IB Biology Extended Essay

Effect of smartphone case material on bacteria growth

"How do the leather, silicone and plastic smartphone cases and the glass back of the phone without any case differ in terms of total bacteria count, measured using the viable cell counting method after a timespan of one week with daily usage of exactly one charge cycle?"

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Abstract

 Mobile phones, especially ones with internet capabilities, have recently entered our lives and become essential parts of our daily lives. As a result, they have become an important factor in the spread of pathogens, especially among teenagers. The aim of this research is to compare smartphone case materials and the glass back of the phone (Apple iPhone 4S**®**), based on their contamination by bacteria after 1 week of usage.

 The research question of this investigation is: *"How do the leather, silicone and plastic smartphone cases and the glass back of the phone without any case differ in terms of total bacteria count, measured using the viable cell counting method after a timespan of one week with daily usage of exactly one charge cycle?"*

 The method used to investigate the research question is the viable cell counting method. The case is first disinfected and used everyday until the phone battery runs out. Culture is taken from the case by a cotton swab after 1 week and then is serial diluted up to the factor of $10⁵$. All dilution factors are incubated in LB agar plates for 24 hours at 37ºC. Bacterial colonies are counted, and the dilution factor with colony count between 30-300 bacteria is used in data analysis.

 The mean results of viable bacteria number per ml of culture (in CFU/ml) are as follows: $8.86x10^5$ for leather, $7.28x10^5$ for silicone, $6.32x10^5$ for plastic and 4.42x105 for glass. ANOVA tests proved that there is indeed significant difference between test groups ($p = 0.015114832$). The conclusion is that all smartphones get contaminated by bacteria, but the glass has the lowest bacteria count showing that smartphone cases should be avoided for hygiene as much as possible. If a case is absolutely necessary, leather ones should not be preferred.

Word Count: 295

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1. Background Information:

 Since the existence of humanity, communication between different individuals and villages has been a necessity. The earliest humans used smoke, pigeons and other natural ways to do the job, but as the human civilization got more and more improved in time, much better ways of communication have evolved.

 The most popular way of interpersonal communication in the 21st century is mobile phones without any doubt. 93.2 percent of Turkey's population owns a mobile phone whereas only a minority of 45.5 percent owns a cable phone 1 . Especially with the development of internet phones and smartphones, communication gadgets have become an indispensable part of everyday life. More features and functionalities have risen the average time of daily interaction with such devices significantly. Apart from the health concerns due to electromagnetic radiations, another risk factor is that they can act as a container of many species of microorganisms.

 Mobile phones are important factors in transmission of many illnesses. They come in contact with hands, face, ears, and even some of the saliva of the owner. This causes many species of bacteria from various flora of human body to be transported onto the phone2.

Table 1: Bacterial genera from the normal flora of human skin, nose, mouth, pharynx and respiratory tract. Bold genera are nearly 100% found in all humans.

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¹ http://www.tuik.gov.tr/PreTablo.do?alt_id=1028

² http://textbookofbacteriology.net/normalflora.html

 Since smartphones operate at warm temperatures and get humid due to contact with body parts, they are suitable environments for bacteria to thrive and form colonies very rapidly. In addition, when they are dropped on the floor, used in an unhygienic way or kept in unsterile places such as bathrooms or pockets, the surface can be contaminated with different pathogenic bacteria and this may possibly reach health threatening levels. Researches even show that mobile phones increase the rate of spread of hospital-acquired infections when they are used by hospital personnel in sensitive areas 3.

 A term that became popular recently with the development of smartphones is phone cases. As phones got more technological and more valuable in time, a demand for casings has started to rise among customers not only to protect them but also to personalize them with different colors and patterns. Millions of phone cases with a wide spectrum of colors and materials are available for sale these days. These cases do a very good job at protecting valuable phones from physical impacts, but how well they perform at protection from harmful bacteria mentioned above is still an unanswered question in the minds of the consumers.

 In this era of communication, teenagers are among the highest risk groups for bacterial infections due to smartphones. They not only daily interact with their own mobile phones, but also frequently come in contact with their friends' mobiles in schools. This is a factor that speeds up the transmission of microorganisms and spread of illnesses among teenagers. Today's teens also own their smartphones in fairly early phases of their lives, which puts them under risk for a longer period of time. I, myself, got introduced to smartphones as early as the age of 15 and used protective cases ever since. It was my first touchscreen phone, so I started worrying about how hygienic it is and wondered how phone cases affected the bacterial flora on the surface. This is how I came up with the topic of this investigation.

