TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

How much time is needed to sterile objects from Escherichia coli DH 5 alpha with Ultraviolet light exposure?

Biology Extended Essay

Supervisor: Vidad ELEMİN ŞEMŞİR

Name of the Candidate: Öykü HAN

Candidate Number: D1129048

Word Count:3999

Table of Contents

1. ABSTRACT

In our daily lives, we sterilize lots of tools like manicure tools and aquarium equipments. Sterilization with Ultraviolet light is a way of getting rid of pathogenic organisms among other ways. By UV sterilization, the nucleic acids of the pathogenic organism's genetic material are damaged. This damage prevents the replication of the organisms and in some cases the UV light also kills the organisms. But the organisms should be under the UV light for a certain time interval for the damage to be done. This time interval changes from organism to organism. In this paper the time interval needed for the tools to be sterile from the pathogenic bacteria, *Escherichia coli DH alpha 5*, is investigated. Because E.coli is a type of bacteria, that is capable of living in both the tools that are sterilized with UV light and the humans. Therefore, the bacteria can be transmitted to the humans by those tools and they can cause illnesses. So it is important for us to use sterile tools to avoid illnesses.

The experiment that is explained in this paper searches the time limit for when the tools we use are sterile for use. It was found that the tools that contain E.coli bacteria should be exposed to the Ultraviolet light for at the very least 60 minutes. But even 60 minutes of UV light exposure isn't sufficient for sterilization because there are still bacteria remaining after the exposure. If the bacteria are left under the UV light below 60 minutes, the tools aren't considered sterile and it is recommended that tools containing E.coli after UV exposure shouldn't be used.

Word count: 271

2. INTRODUCTION

I got the idea of my extended essay when I realized that Ultraviolet Sterilizers were used frequently in our daily lives, such as in sterilizing water,air,food,aquariums or manicure tools. As I realized how much UV light was used to sterilize lots of things, I wondered if it was effective enough to be reliable in sterilizing those tools. After some research, I found out that sterilizing with UV light wasn't very efficient. In addition, I discovered that sterilizing the tools that are used in beauty centers with UV light was mandatory by law in Turkey.[1] So I wondered if UV sterilization is sufficient or some other way of sterilization should be used. Thus,I was really interested in UV light's effect on sterilizing the bacteria and decided to research it on my extended essay for Biology.

"The ultraviolet light kills microorganisms by alterations in the DNA strains, thereby leading to disruption of the genetic material. As a result, the cell becomes inactivated and is no longer able to reproduce. All that the ultraviolet radiation does is damage the cellular DNA and prevent multiplication of cells" [2] Also, for the UV light to be effective on microorganisms there shouldn't be any layer of matter separating the UV light and the object. In addition, the UV light has to be exposed to the object for a certain time interval for the object to be sterile. (see appendix-8) This time differs for different bacteria. Some bacteria are affected by the UV light easier than others. Some aren't affected at all.[3] I choose one of the bacteria that are affected by UV light which is named "*Escherichia coli DH alpha 5*."

[1] "Güzellik va Estetik Amaçlı Sağlık Kurumlarımdaki Yönetmelik" Web. 25.09.2011 <http://www.alomaliye.com/guzellik_estetik_yonetmelik.htm>

[2] Aruna Murthy Anaparti. "How Does UV Light Act As a Sterilizing Agent?" Web. 14.09.2011 <http://www.ehow.com/about_6522834_uv-light-act-sterilizing-agent_.html#ixzz1XZbtBvlV >

[3] Angela Herrie. "UV Lights Effect on Bacteria" Web.14.09.2011 <http://www.ehow.com/facts_5730797_uv-lights-effect-bacteria.html>

I decided to observe the effect of UV light on bacteria by doing an experiment about how much time was needed for bacteria to be under the UV light until the bacteria were ineffective on human health.

So, my extended essay project's research question will be as: "How does being exposed to the Ultraviolet light for different lengths of times, which are 5,10,15,20,25,30,35,40,45,60 minutes, affect the number of *Escherichia coli DH 5 alpha* colonies formed?" This paper will show how to prepare and perform an experiment about this and it will discuss the results.

3. HYPOTHESIS

"Commonly known as E. coli, this class of bacteria usually colonizes the human gastrointestinal system within 48 hours of birth. " [4] It is known to cause bloody or non-bloody diarrhea, abdominal cramps, vomiting and sometimes fever. [5] In addition, E.coli can live between 7ºC to 46ºC. Thus, it can survive in normal human temperature.[6]

Sterilization with Ultraviolet light is believed to lessen the E.coli population in water,food or objects that we frequently use. But it is also stated that the bacteria should be under the UV light for at least 30 minutes for it to be sterile.[7] *"Ultraviolet lamps are used to sterilize workspaces and tools used in microbiology laboratories and health care facilities. UV light at germicidal wavelengths (185 nm and 265 nm) causes adjacent thymine molecules on DNA to dimerize, thereby inhibiting DNA replication (even though the organism may not be killed outright, it will not be able to reproduce). However, since microorganisms can be shielded from ultraviolet light in fissures, cracks and shaded areas, UV lamps should only be used as a supplement to other sterilization techniques."* [8]

So, it can be hypothesized that "*the Ultraviolet light exposure wouldn't be effective on Escherichia coli DH 5 alpha for at least 45 minutes*(see appendix-12), *then the number of the E.coli will decrease as the exposure time increases since the light can properly damage the DNA of bacteria."*

[4]Andrew Hazleton. ""What Types of Bacteria Do UV Destroy?" Web. 13.09.2011<http://www.ehow.com/list_6854450_types-do-uv-lights-destroy_.html >
[5] "Escherichia coli - Characteristics" Web. 16.09.2011<http://www.hi-tm.com/1908/SECTION-2-D-1908.pdf >
[6] Minh Thanh Nguyen. "The effect of temperature on the growth of the bacteria Escherichia coli DH5α" Web. 30.09.2011 <http://homepages.stmartin.edu/fac_staff/molney/website/SMU%20Bio%20Journal/Nguyen%202006.pdf >
[7] Barbara A. Hamkalo and P. A. Swenson. "Effects of Ultraviolet Radiation on Respiration and Growth in Radiation-resistant and Radiation-sensitive Strains of Escherichia coli"Web.21.10.2011<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC250099/ >
[8] Kenneth Todar. "Control of Microbial Growth (page 2)" Web. 05.12.2011 <http://www.textbookofbacteriology.net/control_2.html >

4. METHOD DEVELOPMENT AND PLANNING

I learned the importance of the method development while doing the experiment because I had to perform 3 different methods until the best method was found and the results were logical. That's why, all the factors affecting the experiment should be considered to prevent finding inaccurate data.

