents on the oral bacteria.

$$8.38996 \times 10^{-13} = p$$

$$p < 0.05$$

So, H_1 is accepted.

H_0 is rejected.

EVALUATION

In this experiment, my aim was to show the antibacterial properties of the most-known mouth products, Baticon, Tantum Verde, Andorex, Listerine and lemon (the natural antiseptic) on *Streptococcus mutan* in lab conditions. I made my experiment by using the Kirby-Bauer antibiotic testing method which enabled me to investigate the effect of each products on the same type of bacteria. Also, Kirby-Bauer antibiotic method is a reliable and a fairly easy testing method.

Table 1 shows the results which are recorded just after the experiment. Table 2 shows the calculated values which are count, sum, average, variance and standart deviation. By looking at the Table 1, Tantum Verde had the least antibacterial effect. As the average zone of inhibition, Listerine showed a better antibacterial effect that Tantum Verde on the bacteria. However, Baticon had a larger inhibition zone that Tantum Verde and Listerine which means that it has a better antibacterial effect. The second most effective product was lemon and finally, the most effective antibacterial agent was Andorex. The mean of the diameter of the exclusion zone in Andorex was 33.6. In lemon, the mean 30. The mean result for Baticon 5.4 and for Listerine it was 21.8. The last antibacterial agent Tantum Verde's inhibition zone diameter mean was 13.

In conclusion, the results of the experiment showed that the most effective antibacterial agent is Andorex which is followed by Lemon. Baticon is the 3rd most effective antibacterial. Listerine took the 4th place, followed by Tantum Verde. Thereby, The results of the experiment rejected my hypothesis which was, "Tantum Verde will be the most effective antibacterial product against *Streptococcus mutan*, followed by the other well-known product Baticon. The third most effective product will be the commercial mouthwash, Listerine. Andorex will take the 4th place and finally the natural antiseptic lemon." The results rejected my null hypothesis which was "There is not a significant mean difference between the antibacterial effects of agents on the oral bacteria" and accepted the H_1 .¹²

When comparing the standard deviation of each group, Tantum Verde has the greatest standard deviation value. That simply indicates that the values obtained from Tantum Verde is less reliable than the data obtained from the other agents. Baticon has a lower value of standard deviation however it is still greater than the other 3 products. Lemon has the third greatest standard deviation which is followed by Andorex. Finally, Listerine has the lowest standard deviation which means it is the most reliable data group.

In Graph 1, The error bars are fairly short, yet that doesn't mean they shouldn't be considered. Tantum Verde has the longest error bar because it has a greater standart deviation. Moreover, Listerine has the shortest error bar.

The standard deviation values and the length of the error bars indicated that Listerine is the most reliable data group which is followed by Andorex and Lemon. The 4th most reliable data group is Baticon and the least reliable group is Tantum Verde.

An unexpected result of the experiment was the low antibacterial effect of Listerine. The most popular antibacterial mouthwash nowadays is Listerine. However, It was the 4th most effective mouthwash among the 5 antibacterial agents which are tested in the experiment. It was unforeseen that Lemon would have a higher antibacterial effect than Listerine.

I have carefully planned my experiment method and prepared all the material needs for the Kirby-Bauer testing method in order to have a great reliability of data, however while I was doing my experiment with the laboratory assistants, I realized some errors which could cause the reliability of the data to decrease.

They were:

- **The concentration of the liquid antibacterial agents.** Two drops of each antibacterial agent has located on the center of the each agar plate. The antibacterial agent could have been too potent. Two drops of lemon juice, Listerine, Tantum Verde and Andorex were gathered without any interference. However, Baticon has a higher concentration itself and I didn't mix it with the water in case of any unexpected reactions that could occur. Therefore, the reliability of data decreased since I didn't dilute Baticon in order to lower the concentration according to the other antibacterial agents. Even though its concentration, Baticon was not the most effective antibacterial agent on Oral Bacteria. Moreover, It was the 3rd most effective.
- **Testing with 5 agar plates.** Before the experiment, I had decided to use 5 different plates in order to have a more detailed investigation on the Agar plates. So that, I would be able to measure the distance between the bacteria and the center of the plate (where the antibacterial agent is located) more precisely and the recording of results would be more accurate. During the experiment, the laboratory assistant said that the amount of bacteria on each plate can vary. After he said that, I considered it as an error. The assistant and I paid attention to grow almost the same amount of bacteria on each plate. I will test all the products in the same agar plate in order to fix that error in future experiments.

