What is the best alternative for paraben to prevent the growth of bacteria *Escherichia coli* as a hand-washing antibacterial agent?

Biology Extended Essay

Word count: 3967

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I.Introduction

I wanted to write my extended essay from biology since 9th grade, finding a topic wasn't my strong suit. I started looking at my life as a potential EE topic. Then one day when my best friend wanted her hand moisturizer from her bag, I realized that the rash on her skin was getting worse every day. She said that her dermatologist diagnosed her with eczema from her obsession of washing her hands too often. I was curious about why it only happened at school time and not on summer time. I got curious if it's caused by the cheap soaps that are used in our school.

In my family, we use paraben-free soap in daily use since my sister is allergic to that substance. Paraben [1]is known for estrogen-mimicking properties and with profuse amounts of usage enhancing the chance of breast cancer. Although it has this detrimental effect, it is still widely used in soap manufacture today for preventing yeast and bacterial growth in cosmetics, body washes and even our daily use soaps. 'Normal human keratinocytes and the skin equivalents were cultured in the medium containing methylparaben. The following changes were analysed: proliferating ability, apoptotic cells, morphological changes, mRNA and protein expressions.'[2] which shows that apart from their big picture aftermath as cancer parabens also cause skin irritation such as rashes, allergic skin reactions, contact dermatitis and even eczema which was what my friend was experiencing.

I wondered why it was still used widely with all of this background information on this matter does the other types of chemicals not work as well? I started researching about what were the

[1] Cunningham, Vanessa. "10 Toxic Beauty Ingredients To Avoid." *The Huffington Post*, TheHuffingtonPost.com, 23 Jan. 2014, www.huffingtonpost.com/vanessa-cunningham/dangerous-beauty-products_b_4168587.html.

[2] Ishiwatari , S, et al. "Effects of Methyl Paraben on Skin Keratinocytes." *PubMed NCBI*, 2006, www.ncbi.nlm.nih.gov/pubmed/17186576.

companies using in their soaps to prevent bacterial growth if they are advertising it as paraben free. I realized that there were so many options such as the ones using triclosan - an ingredient added to many consumer products intended to reduce or prevent bacterial contamination[3]- the and the ones that are 100% natural[4]. Methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, isopropylparaben and benzylparaben are the most commonly used ones apart from sodium salts. [5]I wanted to compare different kinds cleaning agents' affects compared to a soap containing mehtlyparaben.

I will use *Escherichia Coli* (also referred as *E-coli*) and cultivate it in a Mueller Hinton Agar. After the cultivation I will use the Kurby Bauer Disk Diffusion Method to embed the independent variables in the culture. The perimeter of the experimentation area will be measured after 48 hours and the data will be collected.

I chose *E-coli* for this experiment because it's a gram-negative bacterium which is generally harmless although it might cause, if contaminated, diarrhea. Also in ATCC website it was categorized in Level 1 danger level which is the most suitable for usage in high school level. It is the most prevalent infecting organism in the family of gram-negative bacteria known as *Enterobacteriaceae.*[6] It is generally not found in the hand flora, but the other types of bacteria were either too dangerous to work with or hard to find as a culture. Also, the medical soap I am using is a product of a company who particularly works with colon cancer, so E. coli is a species they can encounter while changing the stoma bags.

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[3] Office of the Commissioner. "Consumer Updates - 5 Things to Know About Triclosan." U S Food and Drug Administration Home Page, Center for Drug Evaluation and Research, 19 Dec. 2017.
[4] What Makes Antibacterial Soap Antibacterial?" *Illumin - The Quest for the Perfect Racket: Advances in Tennis Racket Design*, Dec. 2007.
[5] The Problem With Parabens." *Savvy Brown*, Apr. 2010.
[6] Clark, Marler. "About E. Coli Food Poisoning." *E. Coli Food Poisoning.*

My aim is to identify the chemical or maybe the natural ingredient that can be used instead of parabens to eliminate the side effects of using parabens. I will use 5 types of soaps – a brand that is 100% natural, antibacterial, common use, medical use and a soap containing methylparaben- and have 5 trials to calculate the mean and the standard deviation of the data.

So, my research question is;

What is the best alternative for paraben to prevent the growth of bacteria *Escherichia coli* as a hand-washing antibacterial agent?

II.Hypothesis

Parabens are used in the preservation of cosmetics and cleaning agents since 1920s as an antibacterial additive. In a previous experiment conducted by Nguyen T[1], Clare B, Guo W, Martinac B. it is indicated that of the previously unidentified mechanisms of action of parabens as antimicrobial agents is via an interaction with the mechanosensitive channels to upset the osmotic gradients in bacteria.[7] This way they alter the water concentration within the cell. This way the organism becomes vulnerable causing its death making it an effective antibacterial agent.