 The aim of this study is to compare the bacterial contamination in a time frame of one week of phone cases made out of the most commonly used materials: leather, silicone and plastic. The experiment is also conducted with the bare glass surface of

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³ http://www.ijic.info/article/view/9933

the phone as a control group (The smartphone used in this experiment is an Apple iPhone 4s**®**). During the period of one week, the phone is used casually for exactly one charge cycle everyday for typical usages such as messaging, making phone calls, playing games, listening to music and surfing the internet. To sum up, the research question for this experiment is:

"How do the leather, silicone and plastic smartphone cases and the glass back of the phone without any case differ in terms of total bacteria count, measured using the viable cell counting method after a timespan of one week with daily usage of exactly one charge cycle?"

 It is worth noting here that nearly none of the commercial 'leather' phone cases are made of actual leather and contain no biological tissue. They are made out of synthesized polyurethane which is shaped to imitate the look and feel of leather. The term 'leather case' will be used in this meaning for the rest of the investigation, for the sake of simplicity.

2. Hypothesis:

 There are many environmental factors that affect bacterial growth. Nature of the surface, temperature, pH, moisture, O2 concentration, nutrient concentration, osmotic pressure etc can all be given as examples of these factors.

 In the experiment, none of the case types (plastic, silicone, leather) or glass have antibacterial effects, so bacteria growth is expected in all of the groups. However, the real difference among the groups will be based on the environmental conditions they create due to the properties of the different materials that the case is made out of.

 An example of these properties is roughness. Leather has a natural shape with numerous small infoldings on the surface, which increases the overall effective surface area of a leather case significantly. As a result, the contact between the case and the external world, including water, nutrients and other bacteria increases, and the infoldings provide a grip4 for microorganisms to hold onto the case. In contrary, the glass back panel of the phone itself has a very polished finish and contains only microscopic bumps. This reduces the surface area and provides less adhesion for bacteria than a rougher surface. As a result, my guess is that less bacteria will grow on the glass back compared to the leather case.

 The plastic and silicone are in between the glass and leather in terms of roughness, but very close to each other. This makes the surface area difference between plastic and silicon is negligible, so other properties of the materials will determine the difference in bacteria count after 1 week, which should be negligible too.

 Due to the reasons above, it is hypothesized that the leather case should have the highest bacteria count, followed by plastic and silicon with very minor difference. The least number of bacteria growth is expected on glass back without casing.

¹ ⁴ http://www.aioseducation.org/PDF/pps/awfp/awfp01.pdf

3. Method Development and Planning:

 While coming up with the research question, I went through two stages. First one was coming up with the topic, and the second one was deciding on the method to investigate my research question.

 Since the very first moment i talked to my supervisor about writing the extended essay on biology, I was certain that the topic would involve technology in some way. I am a "tech geek" in real life and I wanted to do the investigation on a topic that would be interesting and appealing to me.

 Working with animals are very difficult in nature, and also most of the applications on them are considered unethical, so they were never appealing for me to do a research on. Plants and fungi are easy to handle, but require a long time to give satisfactory results and are boring to work with in my opinion. The remaining organisms to study were protista, archaea and bacteria. I chose bacteria among them because they are found literally everywhere, interact with humans in a lot of ways and are easy to study in a lot of labs. My mother works in Ankara University Faculty of Medicine as a medical doctor and has access to a microbiology lab where I can conduct my experiment very easily.

 The hard part left was to what to do with technology and bacteria. My first instinct was to study smartphones, as they have entered our lives pretty recently and have become very important in this little time period. I wanted to compare bacteria growth on different brands of phones at first, but there were some problems with that topic. First of all, it would be very difficult to control many variables, such as the age of the phone, and daily usage time. Also i would have to borrow the phones from other people or buy them, both of which would be equally challenging.

 After thinking about disadvantages of comparing brands, I finally came up with another topic related to smartphones, phone cases. It has the advantage over of the previous topic since it only involves my own phone and I don't need other people's phones. It also makes more sense because most people use their phones with a

case anyway, and it is redundant to compare bacteria growth under the case. I chose to compare plastic, silicone and leather cases in the experiment because they are three of the main case materials that I observed to be the most popular among consumers5.