Firstly, the best bacteria to work with should be found. The bacteria should consist of several qualities. It should be safe to use to avoid serious diseases or complications for the performer. Then, the bacteria chosen should be well-known so information about it can be found easily. In addition, it has to be easy to buy and use. Furthermore, the bacteria should be able to live in room conditions, which is the experiment's medium. Also, the bacteria should be able to live on the human skin and tools that humans came into contact with. Because the reason in investigating the effect of UV on bacteria is to find the time needed for UV to sterilize the bacteria, which can be transferred from those tools. The best bacteria strain that have all of these properties is *Escherichia coli DH 5 alpha.* Therefore, E.coli's properties should be investigated and the optimum conditions for the bacteria should be kept stable during the experiment. (see appendix-1)

Then I had to decide on the type of the agar and I choose Luria Broth (LB agar) for growing E.coli. The main reasons are that it is safe to use and used widely to grow E.coli. Also, it is easy to prepare. In addition, LB agar hardly allowed any other kind of bacteria to grow over E.coli which helped me to decrease errors. If some other brand of bacteria was to grow in it then it would cause the nutrient that is all supposed to be for E.coli, to split up and probably cause a competition between two different bacteria, one being E.coli.[9]

[9]"All About Agar" Web. 16.11.2011<http://www.sciencebuddies.org/science-fair-projects/project_ideas/MicroBio_Agar.shtml >

The next step should be to determine the control and the experiment groups so that the best time interval is found to compare the effect of UV light on the growth of E.coli bacteria. From my research, I found out that the bacteria should be under the UV light for at least 30 minutes.[10] [11] It takes approximately that much time for the bacteria's DNA to be damaged enough so that the bacteria won't be able to reproduce and for the bacteria to even die because of the severe damage to its DNA. But according to the manual of the ProSteril UV light cabin, the tools should be exposed to the UV light for 45 minutes. So, I decided to observe at least one group of bacteria after it was exposed to UV light for 45 minutes. For the rest of the bacteria, I decided that some of them should be left under the UV light longer and some of them shorter than 45 minutes. The time intervals for UV exposure that I selected were: 5, 10, 15, 20, 25, 30, 35, 40, 45, 60 minutes and control group of bacteria that wasn't exposed to UV light so it could be compared with the other groups.

Next, I researched the best possible way to expose the bacteria to the UV light. If there is anything between the UV light rays and the E.coli, then the bacteria won't be affected. So plating the bacteria on the agar seems to be the best way, since the surface area would be bigger and the probability of the bacteria getting the UV light increases. However, I wanted all of the experiment and the control groups to be under the same conditions. So I wanted to expose them to the UV light beginning from the same time to get any unwanted affect of the surroundings to a minimum. But the UV light source cabin doesn't have enough space in it to fit all of the first trials of the experiment and the control groups if the bacteria are plated on the agar first. Then I considered the second best option which is to put the bacteria under the UV light before plating them, when they are still in LB broth. Because in this way, although some of the bacteria would be unaffected by the Ultraviolet light since they are too deep in the liquid, a good portion of the bacteria would be directly affected.

[10] Angela Herrie. "UV Lights Effect on Bacteria" Web.14.09.2011 <http://www.ehow.com/facts_5730797_uv-lights-effect-bacteria.html>

[11] Barbara A. Hamkalo and P. A. Swenson. "Effects of Ultraviolet Radiation on Respiration and Growth in Radiation-resistant and Radiation-sensitive Strains of Escherichia coli"Web.21.10.2011<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC250099/ >

But this will cause uncertainties. In addition, all the first trials would be able to fit in the UV light cabin and they would even be directly under the UV light source with the same angle,90º, and the same distance, 21.5±0.1 cm.

The next step is spread plating the bacteria on the agars. (see appendix-3,4, see table-5) For this, the bacteria should be diluted. Because if it isn't diluted (see appendix 2), lots of colonies will grow in a plate and they will create lawn.(see appendix-9)

After the spread plating and dilution of E.coli, it should be incubated overnight to let it grow. The incubator will be calibrated to the optimum temperature of E.coli, which is 37ºC, and the bacteria will have enough nutrients from the LB agar. So the bacteria will be able to grow and reproduce exponentially.[12] This way the bacteria colonies will form and they would be big enough to see with naked eye. So the colonies will be easily counted with the direct counting method. After counting the entire experiment and control group trials, the performing part of the experiment would be over and comparing and analysing the results would be the only part left.

[12] Alexei Sharov. "Exponential Model"Web.28.12.2011
<http://home.comcast.net/~sharov/PopEcol/lec5/exp.html >

5. Materials

- 10 g LB broth agar (see appendix 5)
- 7.5 g agar
- Weighing machine
- Flask (500 ml)
- Autoclaved and double-distilled water (500 ml)
- Autoclave
- 55 plates
- Freezer
- One loop of *Escherichia Coli DH 5 Alpha* from the stock at -80ºC
- 220 eppendorfs (1,5 ml)
- Marker pen
- Ultraviolet light cabin (Prosteril UV Sterilization Machine[13]) (see appendix-15)
- Chronometer
- Microorganism Flow Hood
- Gloves and solution with 70% ethanol and 30% double-distilled water
- Spreader
- Bunsen Burner
- Thermometer

[13] "ProSteril UV Sterilizasyon Makinesi" Web.21.09.2011
<http://www.kuaforpazari.net/index.php?p=show&pid=29&k_id=0>

6. METHOD

6.1. Preparation of LB Broth

1. To prepare 500 ml of LB, measure 10 g LB broth agar, 7.5 g agar and put them in a flask of 500 ml.
2. Fill the flask with 500 ml of double-distilled water.
3. Put the flask into autoclave to sterilize the agar. (see appendix 6)
4. Pour the LB broth on the plates while it is still hot. There should be 20 ml of LB broth per plate. Gently shake the plate so the broth is equally diffused.
5. Leave the plates to freeze for a day, at the temperature 4ºC.
6. Check the agars for any type of contaminations.

6.2. Activation of Escherichia coli DH 5 alpha

1. Take one loop of Escherichia coli DH 5 alpha from the stock at -80ºC. Put it on a flask which is filled with liquid LB broth. (see appendix-10)
2. Leave the flask of bacteria to be incubated at 125 rpm and 37ºC overnight (18 hours).
3. Take the flask filled with bacteria out and shake it gently. Apply pipetting technique so that the bacteria are homogenous throughout the flask. (see appendix-7)