Methylparaben, also known as methyl ester of *p*-hydroxybenzoic acid with the chemical formula $CH_3(C_6H_4(OH)COO)$[8], is one of the most commonly used preservative in the cosmetic market nowadays. Although it is normally seemed as mildly harmful it can be allergenic and irritating to the skin. Studies indicate that methylparaben applied on the skin may react with UVB, leading to increased skin aging and DNA damage.[9] It is also known that it causes accumulation on breasts causing hormone-positive cancer.

[7]Nguyen, T, et al. "The Effects of Parabens on the Mechanosensitive Channels of E. Coli." *PubMed NCBI*, *The University of Western Australia*, 2005.
[8]Hazardous Substances Data Bank (HSDB)." *U.S. National Library of Medicine*, National Institutes of Health.
[9] Handa O, Kokura S, Adachi S, Takagi T, Naito Y, Tanigawa T, Yoshida N, Yoshikawa T (October 2006). "Methylparaben potentiates UV-induced damage of skin keratinocytes". *Toxicology*. **227** (1–2): 62–72

On the other hand, the antibacterial agents used in different soaps varies into a wide range. There's DMDH, 1,3-Dimethylol-5,5-dimethylhydantoin, which is an antibacterial organic preservative used in the natural soap that I will be using in this experiment. Methylchloroisothiazolinone used in commonly used soap is effective against both gram-positive and gram-negative bacteria although they have some side effects as well. 'These, Methylisothiazolinone (MIT) and Methylchloroisothiazolinone (CMIT), common preservatives are found in many liquid personal care products, and have been linked to lung toxicity, allergic reactions[10] and possible neurotoxicity.[11]' Phenoxyethanol is used in the antibacterial soap that I will be using in this experiment. It is a natural antibacterial also used in many vaccines and bug repellants.

The environment I will conduct the experiment will be produced in a petri dish and use the Kirby-Bauer Disk Diffusion Method which normally a method to examine the durability of a specific bacteria to different kinds of antibiotics, but it is usable for soaps as well. The tested variable creates a circular zone of inhabitation where it is effective to stop the reproduction of the bacteria which will be measured.

These are all widely used antibacterial width all ranges of effectiveness but still even though they are cancerogenic parabens are in the use for nearly a century. There must be a reason why this highly corrosive is still on use so I hypothesized that: **Even though parabens will be highly effective and have a wide area on the petri dish which indicates the efficiency of the antibacterial agent, commonly used soap including more of a chemical based antibacterial factor, Methylisothiazolinone, since it was manufactured to be an antibacterial agent rather than being a natural matter which was altered to be effective**

[10] Rohm & Haas (2002). Acute Inhalation toxicity study in rate (methylisothiazolinone 53.52% active ingredient). Rohm & Haas Chemicals, LLC Report, 06R-1002.

[11] Final report of the safety assessment of methylisothiazolinone. International journal of toxicology,2010.

in that job than the other soap types will also have similar a result. The difference between the radiuses of the highly chemical preservatives and natural ones will be observable. The more natural the preservative is the less affect it has on the bacteria due to their pH range and effectiveness since they only contain oils and salts as antibacterial agents not any chemical produced to be an antibacterial.

III. Method Development and Planning

I used the ATCC biosafety levels (*appendix 1*) as a base and searched for a type of bacteria. My supervisor said that a bacterium must score a maximum of level 1 on the scale, so I ended up with 3 types of bacteria from which I chose *E- Coli* since it was the easiest to obtain at my position. I spoke with my friends who had a biologist parents and one of them said they had the sample in their laboratory and that I can use it for my experiment.

After that I started searching for the types of soaps to use. I went to the supermarkets nearby and spent hours in front of the soap aisle searching every ingredient in the soaps to find the best suitable 5 for the experimentation. First day after searching 4 supermarkets I came home with Olive Blossom Natural Olive Oil Liquid[12](Natural) (*appendix 6)* soap and Activex Antibacterial Liquid Soap (Antibacterial) (*appendix 4)*. I was furious that I was unable to find a brand that included paraben. Upon further research I learnt that parabens were advertised under different names such as nipagin or methyl-4-hydroxybenzoate so that customers wouldn't be alerted by the chemical's name since it is a well-known carcinogen. The next day I started searching again and found Le Petit Marseiliais Mediterranean Honey Liquid Soap *(appendix 5)* including methylparaben and as for common use soap I bought Migros Rose Scented Liquid Soap

[12] The ingridients are in the appendix

(appendix 3). I also asked for a medically used soap from my mother since she is a medical sector, so I decided to use her brand which is Medical Skin Cleanser *(appendix 2)*.

With the whole bacteria and soap dilemma out of the way I started researching methods to map my experiment and conduct it. At first, I thought I would apply all the variables of soaps on top of the cultivated bacteria, but it seemed like a waste of substances by using 25 petri dishes and a lot of bacteria. I came across Kirby-Bauer Disk Diffusion Method and it seemed reasonable for my experiment. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.[13] Even though it is normally for antibiotic resistance testing on different species of bacteria, I thought it would also be suitable for my experiment as well. I decided to soak the filter paper disks that I will prepare before the experiment, in different types of soaps and embed them into my Mueller-Hinton agar with E. coli.

