BIOLOGY EXTENDED ESSAY

INVESTIGATING THE EFFECT OF ISOPROPYL ALCOHOL PERCENTAGE IN DISINFECTANTS ON THEIR BACTERIA KILLING CHARACTER

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Abstract

Disinfectants are used for cleaning in many places because of their bacteria killing features. The focus of this study is observing the effect of the alcohol percentage in disinfectants on their bacteria killing character. It isn't possible to test all kind of alcohols, so isopropyl alcohol is chosen for testing. The aim of this study is to investigate if the percentage of isopropyl alcohol in disinfectants has an effect on the bacteria killing effect of that disinfectant by observing the bacteria formation in the well after mixing same volumes of disinfectants that have different percentages of isopropyl alcohol in it and bacteria-agar suspension. Bacteria (*Staphylococcus aureus*) that are used in the experiment are gathered from a public bus by using swab.

As a conclusion after observing the effect of disinfectant with 25%, 10% and 0% isopropanol on the survival of *S.aureus,* these results have been obtained: 5.3 percentage of bacteria survival is measured when disinfectant with 25% isopropanol (the most concentrated disinfectant) is used, 53.6 percentage of bacteria survival is measured when disinfectant with 10% isopropanol and 80.6 percentage of bacteria of survival is measured when disinfectant with 0% isopropanol (the least concentrated disinfectant). So it can be said that as the percentage of isopropanol in a disinfectant increases (when other alcohol percentages are constant) its bacteria killing effect increases. These results verify the research question of the study which is: "Is the percentage of isopropyl alcohol in the disinfectants, which are 25%, 10%, 0%, affect the survival percentage of *Staphylococcus aureus* when the ethyl alcohol percentage in the disinfectants are constant and equal to 70%?".

word count: 268

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Introduction

When I was a kid whenever we used public transportation, my mother always told me not to touch anywhere because there were many germs on it that could make me ill and as soon as we go home she would make me wash my hands with soup. The soup we use has cleaning affect that is for sure but I got curious about if soup cleans enough then why doctors use disinfectants. When I researched I learned that there are many disinfectants with different alcohol percentages, so I decided to look for what difference would it make if there are different percentages of alcohol in disinfectants.

Disinfection is a process that destroys species and amount of microorganisms, which causes illnesses or makes them ineffective. The substances used for this purpose are called disinfectants.[1] The purpose of the disinfection isn't to kill all microorganisms but to decrease their amount in the environment, so that they can't cause any harm to health of the other living organisms. They contain isopropyl and ethyl alcohol. Most of the disinfectants appeared in the first part of the 20th century; even though the applications like the storage of the water in copper and silver pots, boiling water are unconsciously done, they are the disinfection processes practiced in old ages.[2]

I looked at the affect of disinfectants with different alcohol concentrations because alcohol kills bacteria by disturbing their structure. Hydrogen bonding occurs between amide groups in the secondary protein structure of bacterial cell. Hydrogen bonding between "side chains" occurs in tertiary protein structure in a variety of amino acid combinations. All of these are disrupted by the addition of another alcohol. A 70% alcohol solution is used as a disinfectant on the skin. This concentration of alcohol is able to penetrate the bacterial cell wall and denature the proteins and enzymes inside of the cell.[3]

[1] http://www.mansfield.ohio-state.edu/~sabedon/black12.htm
[2] http://www.bvsde.ops-oms.org/bvsacg/fulltext/desinfeccioneng/chapter9.pdf
[3] http://www.elmhurst.edu/~chm/vchembook/568denaturation.html

I decided to work with *Staphylococcus aureus* in my experiment. *S.aureus* is a facultative anaerobic Gram-positive coccal bacterium that means it makes ATP by aerobic respiration if oxygen is present but is also capable of switching to fermentation, its cell wall is lack the outer membrane and it has a spherical shape. It is frequently found as part of the normal skin flora on the skin and nasal passages.[4] The reason I chose *S.aureus* was that they are part of human skin flora so they are found in public transport vehicles and if they are too much on the human skin they can cause illnesses such as bacterial skin infections.[5]

In this experiment, three disinfectants (Appendix 1) with different percentage of isopropanol in them will be used to observe the resistance of *S.aureus* to different disinfectants with different percentage of alcohol.

I chose to study with these disinfectants because their contents were same except their isopropanol proportions: first disinfectant has 25% isopropanol, second disinfectant has 10% isopropanol and third disinfectant has 0% isopropanol. Because most of the disinfectants in markets have different percentages of isopropanol in them where ethanol doesn't differ significantly.

My aim in this experiment is to find what percentage of alcohol in disinfectants should be used to kill the bacteria found in public transport vehicles so the people who transport with them can ride in more hygienic environment and have less illness.

Therefore, the research question of my extended essay will be as: "How does the percentage of isopropyl alcohol in the disinfectants, which are 25%, 10%, 0%, affect the survival percentage of *Staphylococcus aureus* when the ethyl alcohol percentage in the disinfectants are constant and equal to 70%?" This paper shows how this experiment is done and the discussion of its results.

