TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

EXTENDED ESSAY

*Research Question: Is there a significant mean difference among antiseptics used in medical institutions, in terms of their bactericidal effects on Staphylococous epidermidis in laboratory conditions evaluated by the utilization of the Quantitative Suspension Test Method**?***

Subject: Biology

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Abstract

The aim of this extended essay was to evaluate the bactericidal effects of different brands of antiseptics on the bacterium *Staphylococcus epidermidis.*

My research question was: "Is there a significant mean difference among antiseptics used in medical institutions, in terms of their bactericidal effects on *Staphylococous epidermidis* in laboratory conditions evaluated by the use of Quantitative Suspension Test Method?"

It was hypothesized that there would be a mean difference in between antiseptic properties of various agents. Since the antiseptics include different chemicals, they may have different bactericidal properties in terms of destroying microorganisms.

In order to test the hypothesis and to answer the research question, Quantiative Suspension Test Method was used. *S. epidermidis* colonies were cultivated onto Mueller Hinton Plate. In order to compare the data, a control trial was needed. In my research, *S. epidermidis* suspensions were prepared, by using dilutions and then mixed with different brands of antiseptics. After 60 seconds of preparation the solution was neutralized. Then the suspensions were spreaded on a TSA and incubated for 24 hours at 37°C. Following the incubation the colonies were taken out and counted by using a colored marker and multiplied by the dilution ratio in order to determine the actual number.

The results achieved turned out to prove that the hypothesis was right. The p value was 0.042 on single factor ANOVA. This value shows that there was a mean difference among the bactericidal effects of antiseptics. Monorapid turned out to be the most effective one while Aniosrub was the least. My study demonstrated that, different antiseptics have different bactericidal effects. It is possible that with time, bacteria may gain resistance to commercial antiseptics. I believe in order to prevent the spread of these infections especially in hospital settings it is mandatory to test the antiseptic properties of different antiseptics in regular time intervals.

Word count: 303

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I. Introduction:

My parents are both physicians. I regularly go to their hospital to visit them. During my visits I have seen hand antiseptics that are placed in the corner of each room and on corridors. I saw that my parents were frequently using them, especially following the contact with a patient. After several visits, I noticed the presence of a different brand of an antiseptic. Then I asked my parents "Which antiseptic is the most effective one? "Both of them said "Actually we did not study the real effects of them against bacteria, but the producer companies say that each of them effectively destroy the microorganisms. Meanwhile I heard news on the TV reporting that the hand antiseptics had powerful effects on hospital infections as well as on other infections. The news was focusing on the importance of keeping hands clean in order to avoid having influenza infections and to halt the spreading of hospital infections. It was mentioned that the main cause of spread was due to cross contamination of the microorganisms from patient to patient via doctor and nurses. That made me wonder which antiseptics have the best bactericidal effect on bacteria.

Keeping hands clean is one of the most important steps we can use to avoid getting sick and spreading microorganisms to people around us. Many diseases and conditions spread because of not washing hands with soap and available water. If soap and water are unavailable, it is the best for the individuals to use antiseptics.

Agents applied to living tissue to destroy or inhibit the growth of infectious microorganism are known as antiseptics. Antiseptics come in a variety: ointment, liquid, gel and spray. When using a hand-antiseptic; antiseptic should be placed to the palm of the

hand and hands should be rubbed together so the liquid cover all the surfaces of the hands and fingers and rubbed until they are dry.[1] It takes approximately 30 seconds for hands to get dry.

Companies who make commercial hand antiseptics use different chemicals and formulas. Among the major families of antiseptics, there are alcohol, phenols, chlorine, iodine compounds.[2]Alcohol based sanitizers are more effective at killing organisms than soaps and do not dry out hands as much. [3] Most commonly used are ethanol (60-90%),I propanol (60-70%) and 2-propanol/isopropanol (70-80%) or mixtures of these alcohols (70-80%). One study showed that alcohol based hand sanitizers are more effective at killing microorganisms than soaps and do not dry out hand as much. [4]

So I wanted to evaluate the efficiency of different types of hand antiseptics that contain various compounds in different percentages, which are mainly used in the hospitals in order to destroy microorganisms.