In order to make my experiment scientific and accurate, I will have 5 independent variables and 5 trials to calculate the mean, standard deviation and the variance in my experimentation group. Except my independent variables, types of soaps, everything will be stable through the 5 experimentation groups. I was advised to conduct the experiment both in single and double layers to get an optimum result. So, I decided to try the experiment with both one layer and two layers of filter paper to optimize the soap concentration in the agar plate and get a clearer result. All of them will be kept in the same environment and they will be measured with the same ruler to avoid the random error as much as I can. Also, Mueller Hinton Agar will be used in this

[13]Hudzicki, Jan. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol." *ASMscience*, American Society of Microbiology, 8 Dec. 2009.

experiment since it is an optimized environment for bacterial growth, and it has less chance of error because of the fabrication of agar plates.

Even with all this precaution some problems may occur anyway. There are 6 major factors that affect the bacterial growth nutrition concentration, temperature, gaseous concentration, pH, ions and salt concentration, available water.[14] Even though I will try to stabilize some of these variables, there are still some that I can't control. (I will be conducting the experiment in a sterile laboratory of a local hospital (Ankara Education and Research Hospital *(appendix 7)*) all of these variables will be limited since it is a professional environment so that it can be the most accurate.

I will use a bacterial nutrient broth instead of the solid culture since it is more homogenous and easier to apply to the petri dishes. By this way it will be easier to obtain an equal layer of sample to get more accurate data.

The data will be obtained 24 hours of incubation in 37°C with a millimetric ruler and recorded carefully for all 25 (5 variables x 5 trials) of the samples. 24 hours later after the data collection the change in the diameter will be observed qualitatively this time to understand the range of effectiveness of every variable.

Variables	Name of variable	Method of management and/or measurement
Independent variable	Different types of soap	
Dependent variable	Zone of bacterial incubation	Using the same brand and genus (ATCC *Eschericia Coli*)
Controlled variables	Temperature of incubation zone, petri dish (diameters, material), using the same densicheck calibrator, amount soap in each trial, exact ruler	Using an incubator, same brand of petri dish

Table 1: Table showing the variables in the experiment to figüre out the best alternative to paraben as a hand-washing antibacterial agent.

[14] Karki, Gaurab. "Factor Affecting Bacterial Growth -." *Biology Notes*, 18 Dec. 2018.

IV.Method

a. Materials:

- *ATCC Escherichia Coli* (x5)
- 5x Petri dishes
- 5x Sterile swaps
- 5x Mueller Hinton Agar
- Incubation Unit
- Bunsen Burner
- Densicheck Calibrator
- 50x 1mm diameter filter paper
- Forcep
- Marker
- Millimetric ruler
- Experimental sterile tubes
- Perforator
- Scissors
- Olive Blossom Natural Olive Oil Liquid Soap (Natural soap)
- Rose Scented Liquid Soap (Common Use)
- Antibacterial Liquid Soap
- Mediterrian Honey Liquid Soap (Paraben included soap)
- Skin Cleanser (Medical use soap)
- Gloves

b. Method:

Since it is a long process, I decided to divide the process in 4 parts consisting of;

- Preparing the filter papers
- Cultivating the bacteria
- Embeding the variables
- Collecting data

i.Preparing the filter papers

1. Cut 5 filter papers like a stripe with 15 cm length and 5 cm width.
2. Pour 5 types of soaps in sterile cups and name the cups to avoid further misunderstandings.

3. Put the filter papers in the cups and be sure that every part of the paper is in the soap.
4. Let it soak in and after 10 minutes, take the filter papers with a forcep and place them in a petri dish.
5. Let them dry for 12 hours.
6. After they are fully dried, take out the filter papers and create circular filter papers to use in the experiment with a sterile perforator.

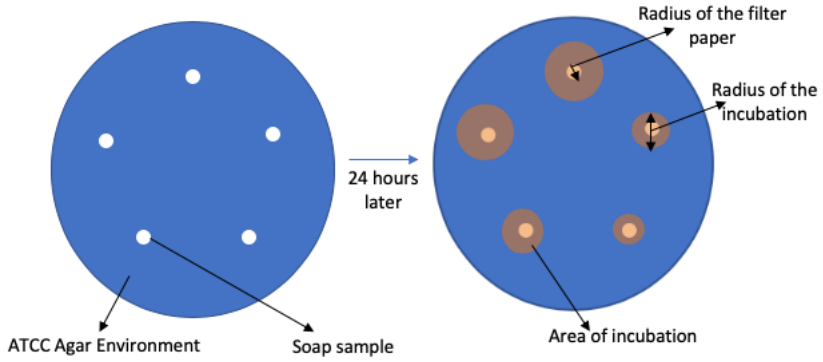
ii. Cultivating the bacteria

1. Take 5 Mueller-Hinton Agar and leave them in the room temperature for 15 minutes while their caps are still closed
2. Light the Bunsen Burner to avoid any contamination that would disturb the experiment and prepare a 0.5 McF ATTC *E-Coli* broth.
3. Take a sterile swap and put into the bacterial broth then swipe it gently horizontally across the agar plate. Once it covers the whole plate turn it 45 degrees and swipe it again for 2 times. Make sure that it covers the whole dish, it must be a uniform layer of bacteria.
4. Put the top of the petri dish back on and label it.
5. Repeat all the steps 4 more times.

