Topical probiotics for reducing infections by multidrug resistant bacteria

BME 301
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Department of Biomedical Engineering
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Abstract

Lactobacillus rhamnosus GG (LGG), a probiotic widely used in various clinical fields, is found to be beneficial in enhancing body health condition. In order to research the effectiveness of applying LGG to the interior of the nose to reduce *staphylococcus aureus* (s. *aureus)* bacteria, a delivery device for LGG is needed to conduct clinical trials. There are three design choices for the delivery device, including the dry powder nasal spray, the liquid nasal spray, and the gel with blister pack applicator. After the design evaluation process, the gel with the blister pack applicator design was chosen as our final design due to a higher preference among the average user along with its ability to better satisfy the design requirements. Testing in the future will be used to figure out the number of bacteria that survive in the nose.

1.0 Introduction

1.1 Problem Statement

Dr. Nasia Safdar, of the UW-Madison Department of Medicine, researches the use of probiotics. Currently, she is researching the efficiency of the probiotic LGG in preventing *staphylococcus aureus (s. aureus)* infections when the probiotics are applied to the interior nasal passage. A device to deliver the probiotic to the inside of the nose is needed to perform clinical trials with the probiotic. The delivery device should allow the accurate delivery of one billion viable LGG organisms to the nose. Also, a solution in which to suspend and deliver the bacteria to the nose needs to be found. The LGG should live inside the nasal passage for at least one day to allow for daily application of the probiotic.

1.2 Background

1.2.1 Probiotics

Probiotics, as defined by the World Health Organization, are “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host” (World Health Organization, 2002). Probiotics can be used to balance the immune system in two major ways. First, probiotics can be used to restore a balance of good bacteria to the body in cases where antibiotics used to kill unfriendly bacteria in the gut also killed those bacteria that are beneficial to the digestive and immune system. The death of beneficial bacteria may lead to side effects, such as cramping and diarrhea, which probiotics can relieve. Second, when disease-causing bacteria, yeast, or other unfriendly microorganisms invade the body, probiotics are able

to stop or suppress the growth of these harmful microorganisms (National Center for Complementary and Alternative Medicine, 2008). LGG is a strain of bacteria and is one of the most common probiotics used in clinical applications. LGG has been proven to be effective to treat and prevent acute diarrhea and antibiotic-associated diarrhea. It is also suggested, but not proven, that LGG may prevent allergies, respiratory infections, dental caries, and nasal colonization of potentially pathogenic bacteria such as *s. aureus* and *streptococcus pneumonia* (Doron et al., 2005).

1.2.2 Staphylococcus aureus

S. aureus is a common type of bacteria that can be found in roughly 30% of the people's nostrils. Normally, s. *aureus* stays inside the nose and the person is considered "healthy." *S. aureus* is able to migrate to other parts of the body in cases where the person has a wound or a compromised immune system due to illness. Once *s. aureus* gets inside the body, they may cause minor infections of the skin, like pimples and boils, or serious infections, such as blood infections and pneumonia, which can be fatal (Center for Disease Control and Prevention). Methicillin-resistant *s. aureus* (MRSA) are s. *aureus* bacteria that are resistant to the antibiotic called methicillin. Three LGG derived peptides, NPSRQERR, VHTAPK and PDENK, have been shown *in vitro* to affect the growth of MRSA bacteria (Lu, 2010). Instead of simply applying these peptides, the establishment of a colony of LGG in the most outer part of the nostrils will be tested in the clinical trials for its ability to prevent MRSA infections in those who have the bacteria present in their nasal cavities.

2.0 Motivation

Since about 30% of people have *s. aureus* naturally in their nose and a wound or a compromised immune system is enough for *s. aureus* to cause an infection, a new method of treating the infection is important. The treatment should pose a minimal chance of creating drug resistant strains of *s. aureus* and should not disturb the body's useful bacteria. Antibiotics applied to the nose have been shown to eliminate the *s. aureus* bacteria, but the antibiotics are harmful to the useful bacteria in the body as well. Antibiotics applied over large areas or for long periods of time create resistant bacteria strains that can cause recurrent infections in the same person or in other people. One example of a developed resistance is seen in the case of MRSA, which is especially dangerous because the antibiotic, methicillin, is no longer effective in curing an

infection. There is an economic burden associated with these bacteria for hospitals, due to increased length of stay of patients, more patient complications, and an increased risk of death. In 2003, the total economic burden of *s. aureus* infection was estimated to be $14.5 billion for all inpatient stays and $12.3 billion for surgical patient stays (Noskin, 2007).

The clinical trials that the delivery device will be used in will be looking at the effect that applying LGG to the nose has on the baseline levels of *s. aureus* in the nose. Probiotics have been studied when taken orally, but since *s. aureus* resides mainly within the nasal passage, the clinical trials will be performed to test the efficiency of LGG in killing or reducing the number of harmful *s. aureus* when LGG are applied to the nasal passage. The use of the probiotic, LGG, instead of the current antibiotics that are on the market will be assessed for their ability to eradicate *s. aureus* from the nose. Using a probiotic instead of an antibiotic minimizes the danger of *s. aureus* developing a drug resistant strain. The probiotic works by secreting peptides that poison the *s. aureus*, whereas an antibiotic targets the cell membrane or enzymes and promotes genetic evolution of resistant strains of *s. aureus*. Therefore, the LGG treatment could be used for long periods of time unlike antibiotics that are confined to shorter treatment periods to decrease drug resistant strain development. The delivery device is needed to successfully complete the clinical trials.

3.0 Design Specifications

3.1 Client Requirements

Figure 1.Culturelle capsules (Walgreens).

The ultimate goal of the project is to design a device for delivering the probiotic bacteria, LGG, to anterior nasal passage. The standardized amount for accurate delivery should be between 10 million and one billion organisms each time, and ideally the device should be used repeatedly for multiple deliveries over a short period of time. The delivery amount of 10 million to one billion LGG is the amount that will be used within the clinical trials. Each Culturelle capsule contains 9 billion organisms (Figure 1), so the LGG in the capsule needs to be diluted or delivered in smaller amounts. The selected solution that suspends the bacteria within the delivery device should be biocompatible with the human body. Once delivered, the bacteria must live in the nose for a minimum of one day in order to obtain reliable observations of the impact that LGG living in the nose has on treating *s. aureus* infections.

