IB Biology Extended Essay

Investigating bacteria growth on different kitchen cleaning equipment

"How do a mop, a kitchen cloth(swab), a towel and a kitchen sponge differ in terms of bacterial colony (*Escherichia coli*) count, found after ten days of daily use and routine cleaning using the viable cell counting method?"

BACKGROUND INFORMATION

All the houses that I have visited, including my own, have a specific kitchen towel or cloth used for cleaning and wiping surfaces at the dining table. My mother always had her favorite brand called “Scotch-Bride” which is a yellow, general purposed wipe, and she always used it to clean our dining table or wipe the excess water at the counter in our kitchen. Dish towels are more practical for cleaning spills on the countertops and general cleanup, such as wiping children's hands and faces. Like kitchen sponges, these cloths can hold harmful pathogens and disperse germs if they are not regularly cleaned. Another germ-holding cleaning equipment is the mop. Mops are used to clean floors while they are wet; hence, they have a higher possibility to contain bacteria. The above applications all have the potential to transmit harmful bacteria.

In a recent study that was presented at the American Society of Microbiology's annual conference, 100 kitchen towels were gathered after a month of use. Results show about half contained bacterial growth such as *Escherichia coli* and *Staph*. According to the study, food poisoning may result from cross-contamination caused by bacteria discovered on multi-use kitchen towels used to handle meat products. "Bacteria can be hazardous in certain situations," some specialists said. Nonetheless, you would anticipate finding these kinds of microorganisms throughout the house.[1]

It has previously been discovered that foodborne germs are frequently present in the home kitchen environment and equipment. Because of frequent use and inadequate cleaning, hand contact sites in the home kitchen often become contaminated. Wet regions in the home kitchen are frequently contaminated with large counts of germs since studies have shown that they are more contaminated than dry places.[2]

Dishcloths and dishbrushes are among the cleaning supplies that are said to be highly contaminated with *E. coli, L. monocytogenes,* and *S. aureus*. As a result, dishcloths in the home kitchen can serve as a reservoir for organisms and contaminate surfaces that come into touch

with food. Foodborne infections may arise from microbial cross-contamination caused by the presence of such pathogens in the home environment.[3]

The bathroom is not the dirtiest spot in the house, despite popular belief! A research commissioned by the Global Hygiene Council found that the kitchen had a higher concentration of coliform bacteria, a sign of possible fecal contamination, than the restroom. In actuality, coliform was detected in 18% of cutting boards, 32% of countertops, 45% of kitchen sinks, 60% of dishcloths, and 75% of dish sponges.[4]

Since this subject is relevant to everyone who uses kitchen cloths or a towel, and considering the bacteria they may harbor, I decided to conduct an experiment on bacterial growth in kitchen cleaning equipment. I set my research question as "How do a mop, a kitchen cloth(swab), a towel and a kitchen sponge differ in terms of bacterial colony (*Escherichia coli*) count, found after ten days of daily use and routine cleaning using the viable cell counting method?"

The aim of this study is to compare bacterial growth in different types of cleaning equipment over a 10-day period. Each item was washed only with soap. During the 10-day period our regular house cleaning was done every three days also a deep cleaning was done too. Regular cleaning involves cleaning the visible and frequently used areas, whereas deep cleaning focuses on areas that are difficult to access and gather dust and dirt.

I am doing this experiment to show how much *E.coli* bacteria grows on common household cleaning supplies. These bacteria can affect human health, and it is important for people to be aware of this subject.

HYPOTHESIS

Environmental elements that affect bacterial development include temperature, humidity, pH, surface, and oxygen concentration, among others.[5] In the experiment none of the equipment is made from antibacterial materials (e.g. microfiber) so bacterial growth is expected in all the test groups. Nevertheless, their material and the surfaces they are used to clean will differ and so is the bacteria growth.

Warm-blooded organisms' lower intestines often serve as home to the bacteria *E. coli*. Some of its strains can cause severe food poisoning, but the majority are harmless.[6] I chose to work on E. coli after reading this World Health Organization article since I discovered that the most of its strains are safe.