 I had the chance to compare the growth of a certain species of bacteria and total number of bacteria on the selected cases. Limiting the species of bacteria was a very reductionist approach and I wanted a holistic view on the topic. Also, quantifying only one species required much more work, which in my opinion is unnecessary.

 There are many methods for counting the total number of bacteria in a sample. I had access to the following options⁶ to choose from:

Table 2: Advantages and disadvantages of different method of counting total bacteria number in a sample

 I selected the viable cell counting method among the others, simply due to its superior accuracy and sensitivity. It was also the only method to distinguish between dead and living cells, which is a very important detail for my experiment.

<u>.</u> ⁵ http://www.ebay.co.uk/gds/Buyers-Guide-to-Fitted-Cases-and-Covers-for-Mobile-Phones- /10000000177589598/g.html

 6 http://textbookofbacteriology.net/themicrobialworld/growth.html

 In my method, I decided to test all the cases for 1 week after disinfection. The main reason for this is because students have different habits on different days of the week. For example, I had chemistry labs on mondays and biology labs on thursdays, handling with chemicals would decrease the bacteria count and handling with living tissues would increase it. This is why I wanted the experiment to cover all my weekly schedule.

 While using the phone, I had to keep the daily interaction time relatively consistent. This is why I set the daily usage time to be exactly 1 charge cycle. I took the phone out of the charger at 6:30 am on weekdays and around 10:00 am on weekends, and used it for daily needs. I am a very intensive user, so my battery ran out everyday on evening around 9:30 pm to 11:00 pm. After my battery ran out, I put the phone on the charger and left there until morning. That way, I managed to control how much I interacted with the phone every day as much as possible.

 After 1 week of usage, I took the culture from the phone using a culture swab. I soaked the tip of the swab with 0.90% saline solution so that bacteria can grip more easily on the cotton. I also did rotating motions and rubbed the tip all over the back of the case in order to better reflect the bacteria contamination all around the back.

 Serial dilution was done on the culture up to a factor of 105, so that it will be easier while counting the colonies and will provide more accurate results. After incubating the culture in all the dilution factors and doing the counting for each of them, I prepared the cultures from rest of the trials with the dilution factor that had the bacteria count range between 30-3007 bacteria in the first trial. This is because doing serial dilution for every trial is a huge waste of materials, energy and time.

 While preparing the culture plates, I decided to use Lysogeny Broth nutritional medium, since it is superior than other alternatives while dealing with a wide spectrum of bacterial cells. Spreading of the culture was done via a glass spreader

¹ ⁷ http://www.bio.fsu.edu/courses/mcb4403L/dilution.pdf

which is kept in 70% alcohol solution for 30 seconds and then ignited over a bunsen burner so that cross-contamination between plates is prevented.

 While spreading the culture on the agar plate, I did zig-zag motions from top of the plate to the bottom with the spreader, rotated the plate 90 degrees, did more zigzags from top to bottom and repeated this until reaching 2 full turns. This is done so that viable bacteria are evenly distributed and colony overlap is minimized. Another benefit is that number of bacteria that stay on the spreader is reduced significantly when dish is rotated.

 Incubation was done at 37°C in order to provide optimal conditions for bacteria growth and reproduction. The cultures are kept inside the incubator for 24 hours so that enough time is given for the bacteria to form countable sized colonies. 24 hours of incubation was preferred over 48 hours to assure that colonies don't get too big and overlap with each other.

 After incubation, I counted the colonies from top of the plate to the bottom, putting a dot on every one I counted with a pen in order not to lose track. I only recorded the results from the dilution factor with 30-300 bacteria since only that culture will be used in the data processing of this investigation.

 I did 5 trials for each case in order to increase accuracy and decrease the effect of randomness on the results.