The solution and materials should allow the bacteria to live for up to two weeks and be refrigerated at 4°C to 5°C to prevent it from overgrowing while in storage. The device needs to be opaque to keep out light and should not contain any food for the bacteria because both the light and food may cause the LGG to grow to excessive numbers within the device. Excessive growth would cause the delivered amount of LGG to exceed the desired amount.

If insertion of the device into the nose is needed, the insertion should not be further than 1 cm into the nasal passage. Any insertion of the delivery device further into the nasal passage may result in the LGG traveling into the sinus cavities and throat, rather than remaining only in the nose. If the LGG makes its way to parts of the body other than the nose, such as the sinus cavities and throat, the LGG themselves may cause a bacterial infection. For the patients' convenience, the device should be easy to transport, with dimensions of less than 7 cm x 7 cm x 2 cm and a weight less than 0.25 kg. The materials for the delivery device must not induce any harm to the user. See Appendix A for further details on the client requirements and design specifications.

3.2 Ethics

To ensure the safety and accurate delivery of LGG, actual testing on human subjects is necessary. While performing the experimental trials, patient confidentiality should be maintained. The device will be clinically tested following proper established guidelines for running such a test. Moreover, it is important to ensure that any input must not be harmful when testing on any human subjects or other living subjects. Substances, whose effects on humans are uncertain, may not be used on human subjects without prior testing that shows their safety when used on humans. Lastly, the product should be carefully designed and evaluated to prevent patent infringement.

3.3 Ergonomics

The probiotic delivery device needs to keep the LGG in storage for up to 2 weeks and still deliver the LGG to the nose in viable condition. It should not damage the LGG or cause harm to the patient. The solution that the LGG are mixed with should not cause the LGG to grow or kill the LGG. The exterior housing for the delivery device should be free of sharp edges so the user is not harmed. The forces applied by the user to the device should not be excessive.

4.0 Existing Devices

There are currently many nasal drugs on the market today; however, none of them use probiotics to treat *s. aureus* infections. Examples of existing devices include nasal sprays and nasal ointments. There are many other types of nasal sprays that solely deliver saline solutions to rinse and moisturize dry noses.

4.1 Nasal Sprays

Afrin (Figure 2) and Flownase (Figure 3) are nasal sprays used to treat discomfort of the nose and sinus area caused by colds and sinus infections. Although Afrin and Flownase relieve pain, they do not treat or cure any disease. Afrin and Flownase come as a liquid nasal spray and Afrin is not recommended for use longer than 3 days (National Center for Biotechnology Information). The average cost of Afrin is approximately $7-$10 US dollars (Walgreens). The average cost of Flownase is between $40 and $80 US dollars depending on insurance coverage (Walgreens).

Figure 2.Afrin liquid nasal spray (Walgreens).

Figure 3.Flownase liquid nasal spray (Walgreens).

4.2 Nasal Ointments

Bactroban (Figure 4) is a prescription antibiotic ointment that is used to treat *s. aureus* infections. Bactroban is commonly used to treat impetigo, skin infections, and fungal infections. Bactroban is applied 2-3 times daily for 1-2 weeks onto the affected area, which is the nose in the case of *s. aureus* infections (National Center for Biotechnology Information). Bactroban nasal ointment has an average cost between $20 and $70 US dollars depending on insurance coverage (Walgreens).

Figure 4.Bactroban nasal ointment (Walgreens).

5.0 Design Proposal Overview

All three designs feature a different solution that the LGG bacteria are suspended in during delivery. Each design has different features that distinguishes itself and provides a unique solution to the problem.

5.1 Design 1: Dry Powder Nasal Spray

The dry powder nasal spray would consist of the LGG without liquid to accompany the powder, so that the bacteria and materials used to encapsulate the probiotic bacteria would land directly on the inside surface of patients' nose. Figure 5 shows a sketch of a device to deliver

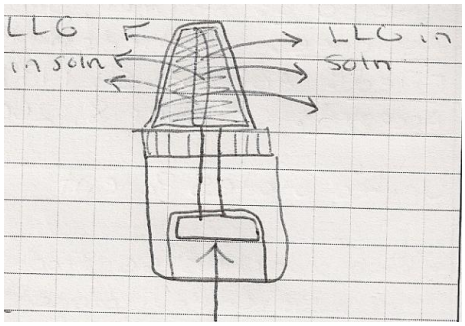

Figure 5.Dry powder nasal spray (illustration by Kimberli Carlson).

powered LGG. The powder would be sprayed out the sides by mixing with the solution of air that is forced out of the bottle when the patient pushes up the pedestal on the bottom of the device. When the pedestal on the bottom of the bottle is pushed up it forces the connected pedestal that resides within the cavity of the bottle, on which the LGG powder is laying, upwards. This upward movement of the pedestal inside the bottle mixes the LGG powder with the air inside the bottle. As the pedestal continues to be forced upwards, the LGG powder suspended in the air is forced out the openings in the cap. The slit shaped openings should help direct lines of powder and air out of the cap in comparison to a larger opening that would result in a cloud of powder escaping.

The Culturelle capsule contains the powder form of the LGG inside the capsule itself. In order to isolate the powder, the capsule needs to be opened and the powder form of the LGG needs to be poured into a spray bottle. The sprayed powder should not travel back into the sinus cavities because LGG can cause bacterial infections in locations within the body other than the anterior portion of the nostrils. An example dosage requirement is two sprays into each nostril to deliver the 1 billion LGG organisms. This requires that each spray of the dry powder would deliver 250 million LGG organisms.

There are several advantages and disadvantages of the dry powder nasal spray design option. A benefit of this design is that the powder spray would be easily delivered by patients since it is very similar to the delivery of liquid nasal sprays used commonly to treat allergies and sinus infections; most patients are familiar with the procedure of using a nasal spray. Another benefit is that the LGG would not have to be encased in anything, such as a liquid or gel that could affect the growth and survival of the bacteria. The LGG would reside inside a bottle as opposed to in a capsule like in Culturelle. Since the Culturelle LGG are in a capsule, there is not an issue with storing the LGG this way for 2 weeks. A downside of this design is that the dry powder may irritate a person's nose and cause them to sneeze. In the process of sneezing the LGG may be expelled from the nose, thus defeating the purpose of applying the powdered LGG in the first place. Another downside of the design is that dry powder is that only 14% of people surveyed chose the dry powder nasal spray as their optimal first choice of nasal treatment delivery.