Among these environmental elements is humidity, which facilitates the spread of microorganisms. Bacterial contamination is more likely in humid environments. Accessible water may also encourage the growth of germs, yeast, and mold—all of which can be dangerous to human health.[7] So, kitchen cloth and towel are expected to have higher bacterial contamination since they are always wet and used frequently. Other factors are also going to determine the difference in bacteria count after 10 days. Each cleaning equipment will retain bacteria at different rates due to variations in its surface texture and material.

Based on the factors mentioned above, the hypothesis is that the mop will have the highest bacteria count, followed by the towel and cloth, with slight differences between them. The lowest bacteria growth is expected in the sponge. No major discrepancies are expected because the experiment was lasted only 10 days, and the equipment was new.

It was discovered that when mops were kept wet, they encouraged the growth of bacteria to extremely high levels and were not sufficiently decontaminated by chemical disinfection.[8] $\boldsymbol{H_1}$ was determined by considering that the mop touches practically every floor in our house.

$\boldsymbol{H_1}$**:** There is a statistically significant difference between the *E. coli* count of different kitchen materials. (mop, towel, cloths, sponge)

$\boldsymbol{H_0}$*:* There is not a statistically significant difference between the *E. coli* count of different kitchen materials.

METHOD DEVELOPMENT and PLANNING

While figuring out what I should choose as a topic I thought this would be more entertaining because odd and shocking outcomes that might change my daily life. I talked to my supervisor and decided to investigate bacteria. Working with animals are considered to be unethical, plants require a long time and patience, which I did not have, and the other organisms to study with were not appealing as much as bacteria. It is a subject we are all familiar with but do not have any idea about how much bacteria grows on the materials we use every day.

The remaining part of what to do with bacterial growth needed brainstorming. But I found my topic when I was cleaning the house for my mum and thought that it would be exciting to do an experiment with daily used cleaning materials. Even after two weeks of use, they began to feel quite unclean. I decided to focus on this topic and carry out my experiment. I looked at the most frequently used kitchen cleaning supplies to choose which products were suitable for the experiment.

Triclosan is one of the antibacterial substances included in many of these products. Unnecessary domestic use may reduce the effectiveness of these substances, which are useful in hospitals and other healthcare settings.[9] On a health channel website, I came across this story on antibacterial cleaning supplies in hospitals. I was inspired by this article to look into the types and quantities of bacteria that can develop in common cleaning supplies that aren't antibacterial. After researching and finding out the non-antibacterial materials I decided on my objects. I had to limit the type of bacteria, me and my supervisor decided on them. Limiting the species of bacteria gave me a more specific view about the topic and kept me from having more data than I could manage. Sponge and dishcloths harbor more *E. coli* and other fecal-based bacteria than any other item in your home. These objects provide the perfect habitat for germs to flourish since they get and remain wet.[10] *E. Coli* grows readily and rapidly in the kitchen products I selected which is another reason I chose it.

There are several plating methods available today, and each has a unique purpose and set of characteristics. Some of these methods involve repeated dilution.[11] Without serial dilution, the bacterial density would be too high, and plating such samples directly would make the bacterial count process almost impossible. By systematically lowering the bacterial density, serial dilution reduces quantification mistakes by enabling accurate colony counting and determination of the initial bacterial population in the sample.

After making the serial dilutions, they needed to be spread to agar plates. I chose the "Spread Plate Procedure: Formation of Discrete Bacterial Colonies for Plate Counts, Enrichment, Selection, or Screening" This technique is used to separate microorganisms contained within a small sample volume that is distributed throughout the surface of an agar plate when the proper concentration of cells is plated. As a result, unique colonies that are uniformly spaced over the agar surface are produced.[12]

E. coli may be found in food and water using a range of bacteriological media, such as bright green bile broth, lauryl sulfate tryptose broth, m-Endo agar/broth, and violet red bile agar. Lactose is the main fermentable sugar in the majority of these medium.[13] I chose Endo agar to use in my experiment because it is used to confirming the detection and enumeration of coliform bacteria.[14]

Many methods can be used to count the total number of bacteria in a sample, and I chose viable cell counting (**Appendix I**). This method was the most accurate and sensitive method for this experiment and bacterium type (*E.coli*) Also, it could differentiate between dead and living cells which could be useful for my experiment.