[4] Kluytmans J, van Belkum A, Verbrugh H (July 1997). "Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks". *Clin. Microbiol. Rev.* **10** (3): 505–20

[5] http://microbewiki.kenyon.edu/index.php/Staphylococcus_aureus

Hypothesis

S. aureus infections can spread through the contact with objects that infected person touched before.[6] Because in public spaces such as busses people touch everywhere, so people can get infection from touching anywhere. Therefore disinfecting public spaces like busses regularly is important for people's health.

"Both ethanol (ethyl alcohol) and isopropanol (isopropyl alcohol) are alcohols that kill bacteria. Alcohols kill bacteria by first making the lipids that are part of the outer protective cell membrane of each bacterium cell more soluble in water so that the cell membrane begins to lose its structural integrity and fall apart."[7]

Eventually it can be said that to protect people from getting infections from public spaces, disinfectants with isopropanol in them can be used as cleaners. So, my hypothesis for this experiment is: "There will be significant difference between the survival percentage of *S. aureus* as the percentage of isopropyl alcohol in the disinfectants changes." The disinfectant with the highest percentage of isopropanol is expected to have the least survival percentage of *S. aureus*.[8]

[6] "*Staphylococcus Aureus* Infection" Web. 21.05.2012

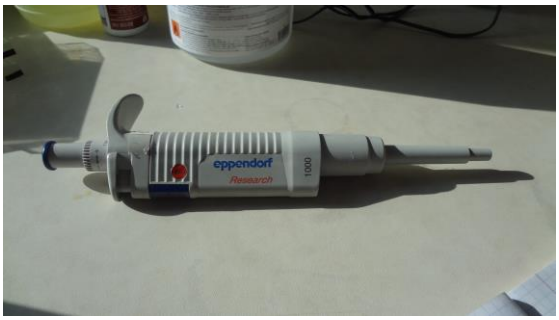
http://www.healthdiscussions.org/hd/index.php?t=staphylococcus+aureus+infection

[7] http://www.ehow.com/how-does_5462404_alcohol-kill-bacteria.html

[8] A. Michelle Caldwell. "How does Alcohol Kill Bacteria?" Web. 21.05.2012 <http://www.ehow.com/how-does_5462404_alcohol-kill-bacteria.html#ixzz1vRs5QswC>

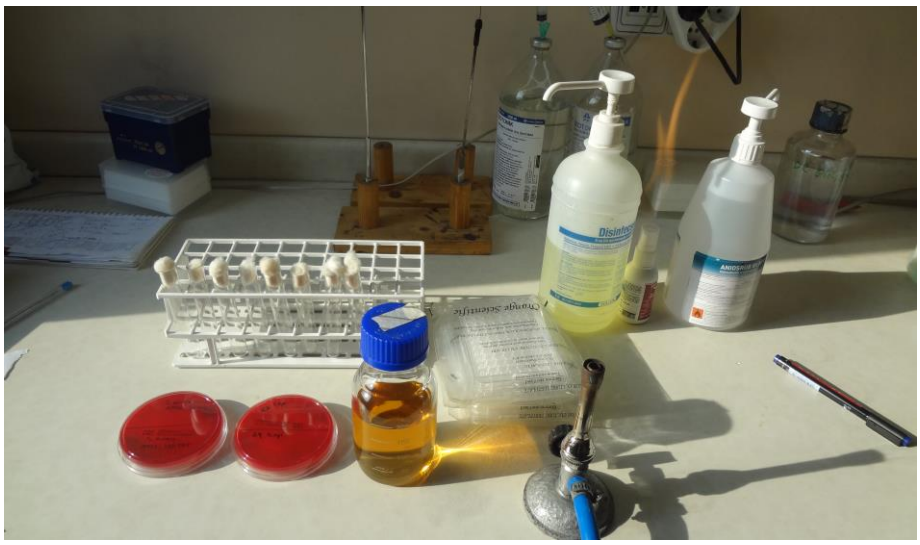
Materials

- *Staphylococcus aureus which is taken from the bus by a swab*
- 54 mL Mueller Hinton Broth agar
- 4.5 mL distilled water
- 2 X Petri dishes with 5% *Ovis Aries* Blood Agar
- Weighting machine
- Heraeus Incubator Swab
- McFarland densitometer
- 6 X micro plates with 96 well
- 15 mL Disinfecsol®



- 15 mL OPA-70®
- 15 mL ANIOSRUB 85 NPC®
- Micro- pipette (100 µL)
- Multichannel pipette (100 µL for each channel)
- Bacteriological Loop

- Bunsen burner
- Goggles
- Gloves
- Lab coat
- 2 test tubes



Method Development and Planning

In order to make statements about the research question “How does the percentage of isopropyl alcohol in the disinfectants, which are 25%, 10%, 0%, affect the survival percentage of *Staphylococcus aureus* when the ethyl alcohol percentage in the disinfectants are constant and equal to 70%?” three disinfectants with 25%, 10%, 0% percentage of isopropyl alcohol and 70% of ethyl alcohol should be used.