Figure 6.The procedure of using a liquid nasal spray (http://familydoctor.org/online/etc/medialib/famdoc/images/100-200/104b.Par.0001.Image.gif.).

5.2 Design 2: Liquid Nasal Spray

The liquid nasal spray would consist of the LGG being dissolved within a 0.9 % saline solution. Figure 6 shows the general procedure of how to deliver a liquid nasal spray to the nose. The nozzle is inserted into the nose and pressed downwards. The depression of the nozzle causes a mist of the liquid inside the bottle to be delivered onto the interior of the nose.

The dry powder nasal spray design requires the capsule to be opened and the powder form of the LGG bacteria mixed with the 0.9 % saline solution. The 0.9% was chosen because it worked to keep the LGG alive during the initial saline survivability test (See Appendix B). It was originally tested because it was the standard percentage of saline used for most applications in the lab where the project's work was done. A short nasal spray nozzle is needed if using this design to prevent the LGG from going too far into the nose and entering the sinus cavities. During the preliminary testing, as described in Appendix B, the capsule originally contained about $1*10^8$ colony forming units per milliliter and at the end of the three days the LGG had only grown to approximately $6 * 10^8$ colony forming units per milliliter. This proved that the LGG can last for at least three days alive within the 0.9% saline solution, which suggests that this design is plausible.

The liquid nasal spray design has advantages and disadvantages in using it. One of the advantages of using the liquid nasal spray is the ease of controlling the output amount per spray. The number of sprays needed per application would need to be relative to the concentration of LGG in the saline solution and the output volume per spray of the spray bottle. For example, if the concentration of the LGG was $6 *10^8$ LGG / mL and the sprayer delivered 0.25 mL / spray, a patient would deliver 1 spray per nostril to deliver $3*10^8$ LGG in total. An advantage of choosing this design is that more people are more familiar with liquid nasal sprays. According to the survey (see Appendix C), the majority of subjects chose the liquid nasal spray as their second choice in nasal drug delivery based on personal preference of nasal drug delivery methods. Although choosing the liquid nasal spray might be more user-friendly, the output distance may be too far, causing the solution to travel into the body, which does not serve the goal of the design. One way to prevent the LGG mist from traveling too far back, we could design the spray to come out of the sides of the cap instead of the top.

5.3 Design 3: Gel with Blister Pack Applicator

The third design option to deliver the LGG to the nasal passage is a gel that is delivered in volumes placed inside a blister pack. The gel would contain the powdered LGG from the Culturelle capsule, suspended in the gel. Since the LGG are thought to be most effective towards the most outer portion of the nasal passage, the gel will be applied close to the edge of the nose. This placement of the gel will prevent the LGG from dripping back into the nose. The gel will be sufficiently thick as to properly adhere to the interior surface of the nose. Preliminary testing showed that a gel would be comfortable and immobile after placement.

To deliver the correct amount of LGG (between 10 million and one billion organisms) a volume of gel with a specific concentration will be placed into blister packs (Figure 7), and are opened by removing a thin metal film. The gel will be scooped out with a finger or a device such as a q-tip and put in the nose.

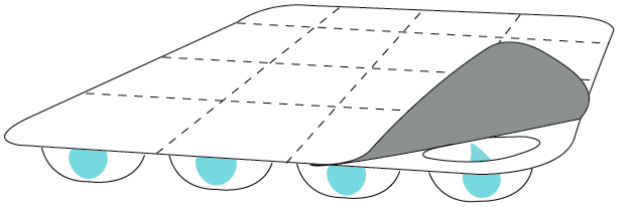

Figure 7.Blister pack that the gel containing the LGG will be placed in (illustration by Wan-Ting Kou).

The gel design has its own specific benefits and obstacles. Since most people are comfortable with using ointments, the gel would allow for a familiar application method to be used. Also, the gel formulation would be comfortable and stay in the location desired. The survey showed that the design ranked first most often was the gel. An obstacle to producing a gel based probiotic solution is finding a gel that maintains the LGG in a viable state, while suspending it within the gel. Along those lines, the gel needs to be formulated with the correct consistency so that it maintains viability of the LGG.

6.0 Design Evaluation

Table 1 below lists the categories that the team used to evaluate the three different designs. The final design would be chosen based on criteria including user preference, accuracy, ease of use, maintaining bacteria viability, and precision of the device. All the qualities were weighted equally because without any one characteristic, the delivery device would not be conducive to safe and productive deliverance of the LGG to the nose by patients. The score in each category is from 1 to 5, where 5 being the highest possible score. Then the total score was added up within each category to determine the final design.

Table 1: Design Matrix for design evaluation

Categories	Dry Powder Nasal Spray	Liquid Nasal Spray	Gel with Blister Pack Applicator
Preference (5)	3	4	5
Accuracy (5)	3	3	5
Ease for use (5)	3	4	4
Bacteria viability (5)	5	4	3
Precision (5)	4	5	4
Total (25)	18	20	21

The first category is the preference of the user. The team conducted a survey (see Appendix C) that asked people which design is the one that they feel most comfortable to use. The result showed that most people would choose gel as their first choice (41%), followed by liquid spray (35%) and then dry spray (14%) so the gel option score the highest point of 5.

The second category is the accuracy of the device to deliver most of the LGG into the front of the nose. Although the right design could be created to aim the spray to the sides of the nose instead of back into the sinus cavities, the risk of LGG escaping into the sinus cavity is still great because the mist would be very fine. All it would take to get the LGG back into the sinus cavity is for the patient to sniff too hard while simultaneously spraying the nasal spray. The dry spray has the same problem as the liquid spray, so these two designs scored only 3 in this category. The gel can be easily applied and restricted to the front area of the nose, so it scored a 5.

The ease of use of the device is based on the ability of the average patient to use the device without having trouble operating the device. Since there are already lots of liquid sprays in the market, this option would be one that everyone is familiar with. Thus liquid sprays scored the highest point in this category. Dry spray operate the same way as the liquid spray, but since it may causes sneezing after use, it only received a 3. The gel option, depending on the users and their preferences, may be unfamiliar to some of the people.