6.3. Plating of Escherichia coli DH 5 alpha and Exposure to the Ultraviolet Light

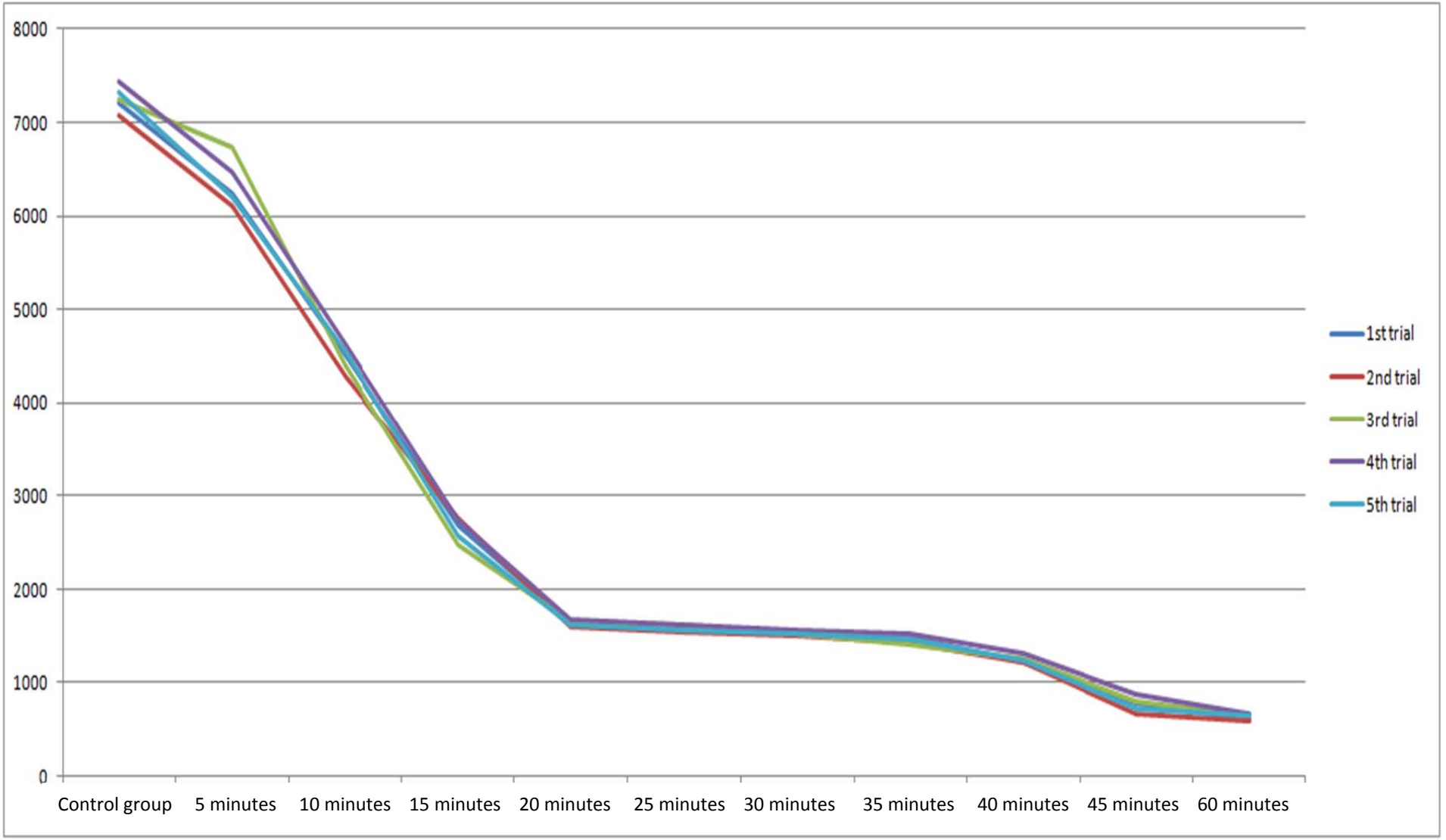
1. Wear experiment gloves and clean your hands with a solution of 70% ethanol and 30% double-distilled water.
2. Check the temperature of the medium with thermometer every 15 minutes and stabilize the temperature by ventilation.
3. Take 55 eppendorfs (one eppendorf per trial). Fill them with 1 ml samples of bacteria from the flask filled with E.coli taken from the stock, with a pipette. Make sure that the pipette is adjusted to 1 ml.
4. Label the eppendorfs with their trial numbers and exposure times.
5. Put the first trials of every experiment and control groups in the UV light cabin so that one trial from 5,10,15,20,25,30,35,40,45,60 minutes experiment groups are exposed to the UV light under equal conditions. Be sure that the eppendorfs' tops are open.
6. Take out the trials from under the UV light when their time is up.
7. Put the bacteria immediately under the Microorganism Flow Hood after taking them out of the cabin.
8. Dilute the bacteria taken out of the UV cabin by 10^{-4} fold with double-distilled water.
9. Clean the spreader with a mixture of 70% ethanol and 30% double-distilled water inside the Hood.
10. Adjust the pipette to 100 µL and take 100 µL of 10^{-4} diluted bacteria with the pipette from one of the eppendorfs taken out of the UV light cabin. Release the bacteria on the LB agar prepared beforehand.
11. Pass the clean spreader from the Bunsen Burner's fire. Cool off the spreader by pressing it on the lid of the plate. Be sure that it is cool enough because the high temperature of the spreader can kill the bacteria.

12. Spread the bacteria released on the agar with “Spread-plate method”. (see appendix-5)
13. Close the lid of the plate and name the plate according to its trial number, dilution and experiment group.
14. Carry out the processes; 9,11,12,13 and 14 for every trial of every experiment and control groups for all of their dilutions.
15. Take the plates into the incubator and leave them in the incubator overnight, at 37ºC.
16. Take out the plates from the incubator 18 hours later. Note down your observations concerning the bacteria colonies.
17. Choose the plates with the same dilutions made according to their ability to be counted. The bacteria colonies in every experiment group should be able to be calculated. The best observation of the bacteria colonies, for this experiment should be gained in the dilutions made by 10^{-4} fold.
18. Put the plates, that won’t be used in counting, to the biological-waste bin.
19. Count the bacteria colonies by direct counting. Record the data you found for every trial of every experiment and control groups.
20. After the counting is done, throw the plates into the biological-waste bin and clean any tool you used.

6.4. DATA COLLECTION AND PROCESSING

	Number of E.coli Bacteria Colonies				
Experiment Group	1st trial	2nd trial	3rd trial	4th trial	5th trial
control group	7210	7080	7250	7430	7320
5 minutes	6230	6110	6730	6460	6200
10 minutes	4510	4270	4390	4630	4550
15 minutes	2680	2750	2480	2740	2570
20 minutes	1630	1590	1650	1670	1610
25 minutes	1570	1540	1620	1610	1570
30 minutes	1520	1500	1530	1560	1530
35 minutes	1480	1430	1410	1520	1460
40 minutes	1220	1210	1260	1320	1240
45 minutes	780	670	800	880	730
60 minutes	610	590	640	670	650