The aim of the present study was to compare the bactericidal effects of hand antiseptics used in medical institutions. The term bactericidal refers to any agent that directly induces the death of bacterium, through disrupting its enzyme mechanisms or else. As the test organism I decided to use *Staphylococcus epidermidis,* (which is a gram-positive bacteria) in order to limit the subject of my extended essay. This bacterium is gram positive and is one the most common skin bacterium found on the human skin. Because it is a

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[1] *Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule* **74** (56). United States Federal Food and Drug Administration. March 2009. pp. 12613–12617.
[2] http://www.ehow.com/info_7865502_chemicals-antiseptic-soap.html
[3] http://www.uoguelph.ca/foodsafetynetwork/alcohol-based-hand-sanitizers
[4] http://www.uoguelph.ca/foodsafetynetwork/alcohol-based-hand-sanitizers

common microorganism; it is usually selected for research purposes in laboratory conditions. My research question is,

Is there a significant mean difference among antiseptics used in medical institutions**, in terms of their bactericidal effects on *Staphylococous epidermidis* in laboratory conditions evaluated by the utilization of the Quantitative Suspension Test Method?**

II. Hypothesis:

Antiseptics, agents applied to living tissue to destroy or inhibit the growth of infectious microorganism are known as antiseptics. Antiseptics are usually used in hospitals or on other facilities. In order to halt the spreading of infections antiseptics are widely used in hospitals as well as in our daily lives. However, with time the chemicals can lose their bactericidal potency due to time or heat, and need to be checked periodically to ascertain their potency and efficiency. Antiseptics usually contain alcohol, phenols, and chlorine, iodine compounds to inhibit the growth of bacteria.[5] Commercial companies usually produce ethanol based and propanol based antiseptics. To be licensed as an antiseptic; antiseptic should be killing 99.9% of the bacteria on hands, 30 seconds after application and 99.999% to 99.999% in one minute.[6] Since all the commercial antiseptics are licensed, we do not expect to see a huge difference in between the antiseptics. Since the chemical properties of antiseptics can vary, it can be foreseen that there may be a difference among the effects of antiseptics. In a study conducted by Jokar and Mohebbi, it was reported that isopropanol based antiseptics had a higher disinfectant effect than ethanol based ones on instruments as well as on skin surface.[7] According to this data, it was hypothesized that there will be a significant mean difference in terms of destroying bacteria in 60 seconds in between different brands of hand antiseptics by the utilization of the Quantitative Suspension Test Method.

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[5] http://www.ehow.com/info_7865502_chemicals-antiseptic-soap.html
[6] http://www.cdc.gov/handwashing/
[7]http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2807625/(Z)

III. Method Development /Planning

While I was trying to find a logical and an appropriate method for my research question, which was, Is there a significant mean difference among antiseptics used in medical institutions, in terms of their bactericidal effects on *S. epidermidis* in laboratory conditions? I confronted with some problems.

A. Bacterium to Use

First of all, my first aim was to determine the appropriate bacterium to be used in the experiment. I needed a bacterium, which can be easily found in the human skin and easily obtained. I carried out an evaluation and realized that *S. epidermidis* may be the ideal bacterium for me to use in my research. First, it was one of the most common bacterium found on the human epithelia and also it is not pathogenic[8] (in the safety class of I at ATCC). This means that it is safe for me to work on it. During my research I decided to use the 2nd subculture of the bacteria, in order for the bacteria to be more active. Additionally, the bacteria that I'll be using will be better than the previous generation since the offspring's of the bacteria has the chance to survive longer. For preparation procedure of 2nd subculture see appendix 2.

B. Antiseptics to Use

[8]Levinson, W. (2010). *Review of Medical Microbiology and Immunology* (11th ed.). pp. 94–99.

Secondly, my aim was to determine which antiseptics should I be using in my experiment? I made an evaluation and saw that the ethanol based antiseptics and propanol based antiseptics were the most commonly preferred ones Therefore I decided to use "Monochol","Monorapid","Steriderm" and "Aniosrub" brands, which are used frequently in hospital settings as well as on other laboratory conditions. The chemicals that the antiseptics are made of can be seen in appendix 1.