It is important that the LGG can survive in the device for 2 weeks, because probiotics need to be live in order to work. The dry spray has the advantage in this category because the source of LGG in our design is from the product Culturelle, which already has LGG in the dry powder form. And since the company of Culturelle tested that LGG can survive in this form for 1 month, the dry spray scored the highest point of 5 in bacteria viability. The team conducted a small experiment that tested if LGG could survive in the saline solution (see Appendix B), and

received positive results that LGG can maintain their viability in the solution for 3 days. Thus the option received a score of 4. With the gel option, since the team hasn't set up any experiment at the time of choosing a final design to test how well the bacteria would live in the gel-type formation; this design receives a 3.

The precision of the device evaluates how precisely the device can deliver 1 billion bacteria every single time. The gel is probably the most precise device to deliver between 10 million and one billion bacteria constantly, while dry spray may have the problem of bacteria being sneezed off and gel may be hard to apply at a consistent amount. The liquid spray may get most of the LGG into the nose, but as discussed earlier, the LGG may not all land at the anterior of the nose. The gel will stay within the nose and the moisture will not cause sneezing as a dry powder would, thus ensuring that most of the LGG deposited by the gel will remain within the nose. See Appendix C for results of the survey of people's method of preference.

7.0 Final Design

The final design is the gel with blister pack applicator, and the specific amount of each component of the sample is shown in Figure 8. According to the several testing in the Testing section, the gel solution is at a ratio of 3 to 1 of the Spectra 360 Electrode Gel to the 0.9% saline solution. Every 10 mL of this solution at this concentration will mix with one Culturelle capsule. Each application to one nostril is around 0.25 mL of the sample, which can deliver approximately 28.5 million of the organisms at the end of 2 weeks, which is still within the desired range of 10^7 to 10^9 bacteria. The dosage should be reapplied daily.

Figure 8. Final design including all components with corresponding amounts to be delivered.

8.0 Testing and Results

8.1 Survivability in saline

To determine the actual amount of LGG in one Culturelle capsule and to see if the LGG survived in 0.9% saline solution, the team set up a saline survivability test. Three capsules of LGG were added and mixed with 10 mL of saline separately. The testing period was three days, and counting of bacteria was performed everyday (see Appendix B for procedure of plating and counting the bacteria).

After completing the culturing and counting, Figure 10 in Appendix B shows the result that there was not a significant decline in the number of surviving bacteria after 3 days in the saline solution.

8.2 Gel comparison

To decide the type of gel that is suitable for our final design, the team compared a variety of gels. Gelatin and peg gel was the first two types of gel tested. However, because both gels melted into liquid when under body temperature, the team concluded that gelatin and peg was not suitable for our design simply because the melting would cause the gel with LGG to drip out of the nose.

The team looked at the Spectra 360 electrode gel and Biopac electrode specifically because both gels can maintain their gel form at room temperature. Biopac is a saline-based electrode gel. Since the LGG survived very well in saline solution, (as determined in the saline survivability test), the Biopac gel was thought to be a good option for our design prior to this test. Spectra 360 gel, on the other hand, was a completely salt-free electrode gel. These two different types of electrode gel were tested to see which gel was better for LGG to survive.

Figure 11 in Appendix B compares the survivability of LGG in two types of gel, Spectra 360 and Biopac. The Spectra 360 gel was mixed 2:1 with 0.9% saline and the Biopac gel was mixed 1:1 with saline. At day 0, when the experiment started, the concentrations of LGG in each gel were roughly the same. This indicated that our method of mixing the gels with LGG was fairly consistent. For over a period of 4 days, LGG was able to survive in the Spectra 360 gel because the concentrations of LGG didn't change significantly. On the other hand, LGG seemed to survive poorly in the Biopac gel because the concentration of LGG went down significantly at day 2 and remained relatively low at day 4.

8.3 Gel consistency in different temperatures

The consistency of the gel solution in different temperatures was tested since the gel solution will be stored in the refrigerator, placed in the room temperature, and applied to the human nostril at body temperature. As a result, we prepared the gel and placed it under the three conditions: 4°C, room temperature, and 37°C. The result of this experiment is promising, showing that the gel solution is consistent under the three conditions.

8.4 Gel solution concentration

Different gels exhibit various levels of viscosity, and this is the main determinant of how to decide the concentration of the gel solution (gel + 0.9% saline). We had to add saline because the gel itself was too viscous. After deciding using the Spectra 360 electrode gel as our delivery mean of the probiotic, different concentrations of the gel solutions were prepared and the team members felt the viscosity of the gel with fingertips and placed it in their noses to test the gel. The three concentrations for comparison were 2:1, 3:1, and 4:1 (gel: saline). The most concentrated gel solution with the 2 to 1 ratio was too thick and sticky, which may result in the inaccuracy of delivery. The least concentrated gel solution with the 4 to 1 ratio was too fluid, which may also result in the LGG running out of the nose. Therefore, the concentration with the ratio of 3 to 1 wins out the competition and the final design will use this concentration for preparation of the gel solution.

8.5 Delivery amount

To determine a proper delivery amount, three different amounts of the gel solution were prepared for comparison. Among three different amounts, the concentration of the gel solution was controlled at the 3 to 1 ratio according to the previous testing. The three amounts are 0.25mL, 0.5mL, and 1.0mL. To obtain the most comfortable delivery amount, the team members also used their fingertips and applied these different amounts of gel solution into the nostril. The results turned out that the amount of 0.25mL is the most reasonable amount to put in one nostril. Figure 9 shows the relative size of the three different amounts.

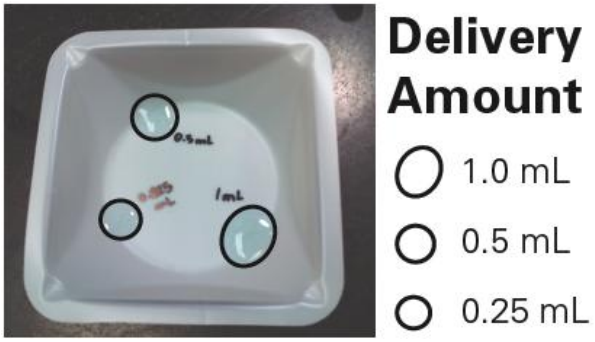

Figure 9. The relative size of the three experimental delivery amounts.
(photo taken and edited by Wan-Ting Kou)

8.6 Bacteria Concentration Comparison

In previous experiments, including saline survivability and gel comparison test, every capsule of LGG was dissolved in 10 mL of either gel or saline solution. If the concentration of LGG is increased in the gel, then less amount of gel is needed for each application. Because the patients may feel more comfortable with less amount of gel applied to their noses, the team decided to test if LGG can survive in a more concentrated environment. Two sets of gel solution (3:1 of Spectra 360: 0.9% saline) that contained different concentrations of LGG in a specific amount of gel were set up. The more concentrated group had one capsule of LGG dissolved in 7 mL of gel solution and the less concentrated group had one capsule dissolved in 10 mL of gel solution.