- **The brands of the antibacterial agent, e.g. Listerine.** I have chosen Listerine for the most popular mouthwash because in oral hygiene industry, It has a great reputation. However, It was not necessarily to pick Listerine for the experiment. That is the reason why the brand is considered as an error as well. The reason why the brand of the antibacterial agent Listerine considered as an error is that it may be seen as a generalization of the commercial mouthwash products when it is only picked for its popularity. There are plenty of mouthwashes which could have the same effect as Listerine or might have a greater effect. Next time, I will test more mouthwash products and pick the convenient one before I start to do my experiment.
- **Only one type of bacterium was grew on Plates.** The bacteria "*Streptococcus mutan*" was grew on the agar plates. There are a great amount of oral bacteria type such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Helicobacter pylori* etc. (The other species are on the introduction section). It is an error because every kind of oral bacteria could have a different reaction to the antibacterial agents.
- **The difference between human mouth and nutritional agar plate.** The nutritional agar plate provides the most suitable environment for the certain bacteria to grow. The reason why they are kept in the incubator is that, as well. However, It doesn't exactly function as a human mouth. That is an error because the aim is to investigate the effects of different antibacterial agents on oral bacteria. However, there is no way to fix that error.
- **The thickness of agar.** The thickness of agar plate could be an error because if the agar plate is too thin, the antibacterial agents wouldn't function as much. So that, the data collected would not be accurate enough. The convenient thickness of an agar is 5 to 6 mm. The thickness of the agar in my experiment was 4 mm. Therefore, It might have decreased the reliability of data. I am planning on giving more attention on the thickness of the agar next time.