After deciding on the antiseptics to work on, I faced up with the problem in choosing the method of my research. I needed a method that would let me to get a precise data with less random errors. Because my research question is based on the comparison of bactericidal effects of different antiseptics; the method I choose should be able to demonstrate the bactericidal properties of different agents. For the procedure, I needed a medium where I would be able to cultivate the bacteria. I made an evaluation and saw that Mueller Hinton agar would be the best one because it is a culture that enables different kind of bacteria to grow on.[9]

C. The Control Trial

Since my aim was to evaluate the bactericidal properties of solutions first I need to find out how the bacterial colonies reproduce in a proper medium with out the presence of any antiseptic. I need a medium where I can obtain the 2nd subculture. Therefore all colonies cultivated here would have a high chance of growth. In order to count the amount of bacteria following the procedure a dilution is needed in order to be accurate. Without the dilution procedure, there may be a large number of bacteria which would made it impossible to count.

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[9]Atlas, R.M. (2004). *Handbook of Microbiological Media*. London: CRC Press. p. 1226. ISBN 0-8493-1818-1.

I used an easy method to find the number of colonies I needed while I was cultivating the bacterium. I decided that the dilution method would be appropriate. If I dilute the bacterial solution I would have the chance of counting the bacterial colonies easily. Consequently; multiplying it with the rate of dilution I would be able to get the actual number of bacteria that is present in the TSA.

Since I'll be planning to dilute the bacterial solution, I needed to choose a diluent that I can use in my research. According to European Union (EU) countries protocol (prEN 13727: April 2009) in order to determine the effect of antiseptic; a specific diluent needs to be used. The materials that make up the diluent is presented in the appendix 3. Then I had to decide on the amount of bacteria that I'll be using on trials. According to the European Union countries protocols the number of bacterium in a suspension should be between 1.5*10^8- 5.0*10^8 cfu/ml. To determine this value a spectrophotometer should be present. In order to get an average value I decided to adjust the value to +0.03 OD. By this way I would be able to determine the amount of bacteria which I will be using in each trial.

To do this, I'll need to make a solution that both contain the diluent and the bacteria. Throughout my trials I'll be naming this solution as the test suspension. Since I needed a small amount of bacteria to find an appropriate number of colonies, I needed to have a test solution that is small in volume. I decided to use 2 ml of diluent and some bacteria in order to reach the value of +0.03 OD. I'll be placing the diluent and the bacteria into a test tube so that they can mix homogeneously. To dilute the test solution I decided to take 100µl of 2ml test solution. By this way I'll be diluting the solution in 1/50=0.02. I'll be adding 100µl of test solution into 4900 µl. I chose the amount of solution as 4900µl because when I'll be once taking 100µl from the solution I'll have the chance to dilute it in the ratio of 1/50. So I

decided to repeat the dilution process for three times. In this way I would be able to dilute the solution in ratio of 1/125000 .

Then I needed a growth medium where I can grow the bacterial colonies after the test. This medium has to have enough nutrients for bacterial growth. So, I chose TSA[10]because it contains soybean which enables the growth of bacteria easily.

Following the dilution process I should place the test solution in a TSA* with the aid of a glass loop; in order to distribute the test solution homogeneously. In order to determine how the bacteria colonies reproduce; an ideal environment was needed. I decided to place them into an incubator, which is set to 37 °C , which would enable the enzymes of bacteria to work properly. In order for the bacterial colonies to grow in an appropriate amount they have to be stored in the incubator at least for 24 hours.

Following the incubation process I would have the chance to count the number of colonies formed. To facilitate the process I decided to count the colonies with a colored marker by starting from the bottom up to the top. Then I would be able to determine the number of colonies that are present in the diluted test solution. In order to obtain the actual number of colonies, I should be multiplying the number of bacteria with the dilution ratio which in this case is 1.25*10^6.

After deciding on the method to determine the number of bacteria present in the test solution, I needed to find a method, which would evaluate the effect of the antiseptic on the bacteria. Since I will be using the antiseptic I should be using the test solution once more.