Figure 12 in Appendix B shows how well the LGG survived over 2 weeks when they were packed with different concentrations of LGG in the Spectra 360 gel. The data indicated that LGG survived better in the less concentrated group. The greater amount of decrease in the more concentrated group may be caused by the limited growth space. However, the concentrations of LGG in both groups were still within the desired range after 2 weeks (See Appendix D for raw data).

9.0 Future Work

There are a few items that will need to be tested in order to have a fully functioning product for the client's clinical trial. The main testing that will need to be done is to determine the number of LGG organisms that colonize the nasal passage in a healthy individual. We will use a finger or Q-tip to apply 0.25mL of gel solution out of the blister pack. We will swab the

nasal passage of this person immediately and every few hours up until 24 hours. The counted cultures will be able to tell us the concentration of the bacteria in the nose. If the value is below 10^7 bacteria, we can either suggest making the gel at an earlier time point (if the gel was greater than 6 days old) or simply by applying more gel. As we expect to lose some gel in the package due to the application, we could give let every blister pack compartment contain more than 0.25mL of gel, between 300 and 350, to make up for this loss. Once this amount has been proven to apply between 10^7 and 10^9 bacteria, we will be ready to test storage conditions in the packaging itself. It is not expected that this packaging will perform any different than the test tubes that we stored the gel in, but if the client requires this testing, she can have it done.

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Appendix A:
Product Design Specification Report
Probiotics Delivery Device

Date: 29 April 2011

Team:
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Problem Statement

Dr. Nasia Safdar, of the UW-Madison Department of Medicine, researches the use of probiotics. Currently, she is researching the efficiency of the probiotic lactobacillus GG in preventing *s. aureus* infections when the probiotics are applied to the interior nasal passage. A device to deliver the probiotic to the inside of the nose is needed to perform clinical trials with the probiotic. The delivery device should allow the accurate delivery of one billion viable lactobacillus GG organisms to the nose. Also, a solution in which to suspend and deliver the bacteria to the nose needs to be found. The lactobacillus GG should live inside the nasal passage for at least one day to allow for daily application of the probiotic.

Client requirements

- Deliver probiotics to anterior nasal passage
- Probiotics to be delivered are the bacteria lactobacillus GG (trade name: Culturelle)
- Accurate and repeatable delivery of 10 million to one billion organisms
- Solution needs to be found to suspend bacteria in
    - Biocompatible with human patients
    - Solution must allow bacteria to live for up to 2 weeks
    - Solution must keep bacteria from overgrowing
    - No food for bacteria will be present
- Daily application: the bacteria must live in the nose for a minimum of 1 day
- Delivery device will need to be able to be refrigerated
- Delivery device should be opaque to keep out light that would promote bacteria growth
- Weigh less than 0.25 kg
- Dimensions less than 7 cm x 7 cm x 2 cm
- Delivery device must prevent insertion of delivery device further than 1-2cm into the nasal passage
- Material of delivery device must not harm user and must be non-abrasive
- Material must not degrade with constant use
    - Lifetime is 2 weeks
    - Use daily
- Material must withstand refrigerated storage conditions
    - 4-5°C
    - 50% humidity

Design requirements:

1. Physical and Operational Characteristics

a. *Performance requirements*: The device will be used daily to deliver the dosage of between 10 million and 1 billion bacteria. The device and bacteria suspended in a solution must allow the bacteria to survive for up to 2 weeks. The device must have ability to secure bacteria inside to prevent contamination of outside surfaces or of bacteria itself.

b. *Safety*: This device must not endanger the user. There must not be toxic materials or sharp edges within the device. There should not be any pathological concerns due to fluids escaping the delivery device. Neither the solution, nor the delivery device should cause harm to the patient.

c. *Accuracy and Reliability*: This device should accurately deliver between 10 million and 1 billion organisms. This delivery should be precise and repeatable for daily delivery up to 14 days. The solution should not cause the bacteria to die or grow excessively.

d. *Life in Service*: The device should have repeatable delivery procedures for 2 weeks. The materials should uphold their features to allow for multiple deliveries of probiotics. The solution should not allow excessive growth or death of bacteria for 2 weeks.

e. *Shelf Life*: The materials of the model should not degrade over time in refrigerated storage for 2 weeks. The solution should not allow bacteria to die within 2 weeks. The solution will be made up to a week before the patient receives it. For the clinical trials, this is a feasible time period because the people in charge of the clinical trial will be packaging the gel themselves. The patient will have it for up to a week for use; the total shelf life is 2 weeks.

f. *Operating Environment*: There will be one device per patient. The delivery will be performed at ambient conditions. The storage will be at 4-5 ^{o}C in a refrigerator.

g. *Ergonomics*: Delivery device should only be used to deliver the prescribed probiotics and should be discarded after use. The probiotic may be discarded in regular trash out of reach of children. The probiotics should be taken only in prescribed dose.

h. *Size*: The device should not exceed a size of 7 cm x 7 cm x 2 cm.

i. *Weight*: The delivery device with the probiotics suspended in the solution should weigh less 0.25 kg.

j. *Materials*: Materials must be safe for use with humans. Any material used should not pose a health risk. Non-radioactive, non-flammable, and non-corrosive materials should be used. Material must not degrade when introduced to the nasal passage. The solution must not be harmful to the bacteria or patient.

k. *Aesthetics, Appearance, and Finish*: The device should be pleasing to the eye. The finish should be smooth and clean looking.