To observe the isopropyl alcohol effect, disinfectants with different isopropyl alcohol concentration but same ethyl alcohol concentration should be obtained. The reason I decided to fix the ethyl alcohol concentration and change isopropyl alcohol concentration is that before deciding on my independent variable, I looked at the contents of the disinfectants that are being used at Gazi University Microbiology Laboratory where I did my experiment and I saw that generally the ethyl alcohol concentration in the disinfectants are constant but isopropyl alcohol concentration changes so, I chose isopropyl alcohol concentration as my independent variable. By fixing the ethyl alcohol concentration and changing isopropyl alcohol concentration, the effect of isopropanol on the survival percentage of *Staphylococcus aureus* can be observed. To find disinfectants with different isopropyl alcohol concentration but same ethyl alcohol concentration I looked for the content of disinfectants that are used at Gazi University Microbiology Laboratory. Among lots of disinfectant kinds I found 3 disinfectants with same percentage of ethyl alcohol (70%) and different percentages of isopropyl alcohol (25%, 10%, 0%). I had a plan-b that if I couldn’t be able to find disinfectants with same ethanol percentage but different isopropanol percentage I would form three different concentrated mixtures of ethanol and isopropanol. I didn’t prefer this way because if I would use disinfectants that are present instead of preparing solutions of isopropanol and ethanol, I would spend less time and less money from founding and getting isopropanol and ethanol solutions.

The bacterium I chose to observe its survival percentage after it is exposed to different concentrated isopropanol, *Staphylococcus aureus,* is obtained from a public transport by rubbing a swab to its pole. *Staphylococcus aureus* is labeled with the machine for automatic classification of bacteria but for labeling there should be

enough amount of bacteria so I planted bacteria in the agar with *Ovis Aries* blood (Appendix 2) for 24 hours in the incubator (the heat of the incubator should be 37°C) and because of the suitable conditions which are the medium with rich nutrient and optimum temperature is provided for bacteria, it reproduced into large amounts. In the experiment agar with *Ovis Aries* blood is used because blood is rich of nutrient for bacteria to use and *Ovis Aries* blood is simple to excess than other mammalian's blood.

I chose to use Mueller Hinton Broth (Appendix 3) in my experiment as nutrient because Bauer, Kirby and Sherris (Appendix 4) recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented medium has been selected by the Clinical and Laboratory Standard Institute (CLSI)1 for many reasons. This medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, and supplies satisfactory growth of most non-fastidious pathogens along with demonstrating batch-to-batch reproducibility.[9] In test tube-1 I put 1.5 mL of Mueller Hinton Broth without any microorganisms in it and in test tube-2, I put 16.5 mL of Mueller Hinton Broth agar and placed *Staphylococcus aureus* in it by using bacteriological loop until it become 0.5 McFarland (Appendix 5) concentrated. I used McFarland densitometer to measure its concentration. By preparing 0.5 McFarland concentrated suspension of these microorganisms and 16.5 mL of Mueller Hinton Broth agar I kept bacteria in a nutritious medium that it wouldn't die because of deficiency of nutrient so it wouldn't affect the results of my experiment. I used 96-well micro plates (Appendix 6); I put 15 mL 0.5 McFarland concentrated suspension of Mueller Hinton Broth containing *S.aureus* in test tube-2 into 10 columns and 15 rows of 96-well micro plates: 100 µL per each well, but because there are only 8 rows in one micro plate I used two micro plates. I put 100 µL of the disinfectant with 25% percentage of isopropanol into 10 columns of the first row.

Because one well of the micro plates is 350 µL and I wanted to put the disinfectants and suspensions with equal amounts to provide an efficient disinfectant

[9] http://www.neogen.com/Acumedia/pdf/ProdInfo/7101_PI.pdf

effect, I put 100 μL to each well. At first I tried it with 150 μL of each to one well but when the solutions nearly filled all of the wells and mixed with each other even after a small touch, I tried it with 100 μL of each to one well and I decided to use 100 μL of each in my experiment.

As for the 11th and 12th columns of the micro plate: 11th column is used as negative control and 12th column is positive control. For positive control I just put 100 μL of solution in test tube-2 into the 15 wells of 12th column with no disinfectants in it. The positive control columns are used for test validity, it ensures that there will be *S.aureus* reproduction when there is no disinfectant added. For negative control I put 100 μL of H_2O and 100 μL of solution in test tube-1, which has no bacterium in, into the 15 wells of 11th column. The negative control columns are to ensure that there will be no reproduction of bacteria when 0.5 McFarland concentrated suspension of *S.aureus* and Mueller Hinton Broth agar is not added.

The change in survival percentage of *S.aureus* is calculated as I gave 1 for each well that has living bacteria in it and 0 for each well that doesn't have any living bacterium in it and I took total scores of 50 wells for each disinfectant; there are 3 trials of them in total. I decided if there are dead or living bacteria by observing the white colored dots in the wells; if there are white dots in the well that means there are living bacteria in that well and if there aren't any white dots and the solution in the well is transparent that means there is no living bacterium in it.

The method will be constant procedure for disinfectants with different percentage of isopropanol in them.