iii.Embeding the variables

1. Using the marker divide the petri dish into 2 equal parts and label them 1 layer and 2 layers.
2. With a forcep pick, place and gently tap – to make sure it is embedded well- 5 filter papers on the petri dish in the 1-layer part that had been divided. *(as shown in figure 1)* (Kirby-Bauer Disk Diffusion Method)
3. Then repeat the same step with 2 layers of soap filters.
4. Repeat the steps 2 and 3 for all the remaining soap types.

5. Flame sterilize the forcep with ethanol and Bunsen Burner in between different types of variables.



Picture 1: Model showing the experimentation process

iv.Collecting the data

1. Wait a day after conducting the experiment.
2. After 24 hours, measure the radius of the circle around the filter papers with a millimetric ruler.
3. 12 hours after the data collection observe the left effectiveness of the soap.

V. Data Collection

Raw data

Soap name	Trial 1 (±0.005 mm)	Trial 2 (±0.005 mm)	Trial 3 (±0.005 mm)	Trial 4 (±0.005 mm)	Trial 5 (±0.005 mm)	Mean	Standard deviation	Variance
	Single layer							
Antibacterial	11.5	11	12.5	12	11	11.6	0.58	0.33
Common use	8.2	9.25	7.2	8.2	6	7.77	1.1	1.19
Natural	15	15	15	15	15	15	0	0
Paraben	14	14	14	14	0	11.2	5.6	31.4
Medical use	0	0	0	0	0	0	0	0
	Double layer							
Antibacterial	9	9	9	10	10	9.4	0.50	0.24
Common use	12.2	13.2	12.2	11.2	13.2	12.4	0.75	0.56
Natural	14	13	14	13	14	13.6	0.50	0.25
Paraben	13	13	15	12	13	13.2	0.98	0.96
Medical use	0	0	0	0	0	0	0	0

Table 2: Table showing the raw data collected by cultivation E.Coli bacteria with 5 different soap samples

After 36 hours

Observational data

Antibacterial: still effective

Common use: No change

Natural: All the effect is lost

Paraben: Slight effect visible

Medical use: No effect at all

Sample Calculations

Mean: $\frac{\sum x}{N} = \frac{11.5+11+12.5+12+11}{5} = 11.6$

These means will be used in while graphing the results and reaching a conclusion.

Standard deviation (σ): $\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$

1: $(x - \bar{x})$= 11.5− 11.6= -0.1
2: $(x - \bar{x})$= 11−11.6= -0.6
3: $(x - \bar{x})$= 12.5 – 11.6 = 0.9
4: $(x - \bar{x})$=12 − 11.6 = 0.4
5: $(x - \bar{x})$= 11 − 11.6 = $= \sqrt{\frac{(-0.1)^2+(-0.6)^2+(0.9)^2+(0.4)^2+(-0.6)^2}{4}}$
=0.58

Standard deviation is used to calculate the precision of the experiment, a low standard deviation means that the calculations are precise

Variance= σ^2= 0.33

Variance shows how far each trial is from the mean.

Area of incubation = Total area of contamination – area of the filter paper (r_2= 6mm)

$= \pi {r_1}^2 - \pi {r_2}^2$
= 103.9 − 28.3
= 75.6 mm^2

This calculation is the find the change in area so the area of the filter paper is removed.

Processed Data

Soap name	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	Standard deviation
	Single layer						
Antibacterial	75.6	66.7	94.4	84.8	66.7	77.6	10.7
Common use	24.5	38.9	12.4	24.5	10.2	22.1	10.2
Natural	148.4	148.4	148.4	148.4	148.4	148.4	0
Paraben	125.6	125.6	125.6	125.6	0	100.5	50.2
Medical use	0	0	0	0	0	0	0
	Double layers						
Antibacterial	35.3	35.3	35.3	50.2	50.2	41.3	7.3
Common use	88.6	108.5	88.6	70.2	108.5	92.9	14.4
Natural	125.6	104.4	125.6	104.4	125.6	117.1	10.4
Paraben	104.4	104.4	148.4	84.8	104.4	109.3	21.0
Medical use	0	0	0	0	0	0	0