2. Production Characteristics

a. *Quantity*: One device is required at this time. However, since the device is to be used on a large scale clinical trial, additional models should be able to be available.

b. *Target Product Cost*: The target manufacturing cost for the product is $10 per delivery device. This target cost includes the bacteria and the solution. The target cost is based on a mass production of the device; the first device will have a target cost of under $150 for the lab supplies, solutions, culture media, and bacteria (the lab is fully equipped and some culture media and solutions will be used from the current stock).

3. Miscellaneous

a. *Standards and Specifications*: This device will require approval by the FDA if this product with the delivery device is mass produced for market use after the clinical trials take place since it will be used with patients. Currently, the device falls under Class I classification and does not require any premarket notification to the FDA regarding the device.

b. *Customer*: The delivery device should adhere strictly to the basic requirements of delivering bacteria to the nasal passage of the patient. The device and the bacteria should be used as prescribed.

c. *Patient-related concerns*: The delivery device will come in contact with patients and therefore should not cause harm to the patient. The patient should not be made sick by the materials of the device or its probiotic contents. Patient confidentiality should be maintained while building and testing the delivery device.

d. *Competition*: There are many types of products that focus on delivering fluid solutions to the nose. Three examples are Afrin, Flownase, and Bactroban. There is not a marketed ointment or sprayer specifically for delivering probiotics to the nasal passage.

Appendix B: Lab Procedure and Testing Results for Culturing Lactobacillus GG

To test the number of organisms that were still living within a solution and gel after the Culturelle capsule contents were placed within that solution or gel, the procedure written below was followed (Gebreselassie, E.):

"1. Take 3 capsules of LGG and vortex mix the contents separately in a sterile saline solution (10 mL).
2. Dilute the suspension serially in 900µL sterile saline solution by transferring 100µL from the suspension.
3. Plate 100µL from dilutions 10^{-4}, 10^{-5}, and 10^{-6} on de Man Rogosa Sharpe (MRS) agar plates and keep the dilutions at 4°C to plate the next day.
4. Incubate plates in anaerobic jar for 48 hours at 37°C and count colonies.
5. Determine the initial number of bacteria in the Culturelle capsule by multiplying with the dilution factors.
6. After 24 hours, plate the same dilutions kept at 4°C, dilutions 10^{-2}, 10^{-4}, 10^{-6} on MRS agar plates and incubate the plates in anaerobic jar at 37° for 48 hours. Count the number of bacteria.
7. Repeat step 6 at 48 hours and see if there is a significant decline in the number of probiotic bacteria."

Saline Survivability Test

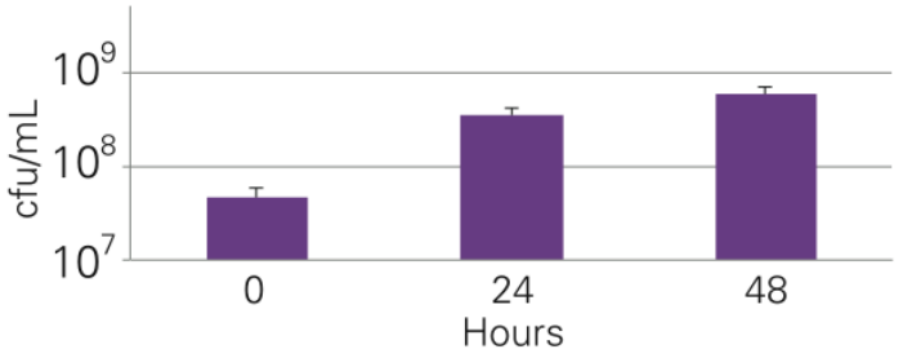

Figure 10. The bacteria re tested in a 0.9% saline solution kept at 4 degrees C at 0, 24, and 48 hours by plating different concentrations on LB plates and grown anaerobically. The plates which contained more than 30 and less than 200 colonies were counted and the initial concentration of colony forming units per milliliter was determined. The bacteria proliferate a significant amount during this time, but not greater than an order of magnitude.

Gel Comparison Test

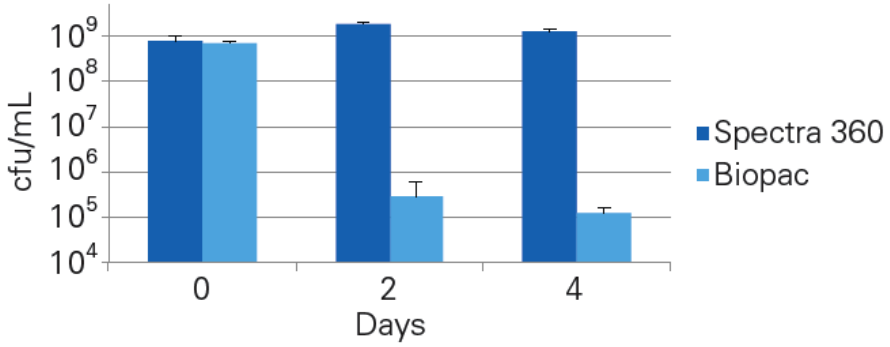

Figure 11.Gel comparison between Spectra 360 and Biopac electrode gel. The experiment is a 4-day trial, and the Spectra 360 electrode gel exhibits the consistent survivability of the bacteria, while there was a significant drop of the survival rate in the Biopac gel.

Gel Concentration Comparison

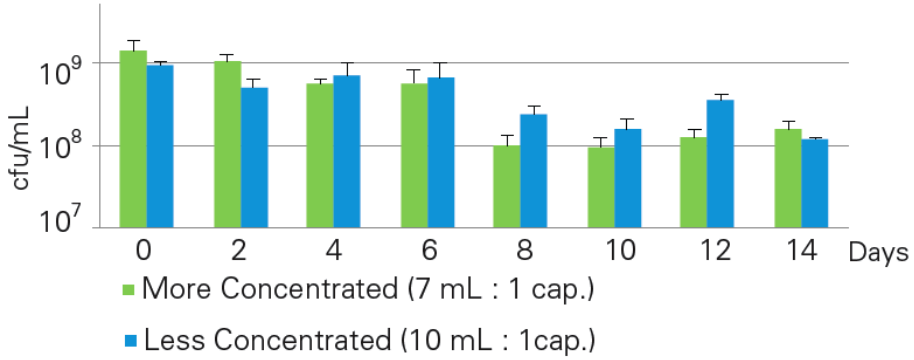

Figure 12. Bacteria concentration comparison.