4. Method:

B. Materials:

- 1. A smartphone (Apple iPhone 4s**®**)
- 2. Plastic case
- 3. Silicone case
- 4. Leather case
- 5. Disinfectant
- 6. 40 x Gloves
- 7. 0.90% saline solution
- 8. 20 x Syringe
- 9. 20 x Culture swab
- 10. 100 x Microcentrifuge tube
- 11. Lysogeny Broth liquid agar
- 12. 100 1000 μL micropipette and tips
- 13. Vortex machine
- 14. 36 x Lysogeny Broth agar plate (Appendix 1)
- 15. 70% alcohol solution
- 16. Bunsen burner
- 17. Glass spreader
- 18. Incubator
- C. Serial dilution and spreading the culture:
	- 1. Put on your gloves and put 5 microcentrifuge tubes in the rack
	- 2. Label their caps with dilution factors 10^1 , 10^2 , 10^3 , 10^4 and 10^5 with a pen.
	- 3. Adjust the 100 1000 μL micropipette to 900 μL.
	- 4. Put the tip on the pipette, and take 900 μL of liquid Lysogeny Broth from its bottle and pour it inside the $10¹$ labelled tube.
	- 5. Fill all the tubes identically with the same method.
	- 6. Adjust the micropipette to 100 μL and take 100 μL of the previously prepared bacterial culture (Appendix 2) from the swab tube and pour it inside the 10¹ labelled microcentrifuge tube.
	- 7. Turn on the vortex machine and mix the tube for 5 seconds.
	- 8. Take 100 μ L of the culture from 10¹ labelled tube and pour it in 10² labelled tube using the pipette.
	- 9. Vortex the 10² labelled tube and continue on diluting the culture until you centrifuge the $10⁵$ labelled tube.
	- 10. Take 5 previously prepared Lysogeny Broth agar plates and label them 10¹, 10², 10³, 10⁴ and 10⁵ with a pen.
	- 11. Take the glass spreader and dip it in 70% alcohol solution for 30 seconds.
	- 12. Turn on a bunsen burner and hover the glass spreader over the tip of the fire for a few seconds so that the spreader is sterilised. Let it cool in your hand and don't let it touch anything.
	- 13. Take 100 μL of culture from $10¹$ labelled tube, take the cover of the $10¹$ labelled agar plate off and pour it inside.
	- 14. Start spreading the culture in the $10¹$ labelled plate with the spreader. Do zig-zag motions and rotate the plate 90° each time for 2 turns so that the culture is evenly distributed. Try not to spread the culture to the edges.
	- 15. Shut the cover of the plate.
	- 16. Pour 100 μL of culture from 10^2 labelled tube to 10^2 labelled plate with the pipette, ignite the loop again, wait for it to cool down and spread the culture. Repeat until all cultures are ready in their corresponding plates.
	- 17. Put the plates inside the incubator at 37°C. Check the time and record it.

D. Counting colonies:

- 1. Take the plates off the incubator after exactly 24 hours.
- 2. There should be visible bacterial colonies as round spots on all of the plates. Put all of the plates on the table in increasing order of dilution factor.
- 3. Start counting the colonies by putting dots on each of them with a pen so that you don't lose track. Start from the top and work your way down in order not to miss any.
- 4. Record your findings for each dilution factor.

E. Notes:

- 1. Repeat the experiment for leather, glass and silicone cases.
- 2. Do 4 more trials for each of the case type for more accuracy in results.
- 3. After the dilution factor which gave the results in the 30-300 bacteria interval is determined with Trial 1 of each treatment, the cultures can be directly diluted to that desired optimal factor for the next trials without the need of serial dilution.

5. Data Analysis:

A. Results:

Table 3: Raw data table of numbers of counted bacterial colonies on LB agar plates after 24 hours of incubation at 37°C, containing cultures from plastic, silicone, leather cases and glass back of the phone after a week of usage.

B. Calculating Number of Viable Cells per ml of Culture:

Number of Viable cells per $mL = \frac{Number\ of\ Colonies\ x\ Dilution\ Factor}{Inoculation\ Volume\ (mL)}$

Since inoculation volume = $100 \mu L = 0.1 \text{ ml}$;

Number of Viable cells per $mL = 10$ x Number of Colonies x Dilution Factor

Table 4: Table of total number of viable bacteria cells per mL of culture (x10⁵ CFU/ml), calculated from raw data from Table 3 using the formula on page 13.