D. Mixing Antiseptics With Test Suspension

* Tryptic Soy Agar

Since bacteria have the chance to kill of %99.999 to %99.99999 within five minutes [11]I need to look at the effect in a specific time period, therefore I decided to take the time span as 60 seconds.

When I placed the antiseptic in the test tube with the test solution I need a neutralizer, which will halt the effects on the antiseptics. According to the Quantitative Suspension Test Method there is a determined neutralizer for use. Hard water needs to be used in order to achieve neutralization. (see appendix 2)So I thought that the volumes I will be using should be similar to the control trial and decided to take 100µl of test suspension and placed it in a glass tube. I added 900µl antiseptic over it, and waited for a minute. Then 100µl of the prepared solution was placed to the tube that contains 800µl neutralizer and 100µl hard water. A minute is needed for the neutralizer to be effective. Then, like in the control trial I should take 100µl and add it on a TSA and place it in the incubator for 24 hours.

Afterwards I should count the number of bacteria as in the control trial. In this trial I diluted in a 0.01 ratio, so I should be multiplying the number with 100. In order to get accurate results I should repeat the trial for five times for each antiseptic to determine the number of bacteria present in the culture without the presence of any antiseptic interaction.

After deciding on the appropriate method I needed a laboratory where I would conduct my research. I made an evaluation and found that Gazi University Medical Faculty Hospital's Microbiology Department have the technical capability and experience to conduct my study.

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[11]http://www.cdc.gov/handwashing/

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IV. Method

Quantitative Suspension Test Method

Quantitative suspension test method (prEN13727.April 2009) is a designated protocol used in European Union countries to determine the bactericidal activity of a chemical disinfectant or an antiseptic against *Staphylococcus epidermidis* .

A) Preparation of the Test Suspension

1.Put on the gloves, and disinfect the table so that no bacteria would contaminate the medium.

2. Take the Mueller Hilton Agar that contains the 2nd bacteria subculture. (see appendix 3)

3. By using the loop take 2 colonies and place it in a test tube.

4. Then with automatic pipette take 2ml of diluent and place it into the test tube.

5.Place the tube in the spectrophotometer, and make sure the value is +0.03 OD. If not adjust it to this value by either adding diluent or adding some bacteria.

6.This way the solution's absorption value will be in the range $1.5 \times 10^8 - 5.0 \times 10^8$ cfu/ml. (Colony Forming Unit/ml)

6.The test suspension is prepared.

B) Estimating The Number of Bacteria Present (CFU) in The Test Suspension

1. 100µl of test suspension is taken from the 2 ml suspension by using a 100µl automatic pipette.

2.Then it is placed in a test tube which contains 4900µl of diluent.

3. By this step, the test solution is 1/50 diluted.

4. The steps between 2-4 are repeated for two more times.

5.This way the test suspension would be diluted for 3 times.

6. After the serial dilution 100µl of test suspension is taken and poured on a TSA

7. By the help of a glass spreader the suspension is spreaded onto the TSA in clockwise direction for two times.

8. Afterwards the TSA is placed in an incubator, which is set to 37°C ±0.5°C

9. The bacterial culture is kept in the incubator for 24 hours.

10. After the incubation process, a colored marker is taken and the white bacterial colonies are counted starting from bottom going to the top.

11.The number of bacteria alive in the diluted suspension is determined.

12.In order to obtain the number of bacteria in the test suspension the number of bacteria is multiplied with the dilution ratio of $1.25 * 10^6$

13.The number of bacteria present in the test solution is determined.

C) Testing of Different Antiseptics By Quantitative Suspension Test Method

1) 100µl of test suspension is prepared in the same steps as in A.
2) It is placed in a test tube.
3) 900µl of antiseptics is added to the test tube in order to mix it with the test suspension.
4) Wait for 60 seconds for the antiseptic and the test suspension to interact at a room temperature of 24°C
5) Following the interaction, 100µl of the mix solution is taken by 100µl pipette.