Method

1. Take samples from button of a public bus and pole of a public bus by rubbing swab to them.
2. Take the swabs to the laboratory and plant the bacteria into agar with *Ovis Aries* (Appendix 2) blood.
3. Hold the planted bacteria in the agar with *Ovis Aries* blood for 24 hours in the incubator (the heat of the incubator should be 37°C) so that the microorganisms will reproduce in it.
4. Take the reproduced microorganisms to the machine for automatic classification of bacteria to label them by bacteriological loop.
5. Place 1.5 mL of the Muller Hinton agar in one test tube and 16.5 mL of the Muller Hinton agar in another test tube.
6. Label these test tubes as test tube-1 and test tube-2.
7. Prepare 0.5 McFarland (Appendix 5) concentrated suspension of these microorganisms and 16.5 mL of Mueller Hinton Broth (Appendix 4) in test tube-2 by using McFarland densitometer. (0.5 McFarland = 1×10^8 CFU)
8. Label the micro plates as micro plate-1 and micro plate-2.
9. Put 100 µL of the Muller Hinton agar that was in the test tube-1 into 15 well of 96 well micro plates' 11^{th} column.
10. Put 100 µL of the Muller Hinton agar that was in the test tube-2 into each well of 96 well micro plates except the wells of 11^{th} columns of both micro plates.
11. Put 100 µL distilled water into 15 well of 11^{th} columns of 96 well micro plates.
12. Put 100 µL Disinfecsol® into first 5 rows of the micro plate-1 except 11^{th} and 12^{th} columns.
13. Put 100 µL OPA-70® into last 3 rows of the micro plate-1 and first 2 rows of micro plate-2 except 11^{th} and 12^{th} columns.
14. Put 100 µL ANIOSRUB 85 NPC® into next 5 rows of the micro plate-2 except 11^{th} and 12^{th} columns.
15. Repeat the steps between 5-14 for two more times.
16. Keep the prepared micro plates in 37°C for 24 hours in the incubator.
17. After a day, observe the bacteria inside of the 96 well micro plates.

18. Note if the bacteria survived in each well or did not by observing the white colored dots in the wells: if there are white dots in the well that means there are living bacteria in that well and if there aren't any white dots and the solution in the well is transparent that means there are no living bacteria in it.
19. Do statistical analysis and calculations to verify or reject the hypothesis.

Data Collection

Well number	25% isopropanol	10% isopropanol	0% isopropanol	N.C.	P.C.
1	0	0	1	0	1
2	0	1	0	0	1
3	0	0	1	0	1
4	0	1	1	0	1
5	0	0	1	0	1
6	0	1	1	0	1
7	0	0	1	0	1
8	0	1	1	0	1
9	0	0	1	0	1
10	0	1	1	0	1
11	0	1	1	0	1
12	0	0	1	0	1
13	0	1	1	0	1
14	0	1	1	0	1
15	0	1	0	0	1
16	0	0	1	0	1
17	0	1	1	0	1
18	0	0	1	0	1
19	0	1	0	0	1
20	0	1	1	0	1
21	0	0	1	0	1
22	0	0	1	0	1
23	0	0	1	0	1
24	0	0	1	0	1
25	0	1	0	0	1
26	0	1	1	0	1
27	0	0	1	0	1
28	0	1	1	0	1
29	0	1	1	0	1
30	0	1	1	0	1
31	0	0	0	0	1
32	0	0	1	0	1
33	0	1	1	0	1
34	0	0	1	0	1
35	0	1	1	0	1
36	0	1	0	0	1
37	0	0	1	0	1
38	0	0	1	0	1
39	0	1	1	0	1
40	0	0	1	0	1
41	0	0	0	0	1
42	0	1	1	0	1
43	1	1	1	0	1
44	0	0	1	0	1
45	0	1	0	0	1
46	0	0	1	0	1
47	0	0	0	0	1
48	0	1	1	0	1
49	0	1	1	0	1
50	0	0	0	0	1
total score	**1**	**26**	**40**	**0**	**50**

Table 1: shows the total survival score of 50 *Staphylococcus aureus* groups that form 1st trial for 25%, 10% and 0% isopropanol where “1” specifies survival and “0” specifies death.

Well number	25% isopropanol	10% isopropanol	0% isopropanol	N.C.	P.C.
1	0	1	1	0	1
2	0	0	0	0	1
3	0	1	1	0	1
4	0	1	1	0	1
5	0	0	1	0	1
6	1	1	1	0	1
7	0	0	1	0	1
8	0	1	1	0	1
9	0	1	1	0	1
10	0	1	1	0	1
11	0	1	1	0	1
12	0	0	1	0	1
13	1	1	1	0	1
14	0	1	1	0	1
15	0	1	0	0	1
16	0	0	1	0	1
17	0	1	1	0	1
18	0	1	1	0	1
19	0	1	0	0	1
20	0	1	1	0	1
21	0	1	1	0	1
22	0	0	1	0	1
23	0	0	1	0	1
24	1	1	1	0	1
25	0	0	0	0	1
26	0	1	1	0	1
27	0	0	0	0	1
28	0	0	1	0	1
29	0	1	1	0	1
30	0	1	1	0	1
31	0	1	0	0	1
32	0	0	1	0	1
33	0	0	1	0	1
34	0	0	1	0	1
35	0	1	1	0	1
36	0	1	0	0	1
37	0	1	1	0	1
38	1	0	1	0	1
39	0	1	1	0	1
40	0	0	1	0	1
41	0	0	1	0	1
42	0	1	1	0	1
43	0	1	1	0	1
44	0	0	1	0	1
45	0	1	1	0	1
46	0	0	1	0	1
47	0	1	0	0	1
48	0	0	1	0	1
49	0	0	1	0	1
50	0	0	1	0	1
total score	**4**	**29**	**42**	**0**	**50**

Table 2: shows the total survival score of 50 *Staphylococcus aureus* groups that form 2nd trial for 25%, 10% and 0% isopropanol where “1” specifies survival and “0” specifies death.