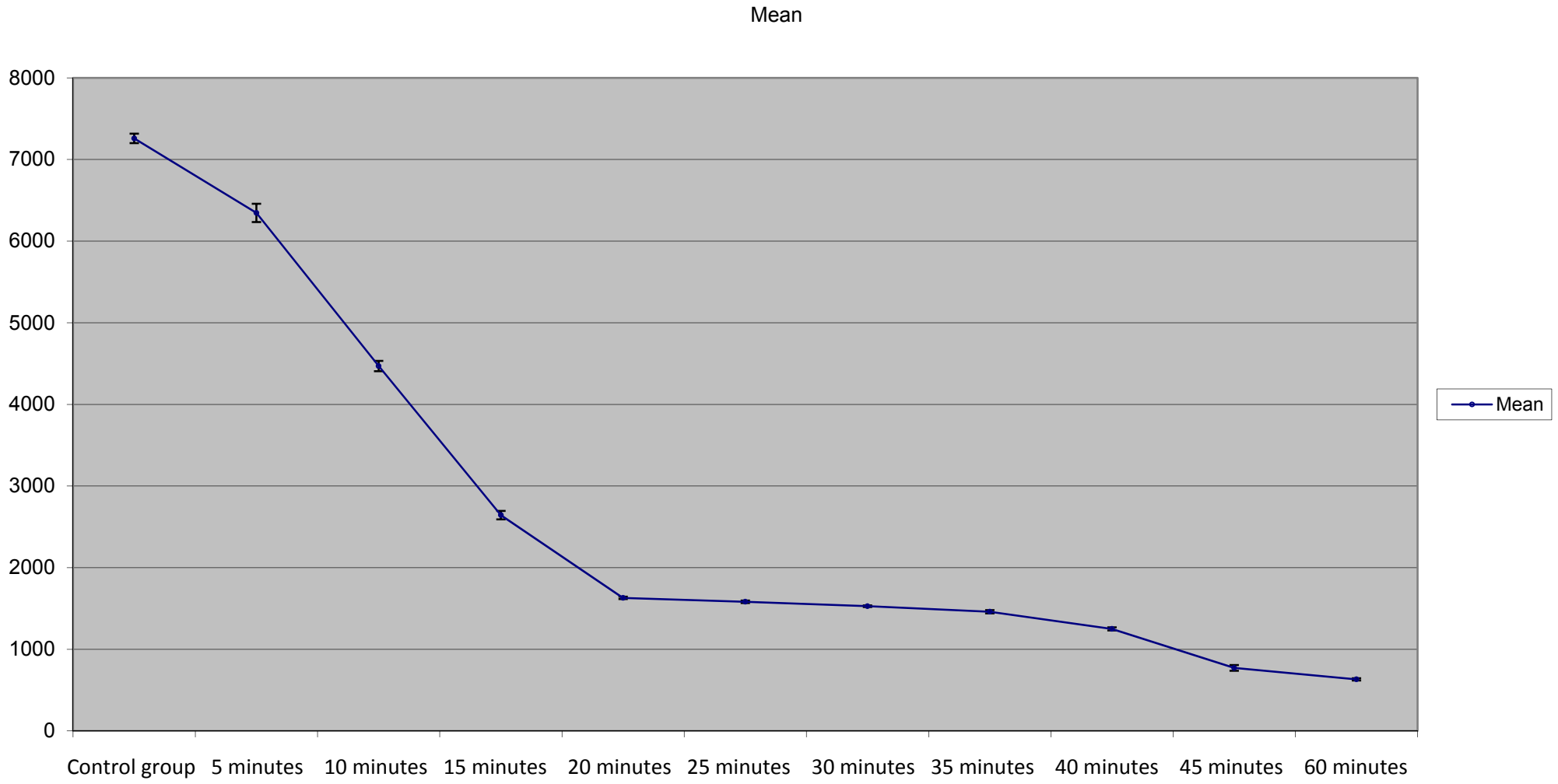
Table 1: The data collection table for the number of Escherichia Coli DH 5 Alpha bacteria depending on the different time intervals that the bacteria was kept in the UV light cabin (wavelength) when all of the bacteria were diluted by 10^{-4} and when all of them were allowed to grow on LB broth agar for 18 hours in the incubator



Graph 1: The graph of the bacteria colony number for all the experiment groups' trials

Experiment Group	Mean Number of E.coli Bacteria Colonies
Control group	7258 ± 58.1
5 minutes	6346 ± 112.0
10 minutes	4470 ± 63.2
15 minutes	2644 ± 52.0
20 minutes	1630 ± 14.1
25 minutes	1582 ± 14.6
30 minutes	1528 ± 9.7
35 minutes	1460 ± 19.2
40 minutes	1250 ± 19.5
45 minutes	772 ± 35.1
60 minutes	632 ± 14.3

Table 2: The mean of the number of E.coli bacteria colonies for all experiment and control groups



Graph 2: The graph of the mean value of the bacteria colony number for all the experiment groups

Experiment Group	Number of Data	Mean	Median	Range	Standard Deviation	Variance	Standard Error	t	%95 Confidence Interval
5 minutes	5	7258	7250	350	129.8845641	16870	58.08614293	2.2622	131.399984
10 minutes	5	6346	6230	620	250.4595776	62730	112.0089282	2.2622	253.3817987
15 minutes	5	4470	4510	360	141.4213562	20000	63.2455532	2.2622	143.0713809
20 minutes	5	2644	2680	270	116.3185282	13530	52.01922721	2.2622	117.6756672
25 minutes	5	1630	1630	80	31.6227766	1000	14.14213562	2.2622	31.99173333
30 minutes	5	1582	1570	80	32.71085447	1070	14.62873884	2.2622	33.09250628
35 minutes	5	1528	1530	60	21.67948339	470	9.695359715	2.2622	21.93242738
40 minutes	5	1460	1460	110	43.01162634	1850	19.23538406	2.2622	43.51346175
45 minutes	5	1250	1240	110	43.58898944	1900	19.49358869	2.2622	44.09756119
60 minutes	5	772	780	210	78.54934755	6170	35.12833614	2.2622	79.46581705
control group	5	632	640	80	31.93743885	1020	14.28285686	2.2622	32.31006688

Table 3: Statistical analysis of data collection table for the experimental values found.

“Anova: Single Factor” test should be done on the data found from the experiment to find whether the exposure time of Ultraviolet light is related with E.coli bacteria growth (number of bacteria colonies) or not.

H_0: $\mu=0$ Null hypothesis states that there is no relation between the exposure time for when the bacteria were exposed to UV light and the growth (number) of bacteria after the exposure.

H_1: $\mu\neq0$ The exposure time of UV light on bacteria has an effect on the growth (number) of bacteria after exposure to the UV light.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
5 minutes	5	31730	6346	62730
10 minutes	5	22350	4470	20000
15 minutes	5	13220	2644	13530
20 minutes	5	8150	1630	1000
25 minutes	5	7910	1582	1070
30 minutes	5	7640	1528	470
35 minutes	5	7300	1460	1850
40 minutes	5	6250	1250	1900
45 minutes	5	3860	772	6170
60 minutes	5	3160	632	1020

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	148179042	9	16464338	1500.304174	1.12553E-47	2.124029264
Within Groups	438960	40	10974			
Total	148618002	49				

Table 4: Analysis of Variances test for the data found from the experiment.

6.4.1. Result of the Anova Test

The P-value from the Anova test for the experiment turned out to be 1.12553×10^{-47}. So, the null hypothesis is rejected whereas the H_1 is accepted. Because if the P-value is smaller than 0.05, it means that there is a significant relation between the growth of E.coli bacteria (number of colonies formed) and the UV light exposure time.

6.5. Qualitative Observations

The relation between exposure time and number of bacteria colonies can also be clearly observed by the qualitative data collected throughout the experiment. After plating the bacteria on off-white coloured agars, the bacteria were left to grow in the incubation. After the incubation, light yellow coloured bacteria colonies formed on the agar. Each bacteria colony could be discerned from each other because the bacteria forming a colony had come together nearly homogeneously on the plate by taking the shape of circle. So each circle presented a bacteria colony. Since the bacteria colonies' colour was different from the LB agar's colour, it can be determined which trial had the most bacteria in it. In the experiment, the agar that contained bacteria that was exposed to the UV light for 5 minutes had the most yellow circles in it which meant that the UV light was the most ineffective on trials of 5 minute. (see appendix-14) It can be observed that the bacteria colonies decreased as the exposure time increased because the yellow circles were denser in the agars with low exposure time and the density of the bacteria lessened as the exposure time increased.