6) It is placed in a test tube where 800µl neutralizer and 100µl hard water are present.
7) Wait for 5 minutes for the neutralizer and the hard water to react against the antiseptic.
8) 100µl of the solution is taken by using a 100µl pipette and placed on a TSA
9) By using a glass spreader the suspension is spreaded in the TSA in a clockwise direction for 2 times.
10) The bacterial culture is obtained
11) Then the culture is placed in an incubator which is set to 37°C and kept for 24 hours.
12) After the incubation process, the number of bacteria on the agar is counted from bottom to the top by using a colored marker.
13)The number of bacteria present in the diluted solution is determined.
14)To calculate the number of bacteria in the solution the number of bacteria present in the diluted solution is multiplied with dilution ratio of 10^2.
15) The steps 1-14 are repeated for each antiseptic.
16)The steps 1-15 are repeated for five times.

V. Data Analysis

Experiment	Trials	Number of Colonies Formed After Incubation
Control	1	287
	2	283
	3	282
	4	286
	5	293

Table 1.1: Table Showing the Number of Colonies Formed on Tryptic Soy Agar in Control Experiment in 24 Hours at 37°C

When the data from table 1.1 used and the number of colonies is multiplied by $1.25 * 10^6$ CFU/ml is obtained for each trial

Experiment	Trials	CFU/ml	Mean (CFU/ml)
Control	1	3.58 *10^8	3.53*10^8
	2	3.53 *10^8	
	3	3.52 *10^8	
	4	3.57 *10^8	
	5	3.66 * 10^8	

Table 1.2: Number of total colonies obtained in the control experiment, Dilution Factor is 1.25*10^6

Trials	Number of Colonies Formed By Diluted Suspension in 1 Minute			
	Monochol	Steriderm	Aniosrub	Monorapid
1	1	2	2	0
2	2	3	3	0
3	1	2	1	2
4	0	1	2	1
5	1	1	4	1

Table 2.1: Raw Data Table Showing The Number of Colonies Formed After Performing Quantitative Suspension Test Method in the Diluted Solution following the mixture of antiseptics with Test Suspension

Descriptive Statistics

1) Mean

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

$\bar{x}$: Number of colonies
n: number of trials
2)Variance:

$$\mathbf{Var}(X) = \frac{1}{n}\sum_{i=1}^{n}(x_i - \mu)^2.$$

n: Number of trials
x_i : Number of colonies
Var(x): Variance
μ : Mean

3) Standard Deviation:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2}.$$

$\bar{x}$: Mean
x_i : Number of colonies
σ: Standard Deviation
n: Number of trials

4) Standard Error:

$$\mathrm{SD}_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

σ: Standard Deviatio
n: Number of Trials

	Monochol	*Steriderm*	*Aniosrub*	*Monorapid*
Mean	1.0	1.8	2.4	0.8
Standard Error	0.3	0.4	0.5	0.4
Standard Deviation	0.7	0.8	1.1	0.8
Count	5	5	5	5
Confidence Level (95,0%)	0.9	1.0	1.4	1.0
Variance	0.5	0.7	1.3	0.7

Table 2.2: Calculated Data Table Showing Descriptive Statistics of Number of Colonies Formed By different antiseptics

Mean Number of Colonies Formed in the Antiseptic-BacteriaSuspension (CFU/ml)			
Monocohol	Monorapid	Steriderm	Aniosrub
100	80	180	240

Table 2.3: Calculated Data Table Showing the Number of Colonies Formed in the Antiseptic-Bacteria Suspension (CFU/ml)

The evaluation method of CFU can be seen on appendix 4.