Prior to conducting this experiment, I checked my school lab for the necessary equipment but since it was unavailable, I got help from Ankara University Microbiology Research Laboratory under the supervision of Dr. Başar Karaca (**Appendix II**).

Gram-negative, facultatively anaerobic, non-spore-forming rods, *E. Coli* bacteria digest lactose strongly to acid and gas at 35 ± 2 °C in 24 or 48 hours.[15]

Controlled Variables	How is it controlled?	Why is it controlled?
Cleaning the materials	Because we used the materials to clean our house they needed to get cleaned. To clean them 5 ml of detergent (Pril dish washing liquid) was used 4 times for 1 minute.	Cleaning them with different antibacterial soaps or detergents could affect the results.
Time	All the equipment was used daily for ten days.	Using them for different periods of time can change my results. They should be used in the same conditions during the same duration.
Temperature	They were all kept in the same temperature while using (our house was set to exactly 27°C and the materials were in a closed room with no heat exchange) and while I was doing the experiment.	Temperature affects bacterial growth, and all the supplies should be kept in the same conditions.
Usage / surface	I used them the way everyone normally does. They did not touch any other surface besides the surfaces they were designed to touch. (e.g. Mop-only floor)	Touching different surfaces can cause new and more bacterial growth.
Agar	Same type of agar was used. (Endo)	While I was doing my experiment, I had access to Endo Agar and figured it was a convenient type for my research.
Incubation time and temperature	The plates for the total coliform count were incubated at 37 °C for 48 hours.	After some research, I found out the appropriate incubation time and temperature for *E.coli* bacterium.

Table 2: Controlled Variables Table

Variables	What is the variable?	How?
Independent	Type of the cleaning materials and the surface they are used on	4 different materials (kitchen cloth, sponge, mop, towel) used in different places of my house were investigated.
Dependent	Total bacteria *(E. coli)* count	The surface and usage of the equipment are expected to affect the total bacterial count.

Table 3: Independent and Dependent Variables Table

MATERIALS and METHOD:

Material	Quantity	(±uncertainty) Unit
Mop	1	
Towel	1	
Kitchen cloth	1	
Detergent	5 ml x4 times in 10 days x4 equipment = 80 ml	± 0.5 ml
Scissors	1	
Physiological serum	20x4x5= 400 ml	± 0.5 ml
Falcon tubes	5	
Vortexer Vortex Mixer)	1	
Micropipette Tips (100µl and 1000µl)	200	$\pm 0.5 \times 10^{-6}$ L
Microfuge tubes	5x5=20	
Incubator	1	±0.5°C
Autoclave or Sterilizer	1	
Sterile Petri Dishes	20	
Bacterial Culture Media	Sufficient for 20 plates	
Test Tubes (Sterile)	20	
Graduated Cylinders	1 (100 mL)	±1 mL
Refrigerator	1	
Dilution Buffer	Sufficient for dilutions	
Sterile Water	Sufficient for dilutions	
Lab Coat	1	
Disposable Gloves	1 box (50)	
Safety Goggles	1 pair	
Permanent Markers	2	
Biohazard Waste Bags	2	
Mueller-Hinton Agar Medium	1 L	±1 mL
Disposable sterile loops	20	
Saline Solution	250 ml	±1 mL

Table 4: The table of materials used, their quantity, and uncertainty.

Materials were used for equal hours to prevent unbalanced and unfair results. Under proper conditions, bacteria may use binary fission to grow to millions in few seconds.[16] Because I wanted to see clear results, the materials were used for equal time periods for 10 days.

Gammaproteobacterial species (*E. coli*) are common members of the sponge microbiota. Food-borne pathogens, like *Klebsiella pneumonia*, which can infect humans from the lungs to urinary tract, also inhabit sponges, along with various viruses and archaea.[17] According to research by the American Society for Microbiology, bacteria may live in damp and porous cleaning

equipment, and some of them may even be harmful to your health.[18] Hence, this bacterium was investigated in this experiment to see how much it can grow in a short time.