CONCLUSION

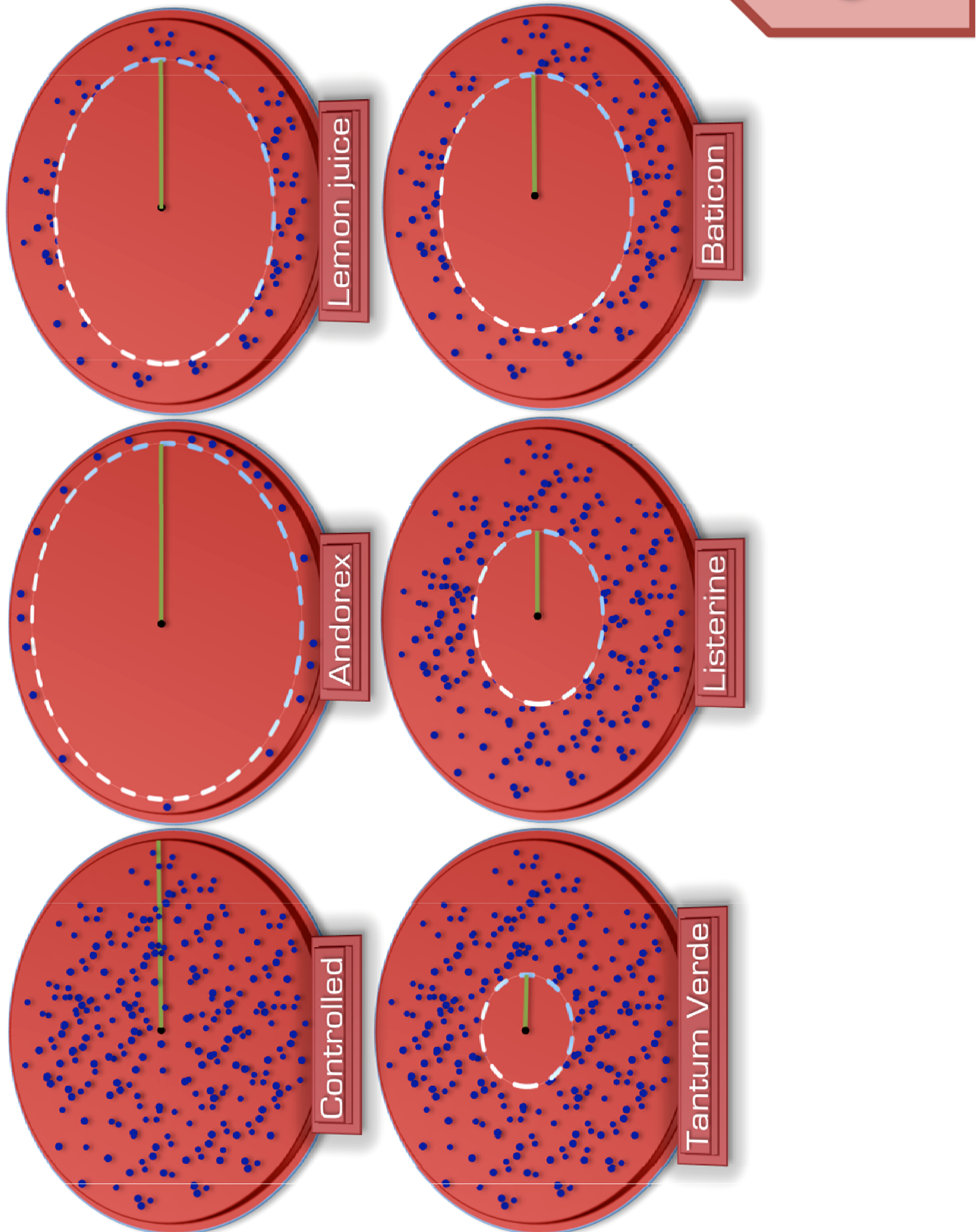
As this study showed, different antibacterial agents can have different effects on oral bacteria. Resultantly, using the popular antibacterial mouthwash does not mean that it is more effective than the other antibacterial agents. The lemon juice was expected to be the least effective agent on oral bacteria. However, the results showed that it has a greater effect than the popular mouthwash Listerine, Baticon and Tantum Verde. So, the results provided a clear answer for my research question "Is there a significant mean difference among mouth antiseptics Baticon, Tantum Verde, Andorex, Listerine and lemon in terms of their antibacterial effects on *Streptococcus mutan* in laboratory conditions?"

I made my study about the oral antibacterial agents because there are products such as Baticon, Tantum Verde, Andorex and natural antibacterial agents such as lemon juice, which are not known by their antibacterial effects on oral bacteria. All of the agents used for the experiment have antibacterial effects for the maintenance of oral hygiene. Usually, they are used after brushing teeth. The main reason why I came up with the topic "Comparison of the bactericidal effects of most-known, medical-level mouth antiseptics, Baticon, Tantum Verde, Andorex, Listerine and lemon on *Streptococcus mutan*" is that Most of the people use the commercial antibacterial mouthwash products without knowing the effect of the other antibacterial agents. There could be other potential products for oral hygiene but they are not known as much as the commercial ones because most people trust the mouthwashes which are advertised on TV. Even though the majority uses the commercial mouthwashes, there are still plenty of people who prefer to use natural products or the other antibacterial agents.

There are many studies on how to maintain the oral hygiene with using different kind of products. However, there aren't any other study that compares the antibacterial agents, including a natural antibacterial, lemon. Basically, I aimed to give an answer to the question "Do we really need to buy artificial agents for oral hygiene instead of using the natural agents? If we need to use artificial antibacterial agents, which one is the most effective." I believe that my study is informative to the people who would like to know the most effective way to maintain oral hygiene.

APPENDIX 1:

Diagram 1: The experimental design.