ANOVA:

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.2	3	2.733333333	3.416666667	0.042965426	3.238871517
Within Groups	12.8	16	0.8			
Total	21	19				

Table 2.4: ANOVA Results of Calculated Values of Number of colonies formed from Table 2.1

Bar graph:

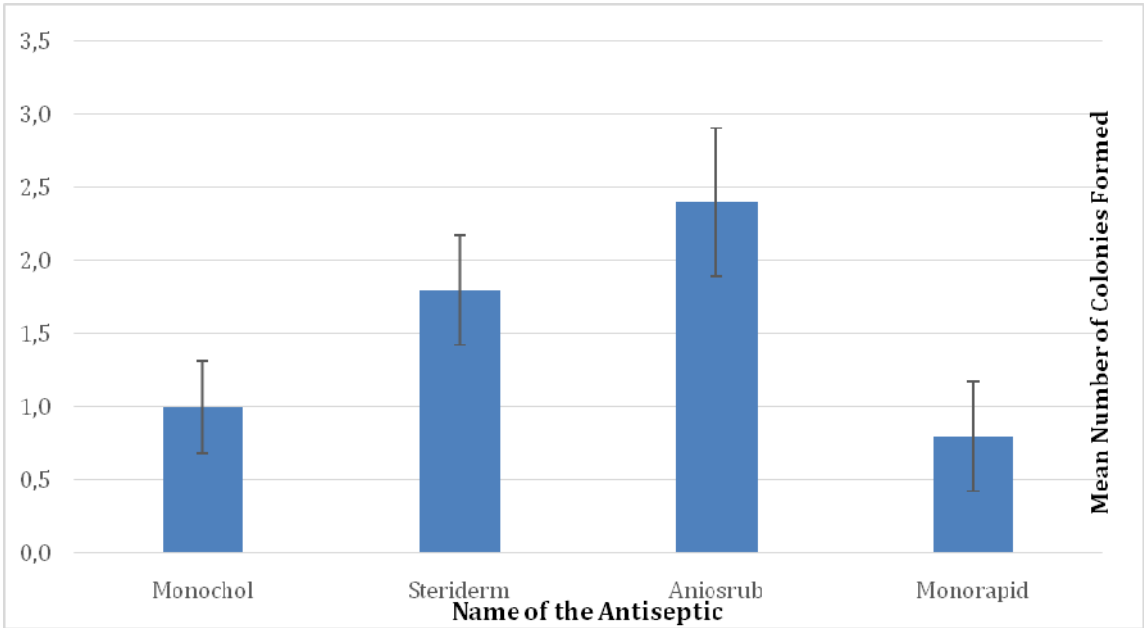

Figure 1 Graph Showing Mean Number of *S.epidermidis* colonies mixed with different brands of antiseptics following 24°C incubation on TSA plates at 37°C

VI. Evaluation

The aim of this study was to find an answer to my research question: **Is there a significant mean difference among antiseptics used in medical institutions, in terms of their bactericidal effects on *Staphylococous epidermidis*in laboratory conditions using Quantitative Suspension Test Method?** It was hypothesized that there would not be a mean difference among antiseptics because the commercial antiseptics should have a specific standard in order to be licensed.

To begin with, my control trial had the mean CFU/ml as 3.53*10^8. According to Quantitative Suspension Test Method the value must be between 1.5*10^8-5.0*10^8. So when the mean value is compared to the determined interval it can be seen that the trials including the number of colonies formed without the presence of an antiseptic were successful**.** This means that there was not any contamination of different bacteria in the test suspension other than *S.epidermidis.*

At the end of the trials, for Monorapid the mean number of colonies formed in the diluted solution was 0.7, for Monochol, 1.0, for Steriderm 1.8 and for Aniosrub 2.4. When comparing the data with the hypothesis, it can be concluded that the hypothesis was correct. It can be seen that there is a difference in the mean number of colonies formed. Since the number of colonies formed by Monorapid is less than the e rest, it can be accepted as the most effective one while the Aniosrub is as the least.

When considered according to the criteria of Quantitative Suspension Test Method all antiseptics were accepted as effective at the end. According to the Quantitative Suspension Test Method evaluation criteria, there must be a difference of 10^5. When we compare the table 2.4 and the 1.2 there is a difference of more than 10^5. All the antiseptics

tested on the trials were successful according to QSTM criteria. So, the antiseptics are appropriate for everyday use.