While the growth of gram-positive organisms is limited on Endo agar, the majority of gram-negative species thrive there. While non-lactose-fermenting organisms (*Salmonella*) create clear, colorless colonies, coliform organisms (*E. coli*) ferment the lactose in this medium and generate a green, metallic sheen. According to this information given, the most suitable culture media was endo agar for *E. coli*.

The following steps were followed:

i. Use your own mop (a in Figure 1), kitchen towel (b in Figure 1), kitchen cloth (d in Figure 1) and kitchen sponge (c in Figure 1) for 10 days to clearly see the bacterial contamination.

ii. Wash them with 5 ml of detergent every 4 times for 1 minute in the 10-day usage period.

iii. After 10 days cut your materials (mop, sponge, cloth, towel) into 5 grams each.

iv. Put the cut materials with 20 mL physiological serum into Falcon tubes. (Figure 2)

v. Prepare a homogeneous suspension to fit the cut parts.

vi. Use a vortexer to shake the immersed Falcon tubes at maximum intensity for 2 minutes.

vii. Take 100 µL of the suspension from each Falcon tube using a micropipette.

viii. Make a serial dilution (10^{-1}-10^{-6}) in microfuge tubes containing 900 µL of physiological serum.

ix. Drop 20 µL of each dilution onto the corresponding culture medium plates and spread with a sterile loop. (Figure 3)

x. Incubate the plates for the total coliform count at 37 °C for 48 hours.

xi. After incubation, count individual colonies by Viable Cell Counting method explained in the method development part.

xii. For all five trials, repeat all the steps (i. to xi.) four more times.

xiii. After the experiment is over, use your biohazard waste bag to get rid of the unnecessary materials and sterilize others.

xiv. Do a statistical analysis.

DATA ANALYSIS

After incubation, individual colonies were counted according to the given formula:

CFU: (N × df × 50 × 20)/5

CFU: Colony forming unit

N: Number of colonies counted

df: dilution factor

50: the number for adjusting the colony forming unit per mL

20: the number for adjusting the colony forming unit to the total volume of the suspension (20 mL)

5: the number for calculating the colony forming unit per gram of each sample.

	Number of *E. Coli* Colonies (CFU)			
Trials	Mop	Towel	Kitchen sponge	Kitchen cloth
First trial	5,00E+08	1,30E+06	2,00E+05	7,50E+07
Second trial	1,00E+09	1,50E+06	7,60E+06	5,00E+06
Third trial	1,50E+09	3,00E+05	1,00E+05	8,50E+06
Fourth trial	1,20E+08	3,70E+05	1,60E+06	1,00E+07
Fifth trial	3,50E+07	1,80E+07	1,40E+05	1,30E+06

Table 6 (Raw Data Table): *E. coli* count of each kitchen equipment for five trials (unit: CFU)

Statistical Data Analysis

Mean and standard deviations of data given in the raw data table.

- The mean was calculated using the formula below:

$$\text{Mean } (\bar{x}) = \frac{\Sigma x}{n}$$

x: measured value

Σx: sum of observed values

n: number of observations.

Example Calculation: Mean of the mop

$$\bar{x} = \frac{5{,}00E{+}08 + 1{,}00E{+}09 + 1{,}50E{+}09 + 1{,}20E{+}08 + 3{,}50E{+}07}{5} = 6{,}31E{+}08$$

- The standard deviation was calculated according to the formula below:

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

s: the sample Standard Deviation

N: number of observations

X_i: value of each observation

$\bar{x}$: the sample mean

To calculate the standard deviation, we need to find the mean and the sum of squares first. Here is an example of the standard deviation of the mop:

Standard Deviation s =617418010

Variance s^2 =3.81205E+17

Count n =5

Mean $\bar{x}$ =631000000

Sum of Squares (SS)=1.52482E+18

$$s = \sqrt{\frac{SS}{n-1}}$$

$$s = \sqrt{\frac{1.52482\text{E}+18}{5-1}}$$

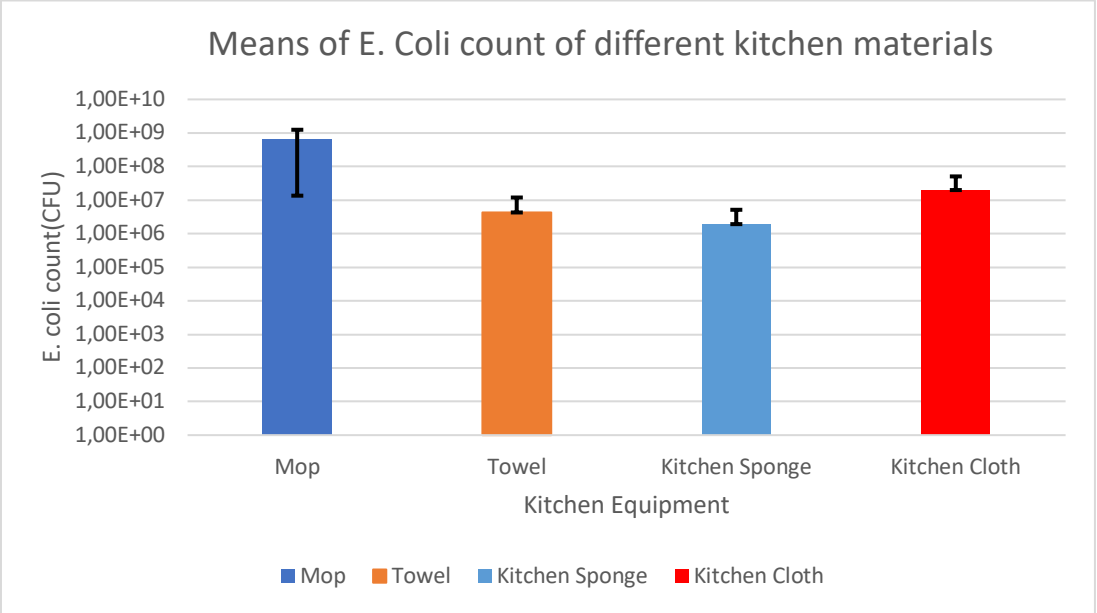
$$s = \sqrt{3.81205\text{E}+17}$$

$$s = 617418010$$

If we do this to all the raw data, we obtain these results:

Cleaning Equipment	**Mean**	**Standard deviation**
Mop	6,31E+08	617418011
Towel	4,29E+06	7680734,34
Kitchen Sponge	2,38E+06	3232788,27
Kitchen cloth	2,00E+07	30951300,5

Table 7: Mean and Standard Deviations of raw data



Graph 1: Bar graph of means of *E. coli* colony count with standard deviations as error bars.

ANOVA Tests

	Mop	Towel	Kitchen sponge	Kitchen cloth	Total
sum $\sum x_i$	3,155,000,000.0000	21,470,000.0000	9,640,000.0000	99,800,000.0000	3,285,910,000.0000
mean $\bar{X}$	631,000,000.0000	4,294,000.0000	1,928,000.0000	19,960,000.0000	164,295,500.0000
sum of squares ($\sum x_i^2$)	3,515,625,000,000,000,000.0000	328,166,900,000,000.0000	60,389,600,000,000.0000	5,823,940,000,000,000.0000	3,521,837,496,499,999,744.0000
sample variance (s^2)	381,205,000,000,000,000.0000	58,993,680,000,000.0000	10,450,920,000,000.0000	957,983,000,000,000.0000	156,946,172,110,263,168.0000
sample std. dev. (s)	617,418,010.7512	7,680,734.3399	3,232,788.2702	30,951,300.4573	396,164,324.6309
std. dev. of mean (SE_x)	276,117,728.5145	3,434,928.8202	1,445,746.8658	13,841,842.3629	88,585,036.0135

Table 8: Statistical data analysis for ANOVA test (sum, mean, sum of squares, variance, standard deviation, standard deviation of the mean.)

	Sum of Squares (SS)	Degrees of Freedom (v)	Mean Square (MS)	F Statistic	p-value
Treatment	1,453,047,559,695,000,064.0000	3	484,349,186,565,000,000.0000	5.0686	0.0118
Error	1,528,929,710,399,999,488.0000	16	95,558,106,899,999,968.0000		
Total	2,981,977,270,094,999,552.0000	19			

Table 9: ANOVA test result which show sum of squares degrees of freedom, mean of squares to derive F statistic and p- value.