APPENDIX 2

Listerine Ingredients ¹³

The active ingredients are Antiplaque and Antigingivitis

- . Eucalyptol 0.092%
- . Menthol 0.042%
- . Methyl salicylate 0.060%
- . Thymol 0.064%

The Inactive Ingredients in Listerine Mouthwash

- . Water
- . alcohol (21.6%)
- . sorbitol solution
- . flavoring
- . poloxamer 407
- . benzoic acid
- . sodium saccharin
- . sodium benzoate

Tantum Verde Ingredients ¹⁴

100 ml of solution containing the active substance: 0.15 g of hydrochloride benzydamine and other ingredients: glycerol, ethanol 96, methyl parahydroxybenzoate, the composition of mint flavor, saccharin, sodium bicarbonate, polysorbate 20, purified water

Andorex Ingredients ¹⁵

Active : Benzydamine/Chlorhexidine

APPENDIX 3

ORAL BACTERIA¹⁶

- 1) streptococci lactobacilli
- 2) staphylococci corynebacteria
- 3) Streptococcus salivarius – Starts to grow in a new-born baby mouth
- 4) Streptococcus mutans - The bacteria in the experiment and also, It colonizes in the mouth at the age of one when the teeth starts to appear.
- 5) Streptococcus sanguinis - They colonize on the dental surface
- 6) Fusospirochetes - When there is bleeding in the oral cavity, the bacteria can cause infection and diseases to oral cavity. Covers the throat area as well.
- 7) Veillonella - It converts the acidic products of other species to less acidic products.
- 8) Aggregatibacter actinomycetemcomitans
- 9) oral pathogen due to its virulence factors and it can cause bone loss.
- 10) Lactobacillus

APPENDIX 4

(This information is taken from the “microbelibrary.org” website) ¹⁷

PREPARATION OF AGAR PLATES

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.
2. If the surface of the agar has visible liquid present, set the plate inverted, agar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).
2. Appropriately label each MH agar plate for each organism to be tested.

PREPARATION OF INOCULUM

1. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 2 ml of sterile saline.
3. Vortex the saline tube to create a smooth suspension.
4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
5. Use this suspension within 15 minutes of preparation.

Organisms to be tested must be in the log phase of growth in order for results to be valid. It is recommended that subcultures of the organisms to be tested be made the previous day. Never use extremes in inoculum density. Never use undiluted overnight broth cultures or other unstandardized inocula for inoculating plates. If the organism is difficult to suspend directly into a smooth suspension, the growth method of preparing the inoculums should be used. However, the recommended organisms listed in this procedure all produce smooth suspensions with little difficulty. See the Clinical Laboratory Standards Institute document (3) for the growth procedure method for preparing the inoculums, if needed.

APPENDIX 5

(This information is taken from the “microbelibrary.org” website) ¹⁷

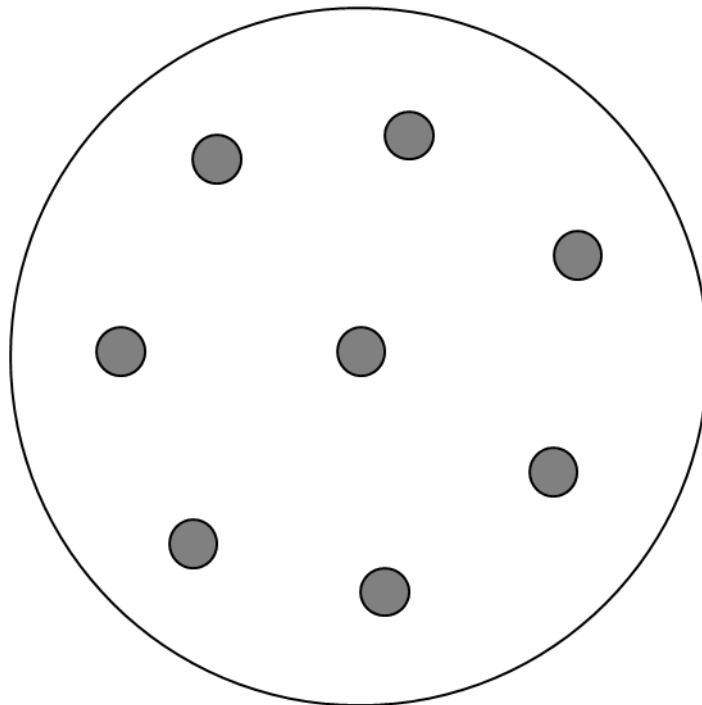
INCUBATION OF PLATES

A temperature range of $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ is required.