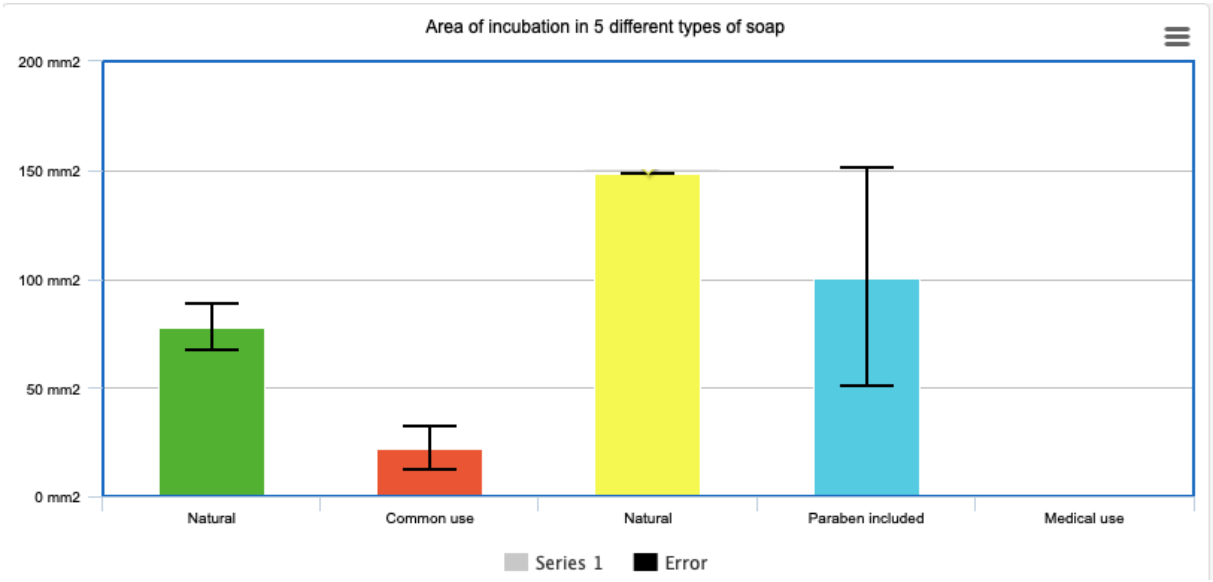
Table 3: Processed data table showing the area of incubation in 5 different soap types.

Source	***SS***	***df***	***MS***	
Between-treatments	71639.9456	4	17909.9864	$F = 26.0975$
Within-treatments	13725.44	20	686.272	
Total	85365.3856	24		

Table 4: Processed data table showing the one way ANOVA variance results in single layer samples

The *f*-ratio value is 26.0975. The *p*-value is < .00001. The result is significant at $p < .10$.

The ANOVA variance is a test that is conducted in scientific experiments to find the significance of the data collected from an experiment. Since my experiment has small data since it focuses on bacterial incubation, it is significant in $p < .10$.



Graph 1: Graph showing the area of incubation in 5 different soaps in single layer trials.

VI. Evaluation

The aim of this study was to identify the best alternative to paraben in daily usage of soaps to decrease its harm to the human body which may even lead to breast cancer. The experiment was set in a laboratory environment with 5 different soap types – one of them including paraben- with ATCC *Escherichia Coli* samples. Since the single layer sample had a less average of standard deviation it was clear that it was a better sample. It was hypothesized that paraben would have an astonishing effect, but the commonly used soap would also be highly effective due to the Methylisothiazolinone, which is a harmful yet powerful chemical, in its mixture.

Although the hypothesis 'Even though parabens will be highly effective and have a wide area on the petri dish, commonly used soap will also have similar a result.' was partially supported in the meaning that the paraben had a sufficient effect it was partly disagreed on the commonly used soap side. The commonly used soap had the least effect after the medically used on actually. It was mentioned that due the chemical values of Methylisothiazolinone it would be a great alternative for paraben -even though it is harmful for male users- when focused on the

effectiveness of the variable rather than the harm. Unexpectedly, the soap with only natural ingredients had the most effect on the bacteria eliminating in average of 148.4 mm^2 *(numerical data is shown in graph 1)* around the impregnated filter papers which was 100.5 mm^2 for paraben included soap, 77.6 mm^2 for antibacterial soap, 22.1 mm^2 for commonly used soap and inadvertently 0 mm^2 for medically used soap.

My null hypothesis which was that the more chemically produced products will have a higher effect on the bacteria was rejected. The soap produced with only natural substances had the most power. Even though when natural soap was compared with chemically produced products, natural one had a less of an effectiveness span since it lost all its area of effectiveness 36 hours after the experiment and the antibacterial soap's effects were still highly observable.