ANOVA Test enables me to compare the data (mean, standard deviation etc.) for the four study groups simultaneously and identify if there are any statistically significant differences between them. The p-value found from the F-statistics resulting from the one-way ANOVA was below .05 (p-value<0.05) implying that at least one treatment and the rest are not at the same level of effectiveness. To determine which specific treatment contrasts are statistically significant post hoc testing will assist in determining which pairs of treatments differ significantly. Tukey's Honest Significant Difference (HSD) test is a widely used post hoc test to assess the significance of differences between pairs of group averages. Tukey HSD is commonly used as a follow-up to one-way ANOVA when the F-test shows that some of the tested groups differ significantly.[19] Research and this quote indicates the best fitting post-hoc test for four groups of data being Tukey HSD.

One or more treatment pairs are likely to be significantly distinct if the p-value, which corresponds to the F-statistic of the one-way ANOVA, is less than 0.05. Since there are four groups in the experiment, six pairs of comparisons must be made. Using the k=4 treatments and v=16 degrees of freedom for the error term, we first determine the critical value of the Tukey-Kramer HSD statistic for significance level α= 0.01 and 0.05 (p-values) in the Studentized Range distribution. We obtain these critical values for Q, for α of 0.01 and 0.05, $Q_{\text{critical}}^{\alpha=0.01,k=4,v=16}$ =5.1924 and $Q_{\text{critical}}^{\alpha=0.05,k=4,v=16}$ =4.0464, respectively.

I next used my sample columns to create a Tukey test statistic, which then I compared to the relevant critical value of the studentized range distribution. For every pair of columns under

comparison, I computed a parameter that we roughly refer to as the Tukey HSD Q-statistic, as follows:

$$Q_{i,j} = \frac{|\bar{x}_i - \bar{x}_j|}{s_{i,j}}$$

The denominator in this equation above is shown below.

$$s_{i,j} = \frac{\hat{\sigma}_\epsilon}{\sqrt{H_{i,j}}} \quad i, j = 1, \ldots, k;\ i \neq j\,.$$

The amount of the harmonic means of the number of observations in columns with the labels i and j is represented by $H_{i,j}$. The common sample size is the harmonic mean of the columns when the sample sizes are equal. When two columns in a pair under comparison have different sample sizes, the harmonic mean falls somewhere in the middle of the two sample sizes. For columns with different sample sizes, the Tukey-Kramer technique cannot be applied without the corresponding harmonic mean. In the predecessor one-way ANOVA process, the Mean Square Error = 95,558,106,899,999,968.0000 was found to have a square root of σ_ϵ = 309,124,743.2672. σϵ remains constant for every pair under comparison. In the calculation of $s_{i,j}$, the denominator—the harmonic means of the sample sizes under comparison—is the sole variable that differs between pairings.

Assessing if $Q_{i,j} > Q_{critical}$—which is based on the degrees of freedom for error v, the number of treatments kk, and the desired level of significance α (p-value)—is the same as determining whether the NIST Tukey-Kramer confidence interval contains zero.

Pairs	**Tukey HSD Q Statistic**	**Tukey HSD p-value**	**Inference**
Mop vs Towel	4,5333	0,025525	p < 0.05
Mop vs Sponge	4,5504	0,02492	p < 0.05
Mop vs Cloth	4,42	0,02991	p < 0.05
Towel vs Sponge	0,0171	0,899995	Insignificant
Towel vs Cloth	0,1133	0,899995	Insignificant
Cloth vs Sponge	0,1304	0,899995	Insignificant

Table 5: It displays the color-coded results of determining whether $Q_{i,j} > Q_{critical}$ for all pertinent pairs (green for significant, red for insignificant). The significance (p-value) of the observed $Q_{statistic}$ and $Q_{i,j}$ are also presented.