Appendix C: Survey Results

To help decide which design should be chosen, a survey was conducted that asked people's opinions on which types of delivery method would be the one that they feel most comfortable to deal with. The participants were mostly composed of the group members' friends or families. The survey had the participants ranked their preferences from which one is the most desired method to use to the least desired method. See (Figure 13 and Table 2) survey results in the table and figure below. Interestingly, most people chose the gel method (41%) or the liquid spray (35%) as their number one option. But on the other hand, many other people didn't like the concept of applying gel to their nose (30%). Liquid drop option was generally the people's last two choices, and the dry spray was not chosen by a lot of people to be their first choice too. Since the gel method received the most 1st choice vote, the team decided to develop the gel method first.

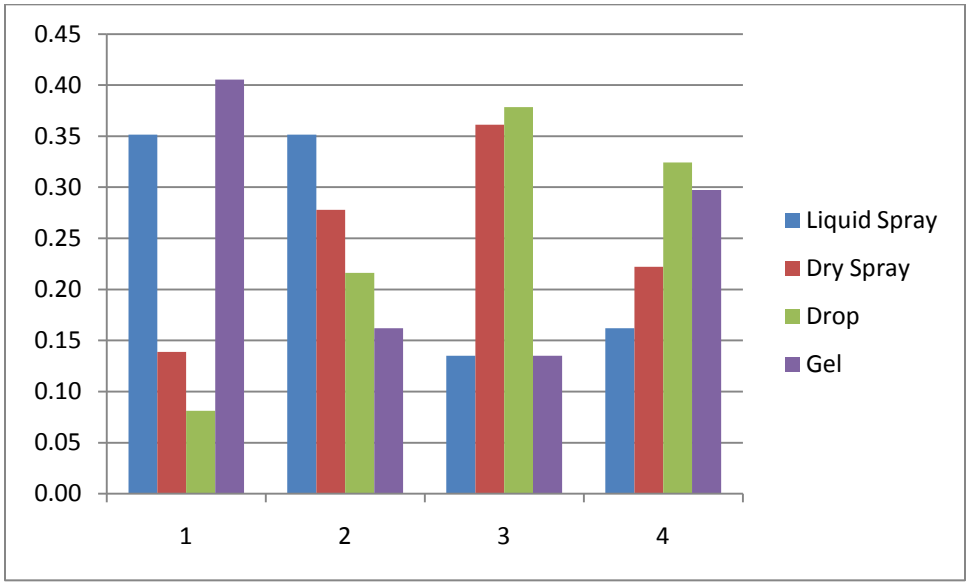

Figure 13. Survey results in chart form. The vertical axis is the fraction of people that chose each category, while the x-axis is the preference of each method with 1 being the most preferred.

Table 2. Table form of results of survey.

	1	2	3	4
Liquid Spray	0.35	0.35	0.14	0.16
Dry Spray	0.14	0.28	0.36	0.22
Drop	0.08	0.22	0.38	0.32
Gel	0.41	0.16	0.14	0.30

Appendix D: Raw Data for Tests Run on Gel

Table 3. Raw data of the Day 0 count for the two types of gel.

0 hrs

Spectra	Conc.	Conc.	cfu/mL
A1A	-6	63	6.30E+08
A1B	-6	102	1.02E+09
A2A	-6	102	1.02E+09
A2B	-6	75	7.50E+08
B1A	-6	56	5.60E+08
B1B	-6	76	7.60E+08
B2A	-6	58	5.80E+08
B2B	-6	59	5.90E+08

Biopac	Conc.	Count	cfu/mL
A1A	-6	71	7.10E+08
A1B	-6	83	8.30E+08
A2A	-6	83	8.30E+08
A2B	-6	71	7.10E+08
B1A	-6	62	6.20E+08
B1B	-6	53	5.30E+08
B2A	-6	64	6.40E+08
B2B	-6	33	3.30E+08

Group	Average	Stdev
Spectra A	8.55E+08	1.97E+08
Spectra B	6.23E+08	9.25E+07
Biopack A	7.70E+08	6.93E+07
Biopack B	5.30E+08	1.42E+08

	Spectra	Biopack
A	8.55E+08	7.70E+08
B	6.23E+08	5.30E+08

Table 4. Raw data of the Day 2 count for the two types of gel

48 hr

Spectra	Conc.	Count	cfu/mL
A1A	-6	209	2.09E+09
A1B	-6	220	2.20E+09
A2A	-6	154	1.54E+09
A2B	-6	148	1.48E+09
B1A	-6	146	1.46E+09
B1B	-6	162	1.62E+09
B2A	-6	176	1.76E+09
B2B	-6	172	1.72E+09

Biopack	Conc	Count	cfu/mL
A1A	-4	0	0.00E+00
A1B	-4	1	1.00E+05
A2A	-4	1	1.00E+05
A2B	-4	0	0.00E+00
B1A	-4	10	1.00E+06
B1B	-4	2	2.00E+05
B2A	-4	5	5.00E+05
B2B	-4	3	3.00E+05

Group	Average	Stdev
Spectra A	1.83E+09	3.70E+08
Spectra B	1.64E+09	1.34E+08
Biopack A	5.00E+04	5.77E+04
Biopack B	5.00E+05	3.56E+05

	Spectra	Biopack
A	1.83E+09	5.00E+04
B	1.64E+09	5.00E+05

Table 5. Raw data of the Day 4 count for the two types of gel

96 hr

Spectra	Conc.	Count	cfu/mL
A1A	-6	110	1.10E+09
A1B	-6	159	1.59E+09
A2A	-6	147	1.47E+09
A2B	-6	127	1.27E+09
B1A	-6	94	9.40E+08
B1B	-6	114	1.14E+09
B2A	-6	124	1.24E+09
B2B	-6	109	1.09E+09

Biopack	Conc.	Count	cfu/mL
A1A	-2	93	9.30E+04
A1B	-2	120	1.20E+05
A2A	-2	105	1.05E+05
A2B	-2	98	9.80E+04
B1A	-2	154	1.54E+05
B1B	-2	164	1.64E+05
B2A	-2	140	1.40E+05
B2B	-2	148	1.48E+05

Group	Average	Stdev
Spectra A	1.36E+09	2.17E+08
Spectra B	1.10E+09	1.25E+08
Biopack A	1.04E+05	1.17E+04
Biopack B	1.52E+05	1.01E+04

	Spectra	**Biopack**
A	**1.36E+09**	**1.04E+05**
B	**1.10E+09**	**1.52E+05**

Table 6. Raw data of the count of every two day over the two-week trial for both more and less concentrated groups.