7. CONCLUSION AND EVALUATION

The results of the experiment showed that there is a significant relation between the growth of *Escherihcia Coli DH 5 alpha* and the exposure time of the bacteria under the Ultraviolet light. When the data found from the experiment is analyzed, it is clearly seen that as the exposure time to Ultraviolet light increases, the number of E.coli bacteria colonies formed on the agars decreases. The number of bacteria colonies indicates the number of bacteria that was able to grow functionally after the exposure of the UV. By exposing the bacteria to the UV light, damage is done to its organic base, thymine, which is a part of its DNA.[14] This damage prevents the replication of the bacteria which stops the increase in number of the bacteria colonies. In addition, further exposure of UV light can also cause the bacteria to die because of the severe damage to their DNA. Therefore, as the bacteria are left under the UV light more and more, most of the bacteria's replication stops and if the bacteria are exposed to the UV light further, then some of the bacteria also starts to die which decreases the number of bacteria on the agars. This can be clearly observed by the experiment. If the Graph 1 and Table 2 are taken into consideration, it is obvious that the number of colonies is decreasing as the time under the UV light increases. For example, the control group which was not exposed to the UV at all, had approximately 7258±58.09 bacteria whereas the number of bacteria colonies that was left 60 minutes under the UV light is averagely 632±14.28. Those data show that there is a dramatic decrease in the number of bacteria colonies after an hour of exposure to the UV light.

Before starting the experiment, some research was done. In the research, the minimum time interval that the E.coli bacteria should be under the Ultraviolet light to reach a safe level was said to be 30 minutes.[15] Whereas on the manual of the Ultraviolet light cabin, the safest time interval to get rid of bacteria was written as 45 minutes. But the time limit in the manual isn't specifically for E.coli bacteria, unlike the

[14] Barbara A. Hamkalo and P. A. Swenson. "Effects of Ultraviolet Radiation on Respiration and Growth in Radiation-resistant and Radiation-sensitive Strains of Escherichia coli"Web.21.10.2011 .<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC250099/>

[15] Angela Herrie. "UV Lights Effect on Bacteria" Web.14.09.2011 <http://www.ehow.com/facts_5730797_uv-lights-effect-bacteria.html>

time interval found by the research. But from the results of the experiment, it turned out that the time that the bacteria needed to be under the UV light was at least 60 minutes, which is more than the time stated both in the research and the manual. There shouldn't be any remaining bacteria. After the exposure of UV light, not all of the bacteria's DNA are damaged. Some of the bacteria go under other bacteria which block the direct exposure of UV light and won't be enough to damage the DNA. Those bacteria would be able to grow and replicate after the sterilization is complete and they would be able to reach the harmful level, as well. All in all, the bacteria shouldn't exist after the sterilization. In the experiment the lowest number of bacteria observed was 60 minutes where approximately 632± 14.28 bacteria colonies existed. So it is safe to say that the bacteria should be exposed to the UV light more than 60 minutes to be considered safe for human health.

On the other hand, the results from the experiment may not be a hundred percent correct because of the scientific errors that occurred throughout the experiment. First of all, the Ultraviolet cabin that was used in the experiment wasn't trustworthy. One of the reasons for the cabin causing error is that its cover couldn't be shut up completely. Therefore, the bacteria were exposed to the environment completely where lots of experiments are done with different types of organisms. If there were any remaining bacteria on the environment, then it was very easy for them to mix with the bacteria used on the experiment. To prevent this error, an UV light cabin whose cover can properly separate the environment of the outside from the experiment environment or the UV light cabin could have been put under the Microorganism Flow Hood which prevented any remaining organism from entering the hood.

Another cause for the error in the experiment was that the lids of the eppendorfs were left open when the bacteria were exposed to the UV light. This freely allowed other microorganisms that were unrelated with the experiment, to mix with E.coli. Those unwanted bacteria could have caused competition with the E.coli, therefore decreased the number of E.coli. In order to avoid this error, another type of container with completely transparent and thinner lid could have been used. This way

the direct exposure of the UV light and the bacteria wouldn't be prevented by anything since the transparent lid wouldn't be able to stop the UV light from reaching the bacteria, whereas if an opaque or semi-opaque lid was used than they would stop the UV light from passing through the lid. In addition to the transparency, the lid also need to be thin enough to let the UV light pass while being thick enough to prevent any unrelated organism from entering the container of E coli.

Furthermore, the bacteria should be under the direct UV light. The bacteria and the UV light source shouldn't be separated by any opaque material. This also includes the bacteria itself as well. For example, the bacteria on the bottom of the eppendorf won't be under the direct exposure of the UV light since the bacteria on top would behave as semi-opaque objects. To avoid this error, the bacteria should have been planted on plates, which increase the surface area, and decreases the possibility of any bacteria preventing another bacteria's the direct exposure of the UV light. Also a bigger UV light cabin would be needed to fit all the bacteria plates from all the trials.

Moreover, error also occurred in counting the bacteria. Since the bacteria after they were left for incubation had formed lots of colonies, it made counting them very hard. Furthermore, because of the colony counting method when the number of bacteria colonies exceed 300 the approximate number is written. For example, if the colony number has 3 digits then it should have 2 scientific numbers in it at the most. For bacteria colony numbers that have 4 digits, at most 3 scientific numbers are needed. The reason for this is the hardness of counting the exact number of bacteria colonies when there are more than 300 colonies. To avoid this type of error, the dilution of the bacteria could have been increased ten more fold because this would decrease the number of bacteria plated on per agar to decrease therefore, there would be less bacteria colonies found after the incubation which will allow counting to be easier.

Another source of error was being unable to expose all of the trials to the UV light in the cabin. Because of this, the environment properties, such as temperature,

changed with each trial and those changes affected the bacterial growth. In order to prevent this, a bigger UV cabin that could fit all the trials of the experiment should be used.

What's more, the pipettes used in the experiment could have caused error as well since sometimes the pipette was unable to empty all of the bacteria on the agar. Because of this, there would have been fewer bacteria on some of the agars which would decrease the number of the bacteria colonies that should have formed if the bacteria were taken into the pipette properly. In order to prevent the error of the pipette, more sensitive pipette that can pour all of the bacteria taken into the pipette, should be used.