The 36 hours observation was to see rather the variable lost its power with time or it remained strong. All of the independent variables had a significantly different result at the end of the experiment. The antibacterial soap was effective in a long duration and the natural soap was more efficient in short term effect. Although the antibacterial agent in antibacterial soap that I have used is Phenoxyethanol which even though don't have such gigantic harm like paraben has to the body, according to the The Material Safety Data Sheet (MDSD) it can cause skin and lung irritation and might have toxic effect to the kidneys, on the other hand natural one nearly has no harm whatsoever since it is made up of all natural ingridients.

The standard deviation of paraben included soap was high both in single-layer and double-layer trials which may be a result of random error or can be caused by the brand or the other components in the soap. Although there was still a specific variable that outshined.

The most surprising result was that the natural soap had such a distinguishable effect, but the fact that medically used soap had no effect at all on the sample *(graph 1).* After the results were analyzed I realized that this might be a result of a random error that I didn't pay attention during the experimentation or an error by the nature of the soap.

Although through the course of the experiment, there were no visible mistakes since it was conducted in a professional laboratory with many advisors, while writing this essay it was clear that there were some systematic error or confounding variables which may have affected the end result. The following points may be the cause of high standard deviation results this research had:

1. All the soaps had different content which may have affected their absorption rate by the filter paper. For example, the medically used soap had more of an alcohol-based composition so it is possible that it may have evaporated during the preparation process of the filter papers which would lead to these negative results towards the product. Even in the experimentation process it was observable that medical soap was the fastest to dry, or fastest to evaporate since it was dried in an open environment.
2. The soaps I had used were all from different brands which changes the concentration of the targeted substance. The concentration of the antibacterial agent affects the overall result in the experiment.
3. The agar environment is not a suitable representation of the skin. After the embedding of the bacteria, the petri dishes incubated in 37°C for 24 hours which is not the regular temperature for normal bacteria growth in human skin.
4. The only bacteria that was used in this experiment was *Escherichia Coli* so there was only one factor of resistance. The other types of bacteria may have different vastness of resistance towards these kinds of antibacterial agents. Also, even though it has the

minimal biosafety level and it has a fast multiplication rate, E.Coli isn't always present in human hand ignoring the circumstance of infection.

5. The viscosity of the soaps was not standard, so the absorption-evaporation ratio was different in each of them. This also may be a reason why medically used soap showed no effect on the bacteria in anyway, since it was the runniest and fluid-like of all of them. It was the first one to absorb and dry. Some of them had to wait overnight but medically used soap was dry in minutes

VII. Conclusion

My research question 'What is the best alternative for paraben to prevent the growth of *Escherichia coli* on the surface of the hand?' was answered that it is the natural soap. Its components are all natural, so it has no harm to the human and it also is the most effective in this sample group having a mean of 148.4 mm^2 *(graph 1)* area. It was pretty surprising that even though it only consists of natural salts as the antibacterial agent it was this highly achieved. The reason I chose this topic was because people around me had different kinds of problems with different types of soaps. I now understand that there is no one way to go with a soap, there are so many variables that make a kind of soap effective. There are so many ingredients that can be used as an antibacterial agent. Although on the other hand the healthiest of these agents can be detected using methods like these.

Even though there are so many alternatives, it is kind of odd that paraben regarding all its harms to the human body is still in use. I It turns out that answering that huge question was out of my abilities as a high school student. Paraben is in use since 1950s and even though it caused a huge polemic in the past years, the producers just change its name to its organic product name and continue the usage.

Soaps are actually hiddenly a huge part of our lives. Every one of us use it daily and if it has any harm to us, it would have a serious impact on our health. The ingredients in a soap can play with one's health without even them noticing. After this investigation, I can surely say that this topic needs a lot more research on it to come up with the best for human health.

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IX. Appendix

I. Appendix 1

ATCC

Product Sheet

Escherichia coli (ATCC® 25922™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Escherichia coli* (ATCC® 25922™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Designation: FDA strain Seattle 1946 [DSM 1103, NCIB 12210]
Deposited Name: *Escherichia coli* (Migula) Castellani and Chalmers
Antigenic Properties: Serotype O6, Biotype 1
Product Description: Does not produce verotoxin. This organism is a CLSI control strain for antimicrobial susceptibility testing. It is used for media testing, as a negative control for LT toxin production, and as a quality control strain for Abbott, API, Autobac, BBL, bioMerieux Vitek, Biosynth, Difco, IDS, Micro-Media, MicroScan™, Roche Diagnostics, and Sensititre products. Used in susceptibility disc testing of neomycin, colistin [colimycin], kanamycin, cephalexin, gentamicins, cefamandole, cephalothin, tetracycline, cephaloglycin, cephaloridine [cephalomycin], nalidixic acid, and chloramphenicol.

Propagation

Medium
ATCC® Medium 18: Trypticase Soy Agar/Broth

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure

1. Open vial according to enclosed instructions.
2. Using a single tube of #18 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #18 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 hours.