CONCLUSION and EVALUATION

This experiment was done to find differences between *Escherichia coli* counts in 4 different kitchen supplies (**Appendix III**). Therefore, I used one-way ANOVA (analysis of variance) with Tukey's post-hoc analysis to figure out differences among *E.coli* colony counts, and $p \leq 0.05$ was considered significant because only ANOVA will indicate the significance but will not tell exactly between which groups, so a following Tukey' post-hoc test was done. The ANOVA analysis also refers to small P-value for both tests, at 5% significance level. Hence, $\mathbf{H_0}$ is rejected and $\mathbf{H_1}$ is accepted indicating that there is a statistically significant difference between the bacterial count of mop compared to other materials. Therefore, an additional Tukey analysis revealed that there were substantial differences between group pair means. ($p < 0.05$). The results of this experiment clearly show a significant difference between mop with other equipment and the number of *E.coli* colonies were the highest within all the groups due to cleaning the kitchen floor which contains more bacteria compared to other surfaces in the kitchen as can be seen from Graph 1 where the mop (dark blue column) has the highest bacterial growth. The standard deviation for the mop was also the highest. This explains that the bacteria count for the mop was further away from the mean compared to others. In addition, it proves that the data for the mop has more variation. Figure 9 below shows the *E.coli* colonies growing on an Endo Agar plate.

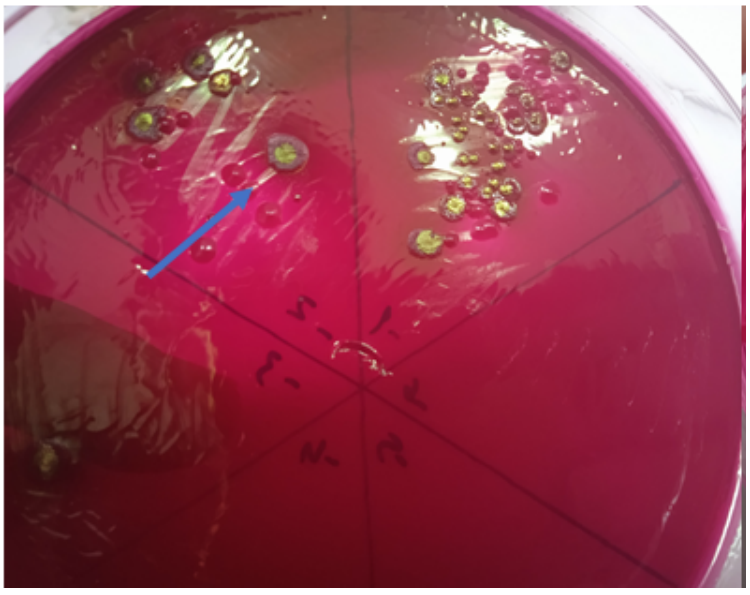


Figure 9. Typical *E. Coli* colonies grown on Endo Agar plates. Blue arrow indicates typical bright green colonies of *Escherichia coli* colonies

In this experiment there are a few limitations. I did five trials of each equipment. More trials can be more adequate because the data varied a lot. I took samples from different parts of the equipment, and it also can lead to false data because some parts may not be equally washed with detergent due to cleaning at home by hand. Consequently, more trials can increase the power of the study. The third limitation is the time of data collection. After some research I decided for my family's health that 10 days was long enough, but more bacteria can grow in a longer period (which is as long as some people use). Three other limitations regarding method are about the vortexer, serial dilution and colony counting method. An overestimate of contamination levels could result from the vortexing step's inability to completely remove microorganisms from porous or densely woven textiles. Particularly at higher dilutions, serial dilution creates the possibility of pipetting errors, which could distort results. Colonies that overlap on the plate may cause counting errors in viable cell counting method. Viable but non-culturable (VBNC) or dead bacteria are not detected by viable cell counting; only culturable bacteria are.

A few suggestions to reduce these limitations can be increasing the number of trials to reduce the impact of outliers and provide more reliable results, extending the duration of the experiment to observe the growth of bacteria over a longer period, provided health risks are minimized, and improve colony counting accuracy by counting dead bacteria etc.