As the effects of E.coli bacteria are found and investigated, efficient ways of sterilizing the tools we use from this bacteria type is also investigated. One of those sterilizing ways is using Ultraviolet cabins. In this paper, the effectiveness of the UV light was investigated according to time. The minimum time interval that the E coli bacteria should be under the exposure of the UV light was found to be 60 minutes. Even though this level of the bacteria was not deemed as safe for human use, the bacteria should be exposed to the UV light at least 60 minutes. Despite the errors that have occurred during the experiments, the experiment results are reliable. This indicates that the users of the UV light cabins to get rid of E. coli bacteria should at least expose the bacteria to the UV light for 60 minutes. If this time interval seems as very long, then other possible ways of sterilizing tools from the E coli, such as autoclaving[16], should be investigated because it is not safe to use tools that aren't exposed to the UV light at the very least 60 minutes. In addition, using a machine whose voltage is 220V for an hour can be expensive when compared with other possible ways. For example using autoclave which works with 220V [17] as well would be less expensive.

[16] Jennifer Klinkel. "Sterilizing Pedicure Tools and Manicure Tools"Web. 25.12.2011 <http://www.atyourfeetnailcare.com/sterilizing-pedicure-tools.html >
[17] "Autoclaves" Web. 13.12.2011< http://www.m-r-c.co.il/Media/Uploads/CD/TableAutoclaves-SPEC.pdf >

8. APPENDIXES

1. Properties of *Escherichia coli*

   Escherichia coli is a bacteria that was discovered in 1885 by Theodor Escherichi and referred shortly as E.coli. *"Commonly known as E. coli, this class of bacteria usually colonizes the human gastrointestinal system within 48 hours of birth. Their roles within the human digestive process are not well understood."* It is known to cause bloody or non-bloody diarrhea, abdominal cramps, vomiting and in some cases fever. In addition, E.coli can survive on both humans and most of the objects that are used by humans because it can live between 7ºC to 46ºC, which includes the normal temperature of human body (36ºC). Also, it can survive very low temperatures without being destroyed and doesn't need air to live, although there will be a slight decrease in its population. Some other factors affecting the growth of E.Coli are pH, salt and water concentration of the medium. If all the properties of E.coli is considered, it is found that E.coli can survive on humans with optimum conditions. [18] [19]

2. Serial Dilution

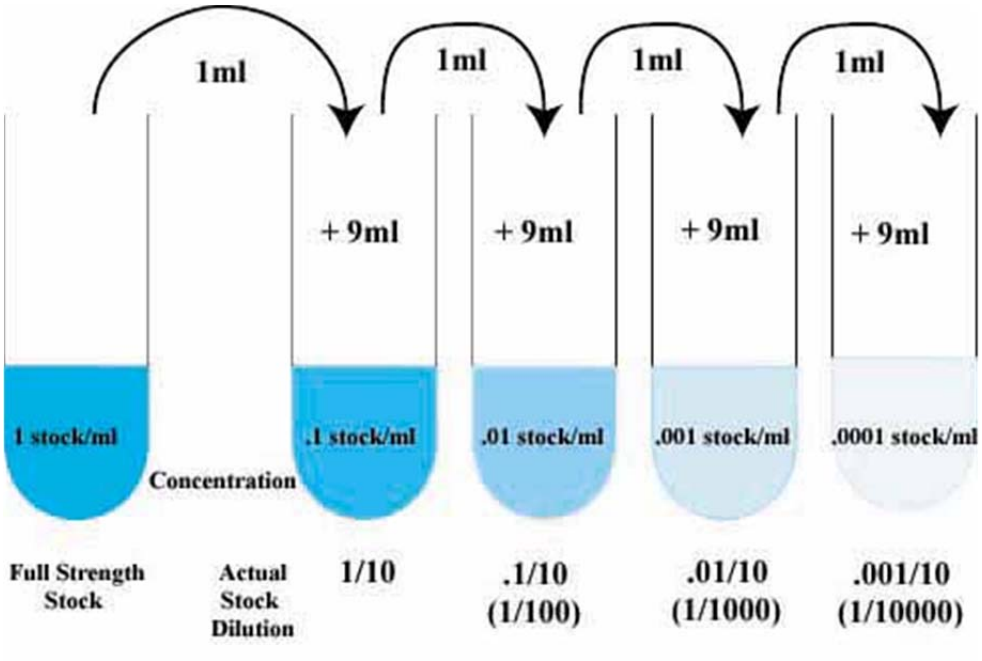
   A serial dilution makes the solution at hand more dilute. The serial dilution dilutes the solution by ten-fold in a logarithmic scale. This done by taking 1 unit of concentrated solution into 9 units of distilled water, the taking 1 unit solution from the newly formed solution into 9 units of distilled water. Therefore the concentration of the solution is progressively decreased by ten-fold with each dilution.[20] [21] (see Picture 1)

[18] Minh Thanh Nguyen. "The effect of temperature on the growth of the bacteria Escherichia coli DH5α" Web. 30.09.2011 <http://homepages.stmartin.edu/fac_staff/molney/website/SMU%20Bio%20Journal/Nguyen%202006.pdf >

[19] FSIS." Risk Assessment of the Public Health Impact of Escherichia coli O157:H7 in Ground Beef" Web. 27.12.2011< http://www.fsis.usda.gov/oppde/rdad/frpubs/00-023n/00-023nreport.pdf>

[20] "Serial Dilution"Web. 07.10.2011<http://en.wikipedia.org/wiki/Serial_dilution >

[21] "Successive Serial Dilutions" Web. 07.10.2011 <http://biology.kenyon.edu/courses/biol09/tetrahymena/serialdilution3.htm >



Picture 1: The procedure of applying dilution on a solution

3. Comparison of Streak-Plate and Spread-Plate Methods

The spread method distributes the bacteria equally over the plate which makes the colonies formed easier to count. However, the streak plate method diffuses the bacteria heterogeneously so it is nearly impossible to count the colonies, since the bacteria are grouped in one part of the plate and the other part of the plate is nearly empty. In addition, the spread plate method is easier to perform than the streak plate method. Because in the streak plate method, sharp edged loops have to be used and those can really easily break the agar so it is hard to use. But in the spread method a glass triangle shaped object called spreader is used and this is very easy to use since it doesn't damage the agar easily. Furthermore, the loop used in the streak method is metal so its temperature rises very quickly and takes longer to cool than the glass spreader. That's why; using the loop would be to my disadvantage. Because I would have to wait much longer and I could easily kill the bacteria from the

heat of the loop after taking it through the Bunsen burner to clean any unwanted organism on it if I don't wait enough.[22] [23]

4. Application of Spread Plate Method

Spread Plate Method is applied by moving the spreader on the agar upside down while turning the agar clockwise. At first, it should feel like the agar is wet because of the bacteria. Go on spreading the bacteria until this feeling is gone and the agar seems like drier. By doing this you guarantee that the bacteria are equally diffused on every part of the plate.

5. Contents of Luria Broth (LB Broth)

The contents needed for the preparation of 1 liter of Luria Broth agar are 10 grams of tryptone, 5 grams of yeast extract and 10 grams of NaCl.[24]

6. Properties of Autoclave

The temperature of the autoclave is 121ºC and you should leave the flask in it for 15 minutes.