C. Statistical Analysis:

1. Mean:

n = number of trials

 x_i = number of viable cells per mL of culture

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

- 2. Variance:
- n = number of trials
- x_i = number of viable cells per mL of culture
- \bar{x} = mean

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

- 3. Standard Deviation:
- n = number of trials
- x_i = number of viable cells per mL of culture
- \bar{x} = mean

4. Standard Error:

$$
SE_{\bar{x}} = \frac{s}{\sqrt{n}}
$$

- s = standard deviation
- x_i = number of viable cells per mL of culture

Table 5: Descriptive statistics of calculated values of viable cell number per mL of culture (x10⁵ CFU/ml) from Table 4.

Table 6: ANOVA results of calculated values of viable cell number per mL of culture (x10⁵ CFU/ml) from Table 4.

D. Bar Graph:

Graph 1: Bar graph of mean number of viable bacteria cells per mL of culture, expressed in CFU/mL. Error bars are included to represent the confidence level (95.0%).

6. Conclusion and Evaluation:

A. Evaluation

 The aim of this research was to investigate the research question: "How do the leather, silicone and plastic smartphone cases and the glass back of the phone without any case differ in terms of total bacteria count, measured using the viable cell counting method after a timespan of one week with daily usage of exactly one charge cycle?". My hypothesis was that the leather case will have the greatest number of total bacteria, followed by plastic and silicone with minor difference, and glass after them with the lowest number of bacteria.

At the end of the experiment, the mean results turned out to be 6.32x10⁵ CFU/ml for plastic case, 7.28x10⁵ CFU/ml for silicone case, 8.86x10⁵ CFU/ml for leather case and 4.42x10⁵ CFU/ml for glass back of the phone. These results show similarities to my initial hypothesis in the way that leather had the highest bacteria count and glass had the least count. All of the groups had significant bacteria growth, which was also expected according to the hypothesis. One thing that my hypothesis failed to predict was the fact that silicone case had more bacteria than the plastic case. This difference is not very significant but should not be omitted nonetheless. Such a difference between plastic and silicone may have risen from differences in surface properties such as water adhesion, heat capacity or elasticity.

The null hypothesis was that for α = 0.05, the difference between the groups are insignificant statistically. Since the p value of the data given by the ANOVA test (p = 0.015114832) is less than the α value, it is proven that there is indeed a meaningful difference between the case types and the back of the phone. This rejects the null hypothesis and further proves my initial hypothesis.

 Even though ANOVA tests proved the difference between the experimental groups, the standard deviation and confidence level (95.0%) values are relatively too high (see Table 5 and Graph 1) for the results to be 100% accurate. This shows that the data from different trials have high dispersion and low precision.

 High dispersion of the data can be explained by the fact that some random errors along with probable systematic errors were involved with the experiment. These errors and some measures that could be taken to reduce these errors include:

- 1. Since this experiment is based on the bacterial contamination of cases in daily life and no two days are identical in a person's life, randomness plays a very significant role for this investigation. This is why number of trials is very important in the accuracy of the results. I now think that I could have increased accuracy by using a group of 4 people as test subjects, and alternating the plastic, silicone, leather and glass groups between them. This way I could essentially quadruple the number of trials I could do in a limited amount of time and decreased the effect of random errors on the results.
- 2. Trial 4 of plastic and trial 2 of glass both gave results lower than the 30- 300 colony interval (28 for both). This means they have lower statistical accuracy than the other data. Even though it was mentioned that serial dilution at every trial is unnecessary, doing so would have increased the overall accuracy of the experiment.
- 3. The silicone, plastic and leather cases were black in color but the phone was white in color. This may have resulted in a difference in the overall mean temperature between groups. In a future experiment I would use cases of the original color of the phone.
- 4. All the bacteria cultures were prepared using LB nutritional agar. Using other alternatives such as sheep blood agar could have yielded different colony counts. For a healthier result, same procedures could be followed multiple times, using different growth media each time.

There are also some limitations to this experiment, including:

1. There are thousands of case manufacturers and case types of different hardnesses, textures and chemical compositions. Therefore the results from this particular set of cases that were used may not be valid for all leather, silicone and plastic cases on the market.

2. Different people deal with different species of bacteria on their daily lives. This investigation reflects the results from the life of a high school student from Turkey, real world results for people with different lifestyles may be different.