Using kitchen equipment without properly sanitizing or changing it regularly causes serious numbers of bacterial growth. Although there were many experiments done before to show how much harmful bacteria can grow in the kitchen without properly cleaning or changing the equipment frequently. Your sponge should ideally be changed once every two weeks, or sooner if you detect an odor. You may reduce the risk and extend the time between replacements by washing your sponge every other day, putting it on a holder to dry, and wringing it out completely after use. It should still be replaced on a regular basis because thorough cleaning with boiling water, microwaving, or vinegar rinse won't get rid of all the bacteria and may even

cause some to grow.[20] Even though most people do not give attention to these it can cause serious health problems. The majority of *E. coli* are safe and found in a healthy digestive system. *E. Coli* aids in vitamin production, food digestion, and germ defense. However, some strains of it can cause sepsis, pneumonia, diarrhea, and urinary tract infections, among other health problems. Hemolytic uremic syndrome (HUS) is a dangerous illness that can result from an infection with Shiga toxin-producing E. coli (STEC). Kidney failure, long-term health issues, and even death can result from HUS.[21] This quote about *E. coli* shows that even most of them is harmless some can cause serious illnesses.

Further research can be done to experiment how long does it take the harmful bacteria to grow significantly or what is the most economical way to clean or replace our kitchen equipment. Thus, the new research question could be "Which household materials can be used to clean kitchen equipment properly, and what techniques are the most efficient?"

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APPENDICES

Appendix I: Picking a cell counting method

Method	Advantages	Disadvantages
Viable cell/colony counting	Only counts living cells, making it the most accurate of all.	Sample needs to be at the right density, takes a long time, cells form into a single colony
Using spectrophotometry(turbidimetric)	Simple to perform and set up, it can be applied to a very small sample size.	Not a direct count more like an estimation, dead cells cannot be distinguished, less accurate with prokaryotic cells
Microscopic counting	Direct, easy to set up	Not sensitive to detect grouped bacteria, dead cells cannot be distinguished, sample needs to be at the right density

Table 1: Cell counting method advantage and disadvantage table

Appendix II: Ankara University confirmation letter

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25 February 2025

To whom it may concern,

This letter is written to confirm the support of Ankara University for the research project by Ms. ~~Duru Oygür.~~

~~Ms. Duru Oygür~~ requested our permission to use our laboratory facilities for her research which will be conducted using basic microbiological equipment and instruments. We hereby confirm that she has been granted permission to use our laboratory which means she will have authorization to use laboratory equipment, and the materials needed for her study.

She will do the experiment under the supervision of Dr. Başar Karaca. She is allowed to use culture media, incubators and other required instruments for her study.

Please do not hesitate if you need any further information.

Yours sincerely,

Associate Prof. Dr. Başar Karaca

Ankara University, Faculty of Science,

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Appendix III: Pictures of the equipment and agar plates during the experiment.

a) b) c) d)

Figure 1. a) Mop b) Towel c) Kitchen sponge d) Kitchen cloth.

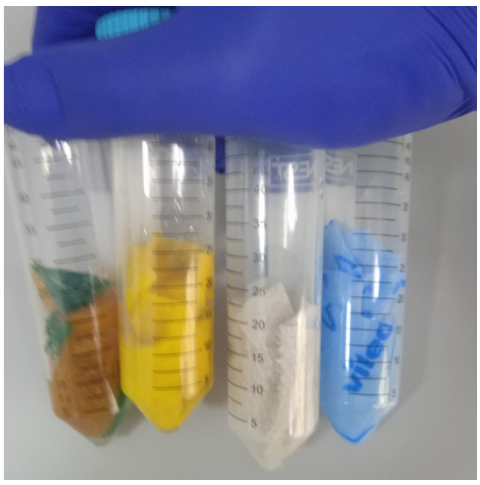

Figure 2: Immersed samples in 50 mL-Falcon tubes containing 20 mL of physiological serum.

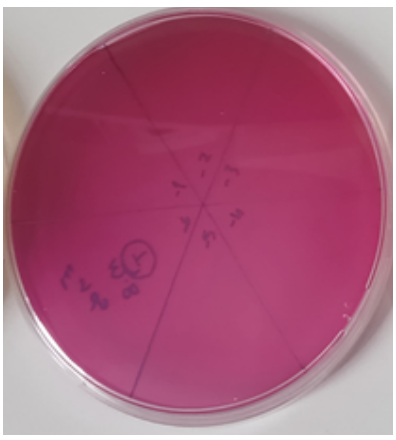

Figure 3: Preferred culture media. (Endo Agar from one of the five trials.