[22] David B. Fankhauser. "SPREADING TECHNIQUE FOR PLATING BACTERIA" Web. 23.10.2011<http://biology.clc.uc.edu/fankhauser/labs/microbiology/yeast_plate_count/spreading_technique.html >
[23] Regina Bailey. "How To Streak a Bacterial Culture"Web. 23.10.2011<http://biology.about.com/od/biologylabhowtos/ht/streak-a-bacterial-culture.htm>
[24] "Lysogeny Broth"Web. 20.10.2011< http://en.wikipedia.org/wiki/Lysogeny_broth#Preparation>

7. Pipetting Technique

Pipetting technique ensures that a certain volume of a solution is added to another solution with precision. This way the mixture of the solutions is homogeneous and the volumes of solutions that are mixed are precise. For pipetting, the pipette should be set on a certain volume. Then a new pipette tip should be set and this tip should be rinsed with the solution that will be taken by the pipette. So that there aren't any other solutions from others remaining on the pipette. Then the solution should be taken into the pipette by pushing the plunger of the pipette down slowly when the tip of the pipette is in the solution. Then the plunger should be released into the other solution. The mixed solution should be taken into the pipette then released again for approximately 8-10 times. This makes the mixture homogeneous.[25]

8. Definiton of "Being Sterile"

The definition of being sterile is being "*free from all live bacteria or other microorganisms and their spores*."[26] In the case of this experiment, it is desired to know the minimum time interval needed to be sterile from E.coli bacteria by exposing the bacteria to UV light. It is very important that there aren't any remaining bacteria after the exposure to the U light because if there are any bacteria left that can replicate itself, then it indicates that the number of E.coli will increase as time passes. So the bacteria would be able to reach a level that is harmful for the human body and cause illnesses or complications.

9. Definition of "Creating Lawn"

If the dilution of the bacteria isn't enough, then a situation called "creating lawn" will be observed. Creating lawn means that after the incubation of bacteria, there will be so many bacteria that it will be impossible to count the

[25] "Introduciton to Lab Techniques" Web.27.10.2011<http://faculty.plattsburgh.edu/donald.slish/introlab.html>
[26] "sterile"Web. 13.09.2011<http://medical-dictionary.thefreedictionary.com/sterile >

bacteria colonies or distinguish one colony from the other since the colonies will be packed so closely.

10. What happens to the LB when E.coli is waited in the LB for a day?

When the E.coli bacteria taken out of the stock are put into incubation inside LB broth, the bacteria starts to consume the nutrients in the LB broth and they start to replicate. Therefore by the end of the incubation, the LB broth solution's nutrient concentration is lower than the beginning. Thus, the LB broth solution doesn't affect the bacteria's growth so much when they are under UV exposure since there is relatively small amount of nutrients left in the broth.

11. Direct Counting Technique

"The basis of a direct count is the actual counting of every organism present in a sub-sample of a population."[27] Direct counting is done by marking the lid of the agar where a bacteria colony can be seen on the agar with a marker. By marking the colonies, it is made sure that one colony isn't counted twice.

12. The Least Exposure Time of UV According to the Manual of the Machine

In the manual of the ProSteril UV Sterilization Machine, it was stated that the manicure tools should be exposed to the UV light for at least 45 minutes to sterilize the tools. According to the manual, the agars with E.coli bacteria would be free of E.coli after 45 minutes.

[27] Stephen T. Abedon . "Supplemental Lecture".Web. 13.12.2011 < http://www.mansfield.ohio-state.edu/~sabedon/biol4038.htm >

13. Comparison of Three Different Methods Tried Out for the Experiment

Before finding the best possible method, two other methods were tried out. The first two methods were not used during the experiment since the results came out as either irrelevant or invalid and inaccurate. But as the third method's results were consistent and accurate, the third method was selected as the method used in this paper. In the table below (Table 5), there is a comparison and analysis of both three methods that were tried out to find the best method for this paper.

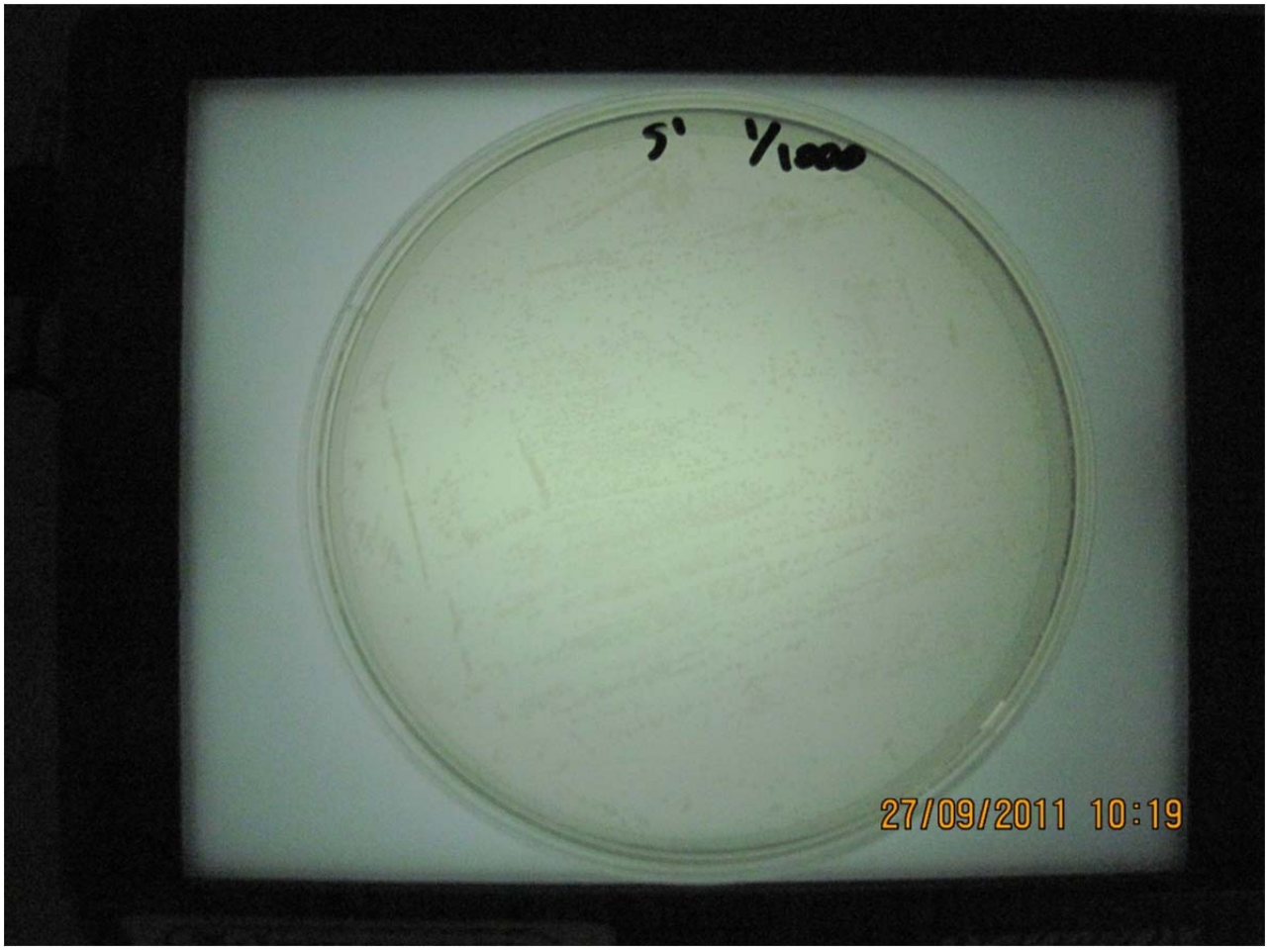
The Comparison Of The Different Method Techniques						
Technique	**First Method**	**Second Method**	**Third Method**	**Analysis Of The Methods**		
				Analysis Of The First Method	**Analysis Of The Second Method**	**Analysis Of The Third Method**
Agar Type Used For UV Exposure	LB agar	LB broth	LB broth	Using agar when exposing the UV light is unefficient becasue the agars from all experiment won't fit in the UV light cabin at the same time. So some of the experiment groups would be exposed to the UV light under different conditions than the others.	The bacteria growth in the broth is greater than agars. As a result, if broth is used for the exposure, the bacteria that waited more in the broth, which are the bacteria that were exposed to the UV light more, replicated more. Therefore, the results were false since the number of the bacteria colonies increased instead of decreasing as the time of exposure increases.	LB broth was also used in this method. But unlike the second method, the bacteria were left to grow on the broth for a day before the UV exposure. This decreased the nutrition in the broth. Thus, there wasn't a drastic difference between the rate of growth when the bacteria were in the agar or in the broth.
Agar Type Used For Incubation	LB agar	LB agar	LB agar			
Plating Technique (see appendix 3,4)	plate streak method	spread plate method	spread plate method	The streak plate isn't useful for counting the bacteria colonies because the streak method doesn't distribute the bacteria evenly through the plate. When this method is used, the bacteria colonies are collected on one part of the plate more than the others. Therefore it is impossible to count the colonies since they create lawn.	The spread plate method is more efficient because the distribution of the bacteria is homogeneous. So the colonies are evenly scattered and can be counted easily.	
Dilution Fold	10^{-3}	10^{-4}	10^{-4}	Since the dilution wasn't enough, the bacteria colonies created lawn.	The dilution fold was enough that the bacteria colonies could be calculated in all of the agars and didn't create lawn while the dilution wasn't too much so bacteria colonies formed in all of the agars.	