Note that temperatures above 35°C may not allow the detection of methicillin-resistant *Staphylococcus*.

Do not incubate plates in CO_2 as this will decrease the pH of the agar and result in errors due to incorrect pH of the media.

Results can be read after 18 hours of incubation unless you are testing *Staphylococcus* against oxacillin or vancomycin, or *Enterococcus* against vancomycin. Read the results for the other antimicrobial disks then reincubate the plate for a total of 24 hours before reporting vancomycin or oxacillin.



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REFERENCES

The references are in MLA style.

1. "Lemon" *Wikipedia: The Free Encyclopedia*. Wikimedia Foundation, Inc., 18 Nov. 2013. Web. 15 Nov. 2013. <<http://en.wikipedia.org/wiki/lemon>>.
- Cheung, Theresa. "15 Hidden Health Secrets of Lemons." *Beliefnet.com*. N.p., n.d. Web. 16 Nov. 2013. <<http://www.beliefnet.com/Health/Physical-Health/Hidden-Health-Secrets-of-Lemons.aspx>>.
- Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López, And J. Perez-Álvarez. "antibacterial activity of lemon, mandarin, grapefruit and essential oils." *wiley online library*. n.p., oct.-nov. 2008. web. 17 nov. 2013. <<http://onlinelibrary.wiley.com/doi/10.1111/j.1745-4565.2008.00131.x/full>>.
- Maruti J. Dhanavade, Chidamber B. Jalkute, Jai S. Ghosh and Kailash D. Sonawane. "Study Antimicrobial Activity of Lemon (Citrus Lemon L.) Peel Extract." *Study Antimicrobial Activity of Lemon (Citrus Lemon L.) Peel Extract* (2011): n. pag. © Maxwell Scientific Organization, 2011, 05 Aug. 2011. Web. 18 Nov. 2013. <<http://maxwellsci.com/print/bjpt/v2-119-122.pdf>>.
2. "Tantum Verde Ingredients." *Eipico.com*. N.p., n.d. Web. 15 Dec. 2013. <<http://www.eipico.com.eg/PRODUCT.ASP?id1=18&id2=0%20&id4=2>>.
3. "Andorex." *Drugs.com*. N.p., 1 Dec. 2013. Web. 17 Dec. 2013. <<http://www.drugs.com/international/andorex.html>>.
4. Okuda K, Adachi M, Iijima K. "The Efficacy of Antimicrobial Mouth Rinses in Oral Health Care." (1996): n. pag. Print.
5. Rogers A H (editor). (2008). *Molecular Oral Microbiology*. Caister Academic Press.
- Fine D H et al. (2008). "Molecular Windows into the Pathogenic Properties of *Aggregatibacter actinomycetemcomitans*". *Molecular Oral Microbiology*. Caister Academic Press.
- Ljungh A, Wadstrom T (editors) (2009). *Lactobacillus Molecular Biology: From Genomics to Probiotics*. Caister Academic Press.
- Okuda K, Adachi M, Iijima K. "The Efficacy of Antimicrobial Mouth Rinses in Oral Health Care." (1996): n. pag. Print.
- "Oral Microbiology." *Wikipedia. The Free Encyclopedia*. Wikimedia Foundation, 15 Dec. 2013. Web. 17 Dec. 2013. <http://en.wikipedia.org/wiki/Oral_microbiology>.
- Okuda K, Adachi M, Iijima K. "The Efficacy of Antimicrobial Mouth Rinses in Oral Health Care." (1996): n. pag. Print.