 The main weakness of this method is that it only gives information about the overall number of bacteria in the culture. Even though highly unlikely, there doesn't have to be a correlation between the total number of bacteria in the culture to number of only pathogenic bacteria. To have a more specific approach on the topic, species specific methods could be used.

B. Conclusion:

 The research question "How do the leather, silicone and plastic smartphone cases and the glass back of the phone without any case differ in terms of total bacteria count, measured using the viable cell counting method after a timespan of one week with daily usage of exactly one charge cycle?" was answered by this investigation. According to experimental results, the total bacteria count increases from glass to plastic to silicone to leather in correct order.

 The most general note that this experiment proved is that smartphones, with or without a case, can act as reservoirs of microorganisms. They are very optimal ways of transportation of natural flora of the owner to other people, not only because they contain large amounts of microorganisms but also because they very frequently come in contact with the owner's relatives and close friends. Spread of pathogens can speed up in public places due to this nature of smartphones in society.

 When it comes to the decision of having a case or not, it is worth noticing here that all case types ended up having significantly more number of bacteria than the glass back of the phone. This shows that by using a case, there is increased risk of raising susceptibility of your body to pathogen bacteria. Even though a case can protect the phone from serious impacts or scratches, it is proven that they are not as effective at protection from biological threats.

 If protection from impacts or scratches is absolutely necessary for the user, choice of case material also makes significant difference. Plastic alternatives should be preferred because they do not host as many organisms as other alternatives such as leather or silicon. Leather, even though looks fancy, should be avoided as much as possible since it turned out to be the weakest group to bacterial contamination according to experimental results.

 The main reason I wanted to study on this topic was that smartphones have very rapidly been implemented to the lives of the people around me recently. Many people started spending hours using their smartphone in every aspect of everyday life, including me. I wanted to investigate and see the risks involved with such an important change in the life of the modern society.

7. Appendices:

A. Appendix 1:

Preparation of LB Agar:

- 1. Weigh 5.0 grams of tryptone, 2.5 grams of yeast extract, 5.0 grams of NaCl and 7.5 grams of agar.
- 2. Mix the ingredients into a powder. 20 grams of premix LB Agar powder (VWR DF0445-17) could be used instead of this mixture.
- 3. Measure 500 mL of distilled water using a graduated cylinder and pour it inside a 1L flask.
- 4. Add the powder mixture into the flask
- 5. On a stirring hot plate, heat the mixture for 1 minute to boil while stirring.
- 6. Let the agar cool to 55ºC.

Preparation of Agar Plates:

- 1. Take sterile Petri dishes off their bags.
- 2. Lift the cover of the plate slightly and pour a thin layer of the prepared LB agar (5 mm, 10 mL).
- 3. Wait for 20 minutes until the plates cool down.
- 4. Flip over the plates and label them with the date.
- 5. Store them in plastic bags in a fridge.

B. Appendix 2:

Preparation of the culture:

- 2. Put on gloves and disinfect the table thoroughly using before starting the process.
- 3. Take a piece of cotton and pour 2 mL of disinfectant on it by pushing the pump on the disinfectant dispenser once.
- 4. Put the plastic case face-down on the table.
- 5. Start rubbing the back of the case with the cotton. Make sure not to leave any unrubbed spots.
- 6. Check the time and date and record it.
- 7. Put the case on the phone and start using the phone normally. Use the phone for exactly one charge cycle everyday. Make sure you wash your hands after you leave the bathroom and after meals. If any sort of antibacterial material or cleaning agent gets spilled on it, start from the beginning.
- 8. After exactly one week, put on gloves again and take the case off the phone.
- 9. Take a few droplets of 0.90% saline solution from the bag by inserting a syringe through the white port.
- 10. Rip the seal of the culture swab, take it out of its tube and soak it with two drops of 0.90% saline solution from the syringe.
- 11. Rub the swab all over the back of the plastic case, doing twisting and rotational motions. Don't let it touch anywhere else.
- 12. Put the swab back in the tube carefully.

C. Appendix 3:

Figure 1: A diagram of spreading of the bacterial culture on a nutritional agar plate with a glass spreader. The process is done 8

times for 2 full turns around the plate.

C. Appendix 4:

Figure 2: A diagram of the process of serial dilution of a bacterial sample. In every step, the

concentration of the culture is multiplied by 10-1.

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