When the Table 2.3 is analyzed thoroughly it can be seen that on the standard error section, the standard error values are smaller than the mean values. However, Standard Error values are significant because the values are relatively close to the mean values. This may mean that there may have been some errors that may have changed the results. As seen in Figure 1; the error bars have high intervals when compared to the mean number of colonies formed in the diluted solution. When the standard deviation values are analyzed; it is obvious that there is a significant difference especially in between Monocohol and Monorapid. Since the mean values and the standard deviation are close relation to each other it can be concluded that once again that there must be some problems that could have affected the results of the experiment.

According to the null hypothesis, there would not be any difference among the data if the α value were less than 0.05. The α was smaller than 0.05, so my null hypothesis was rejected. This shows that there is a significant difference in between each and every antiseptic group.

When ANOVA table is analyzed we see that some standard errors are present. Standard Erorr values of each antiseptic are similar, therefore the errors in the experiment may have effected the outcome of the experiment.

The various compositions of chemicals in each antiseptic may be the reason for this discrepancy. The most effective antiseptic according to our results was Monorapid. Monorapid contains %70 of ispropanol while Monocohol contains %45 of isopropanol. The percentage of isopropanol present on the solution can have an affect on its antibacterial

properties. Steriderm and Aniosrub both contain %70 of ethanol. However, Steriderm has a stronger antiseptic activity, because it contains additional agents other than alcohol which may make it to be more effective. Antiseptics that contain isopropanol as their main component are more effective than the ones who have ethanol as their main component.

During my research, there were no unexpected incidents that may have affected the outcome of my experiment. However, when going through over the data that I obtained; I realized that there might be some errors that may have affected the results.

1. There may have been contamination of the medium. In order to solve this problem more attention should be paid to the surroundings that the study is conducted on.
2. The bacteria that were used were 2nd subculture. There might be a chance that they were not strong enough. So, if I have used 3rd subculture, I would have the chance to increase the possibility of having more active bacteria.
3. Only one type of bacteria was used. There are different types of bacteria that inhabit on the human skin. If different types of bacteria were examined we would have the chance to evaluate the effects of antiseptics on different microorganism.
4. Different types of measuring methods such as spectrophotometric method may have been used.
5. To make a better comparison; different antiseptics with different properties should be used.

6. In order to obtain a more precise number of bacteria; the dilution ratio should be decreased.
7. Different types of bacteria should be used to evaluate the effects of antiseptics on human skin.

VII. Conclusion

My research question "***Is there a significant mean difference among antiseptics used in medical institutions, in terms of their bactericidal effects on Staphylococous epidermidis in laboratory conditions using Quantitative Suspension Test Method?"***is answered with the results that I have obtained from my trials. There is a significant mean difference in between bactericidal properties of different antiseptics. In my study I have seen that the antiseptics that are more concentrated with isopropanol are likely to be more effective than the antiseptics that include ethanol as their main component. Since each antiseptic bactericidal property in Quantitative Suspension Test Method interval, they all can be regarded as successful. Therefore, I considered my study as being accurate.

The main reason why I have chosen to do my extended essay particularly on this topic was to determine the difference in between bactericidal properties of different antiseptics used in medical instutions. However, the topic goes beyond my scope and capabilities. Some studies have been conducted before, which were similar to mine. The studies I have come across were usually drawing attention to the comparison of disinfectants rather than the antiseptics.

People all around the world use antiseptics every day in order to get rid off the bacteria on their hands. Individuals have different choices for antiseptics. The antiseptic market is growing day by day and because each antiseptic has a different composition is hard to conclude which one is superior and recommended for general use.

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IX. Appendices

A)Appendix 1

Materials

* 1000 ml Manorapid(Antiseptica chem.pharm.Produckte GmbH – Germany)
70%(v/v)iso-propanol, 1,3 Butanediol, PEG-75,
Lanoline,water,parfume

*1000 ml Manochol(Gül Bioloji Labaratuarları Sanayi Tic. Limited – Turkey)
Ethyl alcohol l 96%(64-17-5) 45%, Isopropyl alcohol (67-30-0) 25%, odor
Component, moisturizer, de-ioniated water

*1000 ml Anioscrub (ANIOS Laboratoires- France)
%70 Ethanol (700 mg/g i.e 755ml/L-CAS No 64-17-5)