6. "Antibacterial Definition." *Biology-Online.org*. N.p., 3 Oct. 2005. Web. 17 Dec. 2013. <<http://www.biology-online.org/dictionary/Antibacterial>>.

"Antiseptic Definition." *Biology-Online.org*. N.p., 3 Oct. 2005. Web. 17 Dec. 2013. <<http://www.biology-online.org/dictionary/Antiseptic>>.
7. Hudzicki, Jan. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol." *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. N.p., 01 Apr. 2013. Web. 3 Nov. 2013. <<http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol>>.

"KIRBY-BAUER." *KIRBY-BAUER*. N.p., n.d. Web. 01 Nov. 2013. <<http://aminj.myweb.uga.edu/KIRBY-BAUER.html>>.

Rao, Tumulapalli Venkateswara. "Kirby Bauer Antibiotic Sensitivity Testing." *Kirby Bauer Antibiotic Sensitivity Testing*. N.p., 10 Mar. 2012. Web. 1 Nov. 2013. <<http://www.slideshare.net/doctorrao/kirby-bauer-antibiotic-sensitivity-testing>>.
8. "MUELLER HINTON AGAR."
Http://www.neogen.com/Acumedia/pdf/ProdInfo/7101_P1.pdf. Acumedia, 3 June 2011. Web. 1 Nov. 2013.
<http://www.neogen.com/Acumedia/pdf/ProdInfo/7101_P1.pdf>.

"Stuart-SI19." *Stuart Microtitre Plate Incubator SI19*. © Bibby Scientific Limited 2013, n.d. Web. 4 Nov. 2013. <<http://www.stuart-equipment.com/product.asp?dsl=73>>.
9. "Mean, Median, and Mode." *1: Mean, Median, and Mode*. N.p., n.d. Web. 5 Dec. 2013. <<http://edl.nova.edu/secure/stats/lesson1.htm>>.
10. "Standard Deviation." *Wikipedia. The Free Encyclopedia*. Wikimedia Foundation, 9 Dec. 2013. Web. 10 Dec. 2013.
<http://en.wikipedia.org/wiki/Standard_deviation>.
11. "Standard Error." *Wikipedia. The Free Encyclopedia*. Wikimedia Foundation, 16 Dec. 2013. Web. 5 Dec. 2013.
<http://en.wikipedia.org/wiki/Standard_error>.
12. Analysis of One-Way ANOVA
13. "Ingredients of Listerine Mouthwash in Cool Mint." *RSS. Listerine*, Dec. 2011. Web. 1 Dec. 2013. <<http://ingredientpal.com/ingredients-of-listerine-mouthwash-in-cool-mint>>.

14. "Tantum Verde Ingredients." *Tantum Verde*. N.p., n.d. Web. 2 Dec. 2013.
<http://www.polish-european-shopping.co.uk/product_detail.php?product_id=226>.
15. "Andorex." *Generic. Price of Andorex. Uses, Dosage and Description*. N.p., 2003.
Web. 17 Dec. 2013. <<http://www.igenericdrugs.com/?s=Andorex>>.
16. Rogers A H (editor). (2008). *Molecular Oral Microbiology*. Caister Academic Press.

Fine D H et al. (2008). "Molecular Windows into the Pathogenic Properties of *Aggregatibacter actinomycetemcomitans*". *Molecular Oral Microbiology*. Caister Academic Press.
Ljungh A, Wadstrom T (editors) (2009). *Lactobacillus Molecular Biology: From Genomics to Probiotics*. Caister Academic Press.

Okuda K, Adachi M, Iijima K. "The Efficacy of Antimicrobial Mouth Rinses in Oral Health Care." (1996): n. pag. Print.

"Oral Microbiology." *Wikipedia. The Free Encyclopedia*. Wikimedia Foundation, 15 Dec. 2013. Web. 17 Dec. 2013. <http://en.wikipedia.org/wiki/Oral_microbiology>.
17. Hudzicki, Jan. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol." *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. N.p., 01 Apr. 2013. Web. 3 Nov. 2013. <<http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol>>.

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