Well number	25% isopropanol	10% isopropanol	0% isopropanol	N.C.	P.C.
1	0	1	1	0	1
2	0	1	0	0	1
3	0	0	1	0	1
4	0	1	1	0	1
5	0	0	1	0	1
6	0	1	1	0	1
7	0	0	1	0	1
8	0	1	1	0	1
9	0	0	1	0	1
10	0	1	0	0	1
11	0	0	1	0	1
12	0	0	1	0	1
13	0	1	1	0	1
14	0	0	1	0	1
15	1	1	0	0	1
16	0	1	1	0	1
17	0	1	1	0	1
18	0	0	1	0	1
19	0	1	0	0	1
20	0	1	1	0	1
21	0	0	1	0	1
22	0	0	1	0	1
23	0	0	1	0	1
24	0	0	1	0	1
25	0	1	0	0	1
26	0	1	1	0	1
27	0	0	1	0	1
28	0	1	1	0	1
29	0	1	1	0	1
30	0	1	1	0	1
31	0	1	0	0	1
32	1	0	1	0	1
33	0	1	1	0	1
34	0	0	1	0	1
35	0	1	1	0	1
36	0	1	0	0	1
37	0	0	1	0	1
38	0	0	1	0	1
39	0	1	0	0	1
40	0	0	1	0	1
41	0	0	1	0	1
42	0	1	1	0	1
43	0	1	1	0	1
44	0	1	1	0	1
45	1	1	0	0	1
46	0	0	1	0	1
47	0	1	1	0	1
48	0	0	1	0	1
49	0	0	1	0	1
50	0	0	0	0	1
total score	**3**	**27**	**40**	**0**	**50**

Table 3: shows the total survival score of 50 *Staphylococcus aureus* groups that form 3rd trial for 25%, 10% and 0% isopropanol where “1” specifies survival and “0” specifies death.

Isopropanol percentage (%)	Average Survival percentage (%)		Type of used bacteria	Type of used broth	Concentration of bacteria-broth suspension (McFarland) ±0.01 McFarland	Volume of bacteria-broth suspension (1 well) (µL) ±0,1 µL	Volume of disinfectant (1 well) (µL) ±0,1 µL	Temperature of Incubator (°C) ±0,1 °C
25	trial 1	2	*Staphylococcus aureus*	Mueller Hinton Broth	0.5	100.0	100.0	37.0
	trial 2	8						
	trial 3	6						
10	trial 1	52	*Staphylococcus aureus*	Mueller Hinton Broth	0.5	100.0	100.0	37.0
	trial 2	58						
	trial 3	54						
0	trial 1	80	*Staphylococcus aureus*	Mueller Hinton Broth	0.5	100.0	100.0	37.0
	trial 2	82						
	trial 3	80						

Table 1: This table shows the survival percentage of *Staphylococcus aureus* exposed to suspension of disinfectants with different isopropanol percentage and same concentrated *S.aureus* - Mueller Hinton Broth suspension.

isopropanol percentage(%)	25	10	0
trial 1	2	52	80
trial 2	8	58	82
trial 3	6	54	80
mean	5.333333333	54.66666667	80.66666667
Median	6	54	80
Range	8	14	6
Variance	9.333333333	9.333333333	1.333333333
standard deviation	3.055050463	3.055050463	1.154700538
standard error	1.763834207	1.763834207	0.666666667
T	4.30265273	4.30265273	4.30265273
95% CI	7.589166067	7.589166067	2.868435153

Table 2: This table shows the statistics of 3 trials of the *Staphylococcus aureus* mean survival percentage for different percentages of isopropanol: 25%, 10%, 0%. The data above is provided using Microsoft Office Excel 2011.

Analysis of Variance (One-Way)

Summary

Groups	*Sample size*	*Sum*	*Mean*	*Variance*
25	3	16.	5.33333	9.33333
10	3	164.	54.66667	9.33333
0	3	242.	80.66667	1.33333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p-level*	*F crit*
Between Groups	8,784.88889	2	4,392.44444	658.86667	9.31219873E 08	8.05209
Within Groups	40.	6	6.66667			
Total	8,824.88889	8				

Table 3: This table shows the Anova calculations for the groups.

Data analysis

To obtain the values at the table 2 I used the formulas below:

As mean: [10]

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i = \frac{1}{n}(x_1 + \cdots + x_n)$$

where; n is trial number (in my experiment it's 3)

xi is survival percentage value for trial 1

As standard deviation: [11]

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2},$$

where; n is trial number (in my experiment it's 3)

[10] http://en.wikipedia.org/wiki/Average#cite_ref-1

[11] http://en.wikipedia.org/wiki/Standard_deviation

xi is survival percentage value for trial 1

$\bar{x}$ is the mean value which is founded before

As standard error: [12]

$$SD_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where; n is trial number (in my experiment it's 3)

$\bar{x}$ is the mean value which is founded before

σ is the standard deviation of the groups.