Notes

ATCC® 25922™ is a recommended reference strain for antibiotic susceptibility testing. It has been found that passage in broth often results in a change in MIC levels. Therefore, it is best to keep it on agar and to make stocks for storage immediately. Repeated passage is discouraged.
Purified genomic DNA of this strain is available as ATCC® 25922D-5™.
Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and

II. Appendix 2

Information of medically used soap

SAFETY DATA SHEET

SECTION 1 : IDENTIFICATION

Product Name:	**Restore Skin Cleanser**
Product Code:	517210, 7210
SDS Manufacturer Number:	517210, 7210
Manufacturer Name:	Hollister Incorporated
Address:	2000 Hollister Drive Libertyville, Illinois 60048 USA
General Phone Number:	847-680-1000
SDS Creation Date:	November 14, 2014
SDS Revision Date:	May 11, 2015
(M)SDS Format:	

SECTION 2 : HAZARD(S) IDENTIFICATION

Signal Word:	Not applicable.
GHS Class:	Not classified
Hazard Statements:	None.
Precautionary Statements:	None.
Emergency Overview:	WARNING! Irritant.
Route of Exposure:	Eyes. Skin. Inhalation. Ingestion.
Potential Health Effects:	
Eye:	May cause irritation.
Skin:	May cause irritation.
Inhalation:	Prolonged or excessive inhalation may cause respiratory tract irritation.
Ingestion:	Ingestion can cause gastrointestinal irritation, nausea, vomiting and diarrhea.
Target Organs:	Eyes. Skin. Respiratory system. Digestive system.

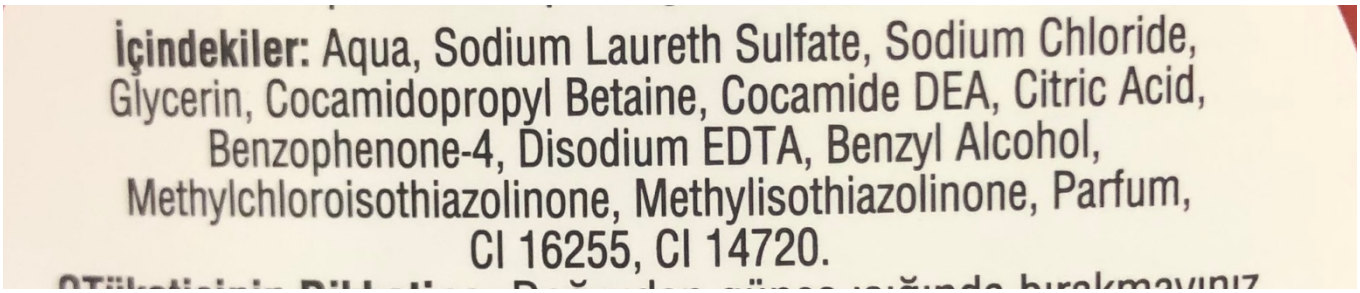
SECTION 3 : COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS#	Ingredient Percent	EC Num.
Non hazardous	No Data	75 - 80 by weight	
Polysorbate 20	9005-65-6	5 - 10 by weight	500-019-9
Propylene glycol	63625-56-9	1 - 5 by weight	226-775-7
Citric Acid	77-92-9	1 - 5 by weight	201-069-1
2-phenoxyethanol	122-99-6	0.1 - 1 by weight	204-589-7