Table 5: The table of comparison and the analysis of all three methods that were tried for the experiment

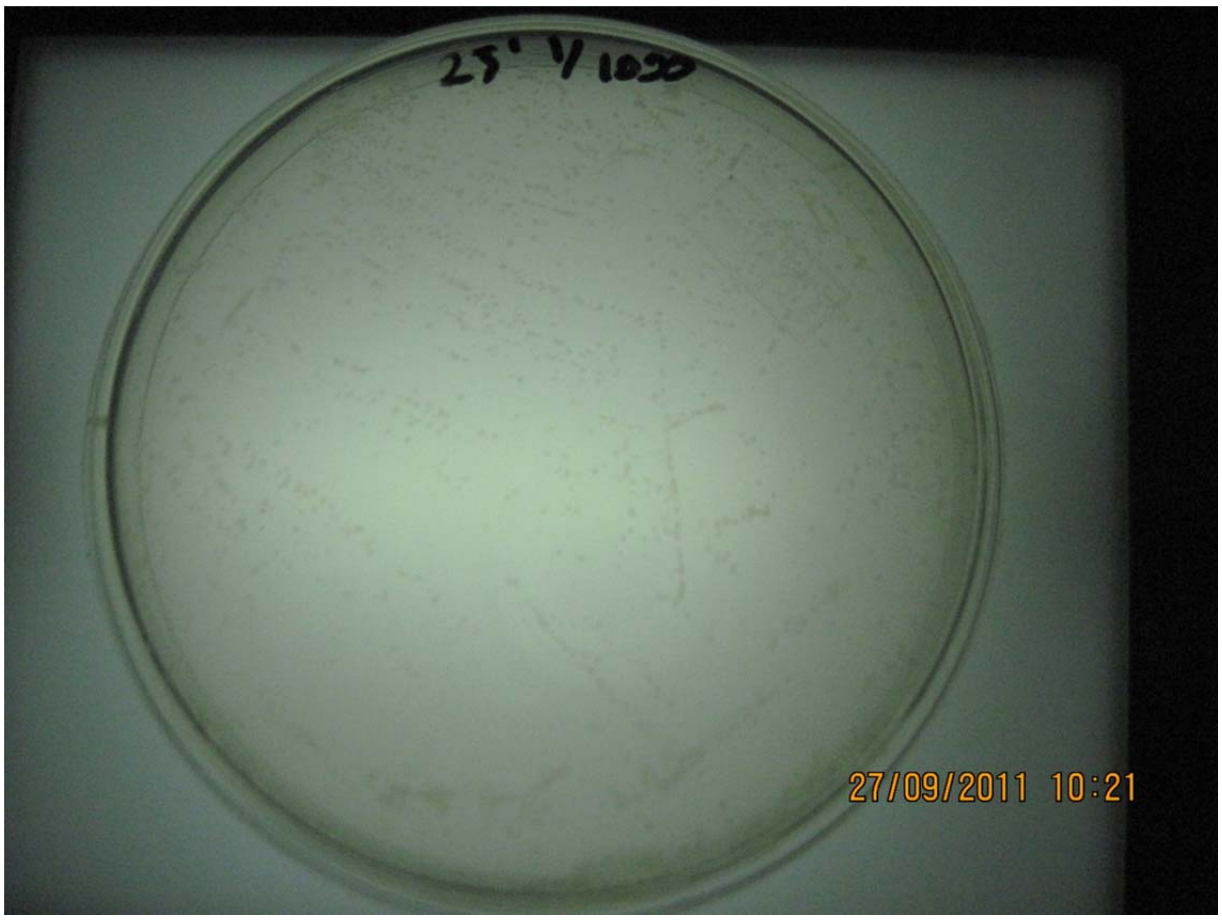
14. Comparison of Agars with E.coli That Were Exposed to the UV Light For 5 and 25 Minutes

Since both the agar and the bacteria colonies were light coloured, only some of them could be seen on the pictures taken of them. One of the trials from 5 minutes exposure and 25 minutes exposure showed the bacteria colonies best. So the effect of time of exposure of UV light on E.coli bacteria colonies formed can be seen on those pictures the best.

As it can be seen in Pictures 2 and 3, as the exposure time to the UV light increases the number of bacteria colonies, which can be seen as light yellow dots, decreases.



Picture 2: A plate with Luria Broth agar and E.coli bacteria colonies formed after overnight incubation that was exposed to the UV light for 5 minutes and diluted 10^{-4} fold.



Picture 3: A plate with Luria Broth agar and E.coli bacteria colonies formed after overnight incubation that was exposed to the UV light for 25 minutes and diluted 10^{-4} fold.

1. ProSteril UV Sterilization Machine

   The UV Sterilization Machine is used for sterilizing manicure tools and it works with 220V. On the manual of the machine, it is stated that it should be used for at least 45 minutes to be effective.

Picture 4: ProSteril UV Sterilization Machine used in sterilizing manicure tools.