As range:

In the descriptive statistics, the range of a set of data is the difference between the largest and smallest values. [13]

$$X_r = X_2 - X_1$$

where; X_r is range

X_2 is the largest value

X_1 is the smallest value

As median:

In statistics and probability theory, median is described as the numerical value separating the higher half of a sample, a population, or a probability distribution, from the lower half. Arranging all the observations from lowest value to highest value and picking the middle one can find the median of a finite list of numbers. If there is an even number of observations, then there is no single middle value; the median is then usually defined to be the mean of the two middle values. [14] [15]

For example, if a < b < c, then the median of the list {a, b, c} is b.

[12] http://en.wikipedia.org/wiki/Standard_error

[13] **^** George Woodbury (2001). *An Introduction to Statistics*. Cengage Learning. p. 74. ISBN 0534377556.

[14] ***a b*** Weisstein, Eric W., "Statistical Median" from MathWorld.

[15] http://www.stat.psu.edu/old_resources/ClassNotes/ljs_07/sld008.htm Simon, Laura J.; "Descriptive statistics", *Statistical Education Resource Kit*, Pennsylvania State Department of Statistics

As variance: [16]

$$\sigma^2 = \frac{\sum (X - \overline{X})^2}{N}$$

Where; σ^2 is variance

x is survival percentage value for trial 1

$\overline{x}$ is the mean value which is founded before

N is number of scores

As t value: [17]

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

where ; $\overline{x}_1$ is the mean value of 1st data set

$\overline{x}_2$ is the mean value of 2nd data set

S_1^2 is the standard deviation of 1st data set

S_2^2 is the standard deviation of 2nd data set

N_1 is the number of scores in the 1st data set

N_2 is the number of scores in the 2nd data set

[16] Cited by Yue Yin from Welkowitz, J, Ewen, R & Cohen, J <Introductory Statistics for the Behavioral Science> page 56-58.

[17] http://ncalculators.com/math-worksheets/how-to-calculate-t-test.htm

As %95 confidence interval: [18]

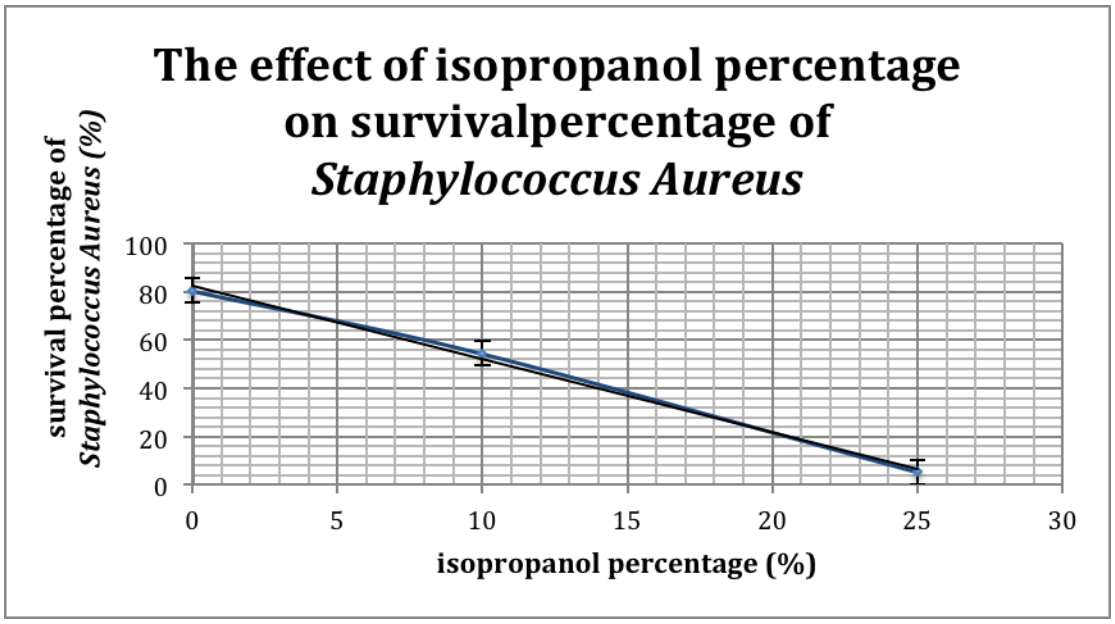
$$\bar{x} \pm t \bullet \frac{\sigma}{\sqrt{n}}$$

where; $\bar{x}$ is the mean value which is founded before

t is t value which is founded before

σ is standard deviation

n is number of scores



Graph 1: This graph shows the relation between the isopropanol percentage in a disinfectant and the survival percentage of *Staphylococcus aureus* exposed to it. The error bars are drawn with 95%-percentage error.