*1000 ml Steriderm (Kimpa İlaç Lab ve Tic. LTD. Şt- Turkey.)
70% h/h ethanol(CAS:64-17-5),Chlorhexidine digluconate (CAS:18472-51-0),
1-3 butandiol

B) Appendix II

Solutions Used In Quantitative Suspension Test

Diluents:

-Tryptone 1.0 g
-Sodium cloride 8.5 g
-Distilled water 1000ml

Neutralizer:

-Tween 80 30 g
-Saponin 30 g
-L- histidine 1g
-Lecithin 3 g
-Nathiosulfate 5 g
-Diluents 1000ml

Hard Water

Solution A
$MgCl_2$ 19.84 g

$CaCl_2$ 46.24 g.

Distilled water 1000ml

Sterilized in autoclave, and can be stored in refrigerator for a month.
Solution B

$NaHCO_3$ 35.02 g

Distilled water 1000 ml
Sterilized with filtration and can be stored in refrigerator for a week.

Hard Water Working Solution

Solution A 6 ml
Solution B 8ml
Distilled water 1000 ml

pH should be 7. Should be freshly prepared for every test and consumed with in 1-2 hour of preparation.

C) Appendix III

Preparation of Tryptic soy agar

Contents of the medium:

Casein peptone 15g/L
Soybean peptone 5 g/L
Sodium chloride 5 g/L
Agar 15 g/L
pH 7.3±0.2

40 gr of powdered medium and 960 ml of distilled water are sterilized in autoclave at 1 atmospheric pressure on 121 °C for 15 minutes..

Samples each containing 25 ml of mixture are poured to a sterile Petri dish so that the thickness is 4 mm when became hard, and stored at 4 ºC on refrigerator until consumed.

Preparation of Mueller Hinton Agar

Contents of the medium

Cattle infusion 300 g/L
Casein acid hydrolysate 17.5 g/L
Starch 1.50 g/L
Agar 17.00g/L
pH 7.3±0.2

38 gr of powdered medium and 960 ml of distilled water are sterilized in autoclave at 1 atmospheric pressure on 121 °C for 15 minutes..

Samples each containing 25 ml of mixture , poured to a sterile Petri dish so that the thickness is 4 mm when became hard, and stored at 4°C on refrigerator until consumed.

Mandatory Contact Interval

Mandatory contact interval for surface disinfection is 5 or 60 minutes, and 60 seconds for hygienic and 5 minutes for surgical hand washing. If contaminated material of the patient is present on the surface ; proposed contact interval for disinfection is 5 minutes. If the

product would be used in clean conditions ; in those circumstances 60 minutes would be used as a mandatory contact interval

Water Used In Experiments

Water should be clean distilled water, not demineralized water.

Product Test Solutions

These disinfectants are used according to the concentration proposed by the manufacturer.

Bacterial Suspensions

Test suspension; bacterial suspension is the suspension used to determine the bactericidal activity of disinfectants.
Stock cultures were obtained by the microorganisms passaged to Mueller Hinton plaques which were stored at -20°C . Second subcultures were obtained from this stock cultures following the re-passage of cultures to Mueller Hinton plaques. 2nd subcultures of the bacteria were used in our experiment.

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D) Appendix 4.

Colony Forming Unit(FCU) Evaluation

In the test method bacterial suspension (N) is calculated as $1,5x10^8$-$5x10^8$ CFU/ml and this suspension is used in the experiment. According to the test procedure 100 µl of liquid from the suspension is processed with 900 µl of disinfectant. During this stage number of bacteria in the suspension is decreased by a ratio of ten because of dilution; while the Colony Forming Unit (CFU) in the suspension is between $1,5x10^7$-$5x10^7$. When 100 µl of this mixture is added to 800 µl neutralizer and 100 µl hard water, it would be diluted 10 times more. 100 µl from this final solution when inoculated to the plaques it is re-diluted as 10. The numbers of colonies that are grown in the plaques are multiplied by the dilution ratio of 100. The bactericidal activity of a disinfectant is considered as effective; if there is a decrease of 10^{-5} or more on the number of colony forming unit (CFU)