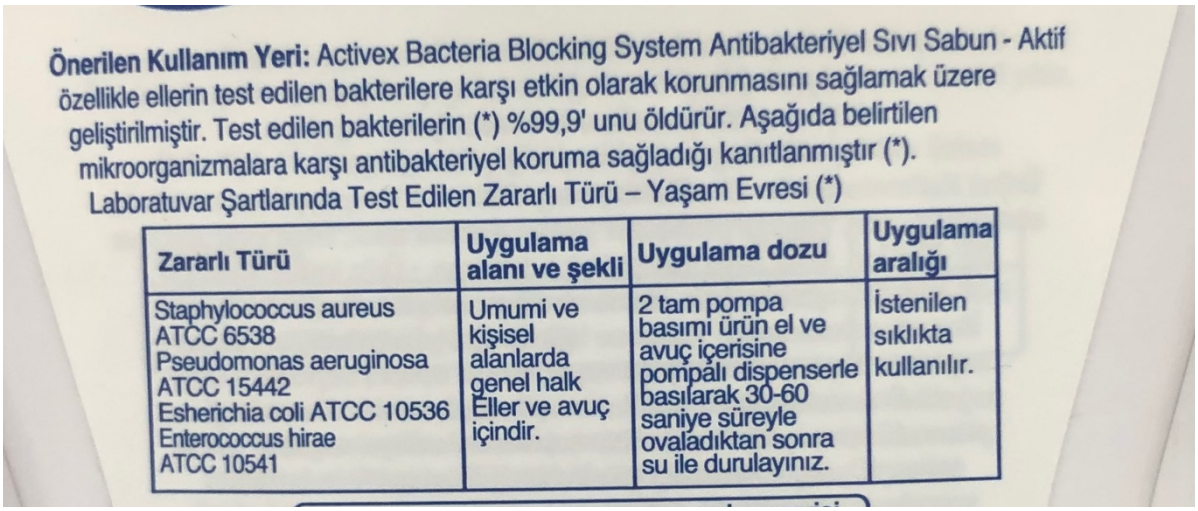
SECTION 4 : FIRST AID MEASURES

Eye Contact:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
Skin Contact:	No effects anticipated. If symptoms develop. Wash skin with soap and plenty of water. Get medical attention if irritation develops or persists.
Inhalation:	No effects anticipated. If symptoms persist, call a physician.
Ingestion:	If swallowed, do NOT induce vomiting. Call a physician or poison control center immediately. Never give anything by mouth to an unconscious person.

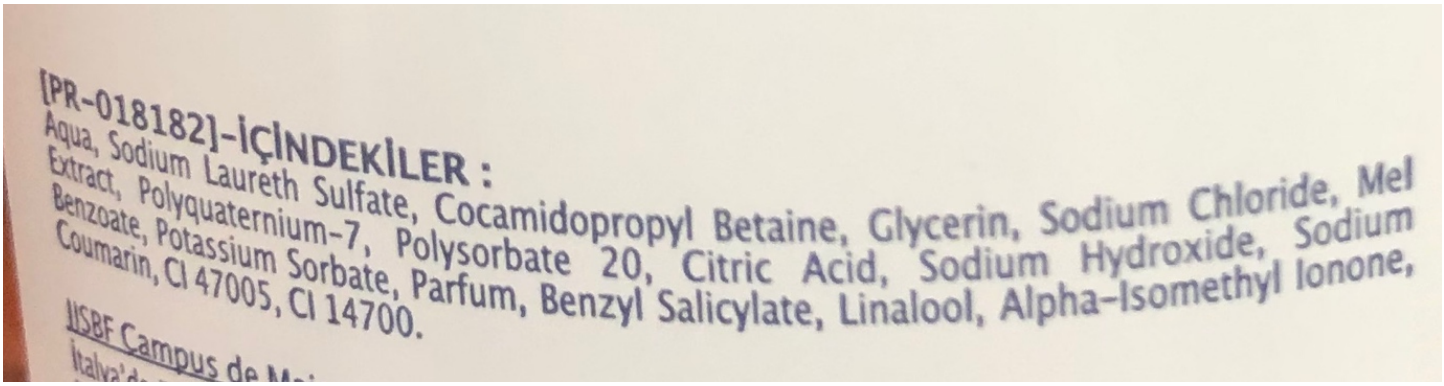
III. **Appendix 3 -Ingredients for daily used soap**



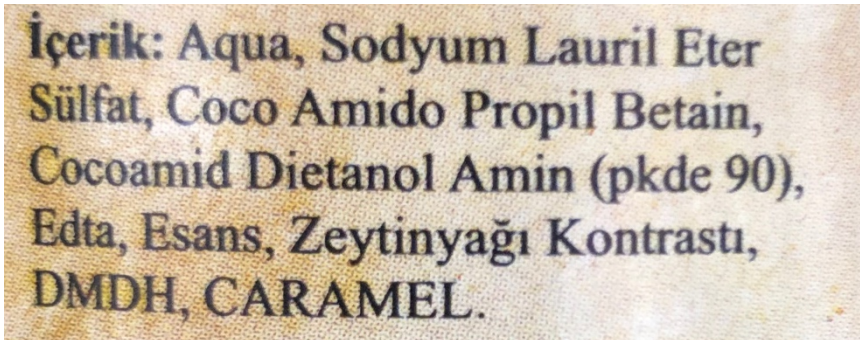
IV. **Appendix 4 – Ingredients for antibacterial soap**



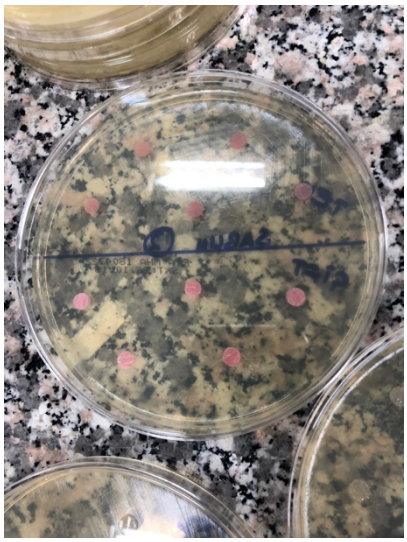
V. **Appendix 5- Ingredients for paraben included soap**



VI. **Appendix 6- Ingredients for natural soap**



VII. Appendix 6- Photos from experiment



VIII. Appendix 7

The student has performed their experiment in Ankara Training and Research Hospital Department of Microbiology themselves with supervision from our staff.

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