[18] http://www.stat.yale.edu/Courses/1997-98/101/confint.htm

Conclusion and Evaluation

In this experiment, the effect of the percentage of isopropyl alcohol in the disinfectants on survival percentage of *Staphylococcus aureus* is investigated. It was hypothesized that as percentage of isopropanol increases in a disinfectant, the survival percentage of *S.aureus* when it is exposed to the disinfectant decreases. So, three disinfectants with different percentages of isopropanol in them were founded to investigate the survival percentages of *S.aureus* after exposing them to these disinfectants.

The mean values of the survival percentage of *S.aureus* as the result of my experiment are 5.3, 53.6 and 80.6 when they are exposed to disinfectants with 25, 10, 0 percentages of isopropanol respectively. The most effective disinfectant used in the experiment is the one with 25 percentage of isopropanol in it and the least effective disinfectant used in the experiment is the one with 0 percentage of isopropanol in it. So, it can be said that as the percentage of isopropanol in a disinfectant increases, the survival percentage of the bacteria that is exposed to it decreases. Also the p value is too small ($p = 6.10111208E-09 < \alpha = 0.05$), so my hypothesis is valid. “The higher level of alcohol percentage or "proof" the more effective it is.” [19] As it is mentioned in the quoted sentence above, when the alcohol percentage is increased its effects as a disinfectant also increase, so this sentence’s idea verifies my experiments results.

“Standard deviation is a widely used to measure the variability or diversity used in statistics and probability theory. It shows how much variation or "dispersion" exists from the average. A low standard deviation indicates that the data points tend to be very close to the mean, whereas high standard deviation indicates that the data points are spread out over a large range of values.”[20] The standard deviation of 3 trials for the survival percentage of *S.aureus* after it is exposed to the disinfectant with 25 percentage of isopropanol in it is 3.05, with 10 percentage of isopropanol in it

[19] http://www.beautifuldragons.com/Disinfectants.html
[20] http://www.princeton.edu/~achaney/tmve/wiki100k/docs/Standard_deviation.html

3.05, with 0 percentage of isopropanol in it is 1.15. The standard deviations of the survival percentage of *S.aureus* are low so it can be said that the data found in 3 trials are close each other, they aren't spread and the variability of the data found in 3 trials is few.

"The standard error is the standard deviation of the sampling distribution of a statistic so that when the standard deviation is divided to sample count the error for mean values for 3 trials of the survival percentage of *S.aureus* after it is exposed to 3 different disinfectants with different percentages of isopropanol in them."[21] The standard deviation in this experiment is low, so the standard error is also low. The standard error of 3 trials for the survival percentage of *S.aureus* after it is exposed to the disinfectant with 25 percentage of isopropanol in it is 1.76, with 10 percentage of isopropanol in it 1.76, with 0 percentage of isopropanol in it is 0.66.

Because the standard error and standard deviation of the results of the experiment is low, it can be said that this experiment is precise and accurate so, its results are reliable. In the experiment to minimize the cause of error, the following factors kept constant:

- Concentration and volume of bacteria-broth suspension is kept constant in each trial so the amount of bacteria that will be affected from disinfectant will be same. In all trials 0.5 McFarland concentrated 100 μL bacteria-broth suspension is prepared by using McFarland densitometer.
- Type of bacteria used in the experiment is constant; the bacteria samples that brought from public transport is labeled by using machine for automatic classification and S.aureus is separated from others and used in this experiment. By using one kind of bacterium I prevented the changes in my results due to the difference of immunity of bacteria.
- The temperature of the incubator (37 °C) and the type of used broth in the experiment (Mueller Hinton Broth) is constant. By that all bacteria have the

[21] Everitt, B.S. (2003) *The Cambridge Dictionary of Statistics*, CUP. ISBN 0-521-81099-X

same conditions for growth so there won't be any changes in the results of this experiment because of this.

- Volume of the disinfectants is kept constant for each trial so that the amount of disinfectants affect certain amount of bacteria will be same, hence the result of the experiment will not be affected by it.

Even though I minimized the errors by stabilizing constant variables, it doesn't mean there are no errors in the experiment. There still can be errors like:

- Even though I used Bunsen burner in order to ensure a sterilized place to work, there are many bacteria and viruses in air and when I was doing my experiment. So, some of them might have contacted with my bacteria-broth suspension and the result of my experiment could have changed because of it.

If more sterilized medium was provided for this experiment my results would be more accurate. But with my facilities using Bunsen burner was the best way for me to ensure sterilization so, there are some errors in my experiment.

- When sterilizing place and materials used in the experiment by using Bunsen burner the survival percentages of bacteria I used can be affected by the heat so, my experiment's results can be affected.

In order to avoid the changes in the survival percentage of the bacteria used in the experiment that is caused by the heat produced during sterilization, the materials used in experiment can be sterilized by different kinds of chemicals with sterilization effect and I could work under a fume hood.

After observing the results and accessed an answer to the research question "How does the percentage of isopropyl alcohol in the disinfectants, which are 25%, 10%, 0%, affect the survival percentage of *Staphylococcus aureus* when the ethyl alcohol percentage in the disinfectants are constant and equal to 70%?" another question comes to light: Does the type of alcohol in the disinfectants affects the

survival percentage of *Staphylococcus aureus?* Some researches can be arranged in order to investigate the effect of different kinds of alcohol with same concentrations in disinfectants on survival percentage of *S.aureus*.

Also investigating the effect of different isopropanol concentrated disinfectants on bacteria other than *S.aureus* can also be done. There are plenty of bacteria kinds around us all day.

Disinfectants are being used in our lives all the time because of their destroying effect on microorganisms such as bacteria. As we use disinfectants to kill organisms it is conferring benefit to know what properties of disinfectants makes it more effective to kill microorganisms. In this research it is found that the percentage of certain kind of alcohol in disinfectants changes the disinfectant's killing effect. As the optimum percentages of alcohols in disinfectants are found, people can use disinfectants more efficiently.

APPENDIX

Appendix 1:

The names of these 3 different disinfectants are **Disinfecsol, OPA-70** and **ANIOSRUB 85 NPC.** These disinfectants are being used in hospitals and biology laboratories for hand and skin cleaning.

- The disinfectant **Disinfecsol** consists in 100 mL for mass/volume 50 g ethyl alcohol, 20 g isopropyl alcohol, glycerin, emollient, conservatives, perfume, coloring and pure water.
- The disinfectant **OPA-70** consists 70% ethyl alcohol, 10% isopropanol, glycerin, emollient agents, and coloring.
- The disinfectant **ANIOSRUB 85 NPC** consists 70% ethanol, moisturizing and emollient agents and water.

Appendix 2:

Sheep (*Ovis aries*) are quadrupedal, ruminant mammals typically kept as livestock. Like all ruminants, sheep are members of the order Artiodactyla, the even-toed ungulates. Although the name "sheep" applies to many species in the genus *Ovis*, in everyday usage it almost always refers to *Ovis aries*. Numbering a little over one billion, domestic sheep are also the most numerous species of sheep.[22]

Appendix 3:

Mueller Hinton Broth is a general-purpose medium that may be used in the cultivation of a wide variety of fastidious and nonfastidious microorganisms. This formulation has not had its calcium and magnesium ion concentrations adjusted to make it suitable for use in quantitative procedures for antimicrobial susceptibility testing.[23]

Appendix 4:

The lack of standardization for the determination of bacterial susceptibility continued to be a problem throughout the early 1960s. Kirby and his colleague, A. W. Bauer, extensively reviewed the susceptibility testing literature. They consolidated and updated all the previous descriptions of the disk diffusion method and published their findings[24]. This publication led the World Health Organization to form a committee in 1961 to lay the groundwork for the development of a standardized procedure for

[22] http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=552475

[23] http://www.bd.com/ds/productCenter/297220.asp

[24] Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 36:493-496

single antimicrobial disk susceptibility testing[25]. The result was a standardized procedure for the disk diffusion susceptibility test, henceforth called the Kirby-Bauer disk diffusion test.

Currently, the Clinical Laboratory Standards Institute (CLSI) is responsible for updating and modifying the original procedure of Kirby and Bauer through a global consensus process. This ensures uniformity of technique and reproducibility of results as pathogens develop new mechanisms of resistance and new antimicrobials are developed to fight these organisms.

John C. Sherris, M.D., Professor Emeritus, Department of Microbiology, University of Washington Medical School, Seattle. He was a driving force behind the development of standardized susceptibility testing, culminating in the standardized disk diffusion test, now known as the Kirby-Bauer or Bauer-Kirby test. His achievements are recognized internationally, through his years of service with the World Health Organization's Expert Advisory Panel on Biological Standardization and the International Collaborative Study on Antibiotic Sensitivity Testing. He also holds an honorary medical degree from the Karolinska Institute, Stockholm, Sweden.[26]

Appendix 5:

In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range.

Original McFarland standards were mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4).

Now there are McFarland standards prepared from suspensions of latex particles, which lengthens the shelf life and stability of the suspensions. The standard can be compared visually to a suspension of bacteria in sterile saline or nutrient broth. If the bacterial suspension is too turbid, it can be diluted with more diluent. If the suspension is not turbid enough, more bacteria can be added.[27]

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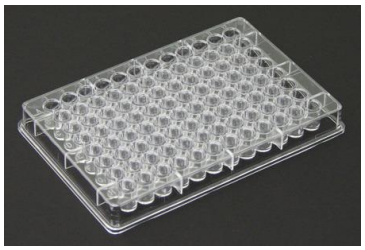
[25] Jorgensen, J. H., and J. D. Turnidge. 2007. Susceptibility test methods: dilution and disk diffusion methods, p. 1152–1172. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, D.C.

[26] http://scienceblog.com/community/older/2004/1/2004940

[27] THE NEPHELOMETER:AN INSTRUMENT FOR ESTIMATING THE NUMBER OF BACTERIA IN SUSPENSIONS USED FOR CALCULATING THE OPSONIC INDEX AND FOR VACCINES. JOSEPH McFARLAND, M.D. JAMA. 1907; XLIX(14):1176-1178.

Appendix 6:



A Microtitre plate (spelled microtiter in the United States) or microplate or microwell plate, is a flat plate with multiple "wells" used as small test tubes. The microplate has become a standard tool in analytical research and clinical diagnostic testing laboratories.[28]

[28] http://www.corporeality.net/museion/category/medical-scientific-instruments/

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