0 Day							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-6	62	6.20E+08	A1A	-6	73	7.30E+08
A1B	-6	78	7.80E+08	A1B	-6	85	8.50E+08
A2A	-6	116	1.16E+09	A2A	-6	88	8.80E+08
A2B	-6	124	1.24E+09	A2B	-6	92	9.20E+08
B1A	-6	167	1.67E+09	B1A	-6	89	8.90E+08
B1B	-6	144	1.44E+09	B1B	-6	104	1.04E+09
B2A	-6	190	1.90E+09	B2A	-6	112	1.12E+09
B2B	-6	212	2.12E+09	B2B	-6	91	9.10E+08
2 days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-6	109	1.09E+09	A1A	-6	46	4.60E+08
A1B	-6	114	1.14E+09	A1B	-6	52	5.20E+08
A2A	-6	86	8.60E+08	A2A	-6	68	6.80E+08
A2B	-6	67	6.70E+08	A2B	-6	63	6.30E+08
B1A	-6	139	1.39E+09	B1A	-6	50	5.00E+08
B1B	-6	101	1.01E+09	B1B	-6	38	3.80E+08
B2A	-6	113	1.13E+09	B2A	-6	30	3.00E+08
B2B	-6	99	9.90E+08	B2B	-6	44	4.40E+08

4 days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-6	52	5.20E+08	A1A	-6	72	7.20E+08
A1B	-6	57	5.70E+08	A1B	-6	56	5.60E+08
A2A	-6	48	4.80E+08	A2A	-6	119	1.19E+09
A2B	-6	50	5.00E+08	A2B	-6	91	9.10E+08
B1A	-6	62	6.20E+08	B1A	-6	73	7.30E+08
B1B	-6	61	6.10E+08	B1B	-6	67	6.70E+08
B2A	-6	53	5.30E+08	B2A	-6	31	3.10E+08
B2B	-6	61	6.10E+08	B2B	-6	34	3.40E+08
6 Days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-6	78	7.80E+08	A1A	-6	82	8.20E+08
A1B	-6	66	6.60E+08	A1B	-6	87	8.70E+08
A2A	-6	473	4.73E+09	A2A	-5	321	3.21E+08
A2B	-6	480	4.80E+09	A2B	-5	333	3.33E+08
B1A	-6	80	8.00E+08	B1A	-6	103	1.03E+09
B1B	-6	69	6.90E+08	B1B	-6	114	1.14E+09
B2A	-5	238	2.38E+08	B2A	-5	408	4.08E+08
B2B	-5	195	1.95E+08	B2B	-5	274	2.74E+08
8 Days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-5	147	1.47E+08	A1A	-5	240	2.40E+08
A1B	-5	118	1.18E+08	A1B	-5	205	2.05E+08
A2A	-4	860	8.60E+07	A2A	-5	257	2.57E+08
A2B	-4	400	4.00E+07	A2B	-5	360	3.60E+08
B1A	-5	85	8.50E+07	B1A	-5	123	1.23E+08
B1B	-5	113	1.13E+08	B1B	-5	215	2.15E+08
B2A	-5	132	1.32E+08	B2A	-5	157	1.57E+08
B2B	-4	656	6.56E+07	B2B	-5	256	2.56E+08
10 Days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-5	55	5.50E+07	A1A	-5	223	2.23E+08
A1B	-5	65	6.50E+07	A1B	-5	239	2.39E+08
A2A	-5	82	8.20E+07	A2A	-5	111	1.11E+08
A2B	-5	86	8.60E+07	A2B	-5	125	1.25E+08
B1A	-5	112	1.12E+08	B1A	-5	101	1.01E+08
B1B	-5	93	9.30E+07	B1B	-5	136	1.36E+08
B2A	-5	138	1.38E+08	B2A	-5	164	1.64E+08
B2B	-5	129	1.29E+08	B2B	-5	141	1.41E+08
12 Days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-5	93	9.30E+07	A1A	-5	395	3.95E+08
A1B	-5	139	1.39E+08	A1B	-5	398	3.98E+08
A2A	-5	159	1.59E+08	A2A	-5	225	2.25E+08
A2B	-5	154	1.54E+08	A2B	-5	322	3.22E+08

B1A	-5	134	1.34E+08	B1A	-5	337	3.37E+08
B1B	-5	129	1.29E+08	B1B	-5	450	4.50E+08
B2A	-5	93	9.30E+07	B2A	-5	300	3.00E+08
B2B	-5	88	8.80E+07	B2B	-5	300	3.00E+08
14 Days							
More	dilution	count	Cfu/mL	Less	dilution	count	Cfu/mL
A1A	-5	144	1.44E+08	A1A	-5	111	1.11E+08
A1B	-5	143	1.43E+08	A1B	-5	103	1.03E+08
A2A	-5	137	1.37E+08	A2A	-5	132	1.32E+08
A2B	-5	148	1.48E+08	A2B	-5	127	1.27E+08
B1A	-5	72	7.20E+07	B1A	-5	120	1.20E+08
B1B	-5	136	1.36E+08	B1B	-5	105	1.05E+08
B2A	-5	214	2.14E+08	B2A	-5	102	1.02E+08
B2B	-5	225	2.25E+08	B2B	-5	110	1.10E+08

Table 7. Data of the average amount of the A and B groups over the two-week trial of the less concentrated group.

More								
Average	0 hours	2 days	4 days	6 days	8 days	10 days	12 days	14 days
A	9.50E+08	9.40E+08	5.18E+08	7.20E+08	9.78E+07	7.20E+07	1.36E+08	1.43E+08
B	1.78E+09	1.13E+09	5.93E+08	4.81E+08	9.89E+07	1.18E+08	1.11E+08	1.62E+08
Stdev								
A	297769485.8	217408984.8	38622100.75	84852813.74	45857569.35	14537308.32	30059662.89	4546060.566
B	293072801	184028983.2	41932485.42	308915711.3	29419494.67	19849433.24	23846732.83	71760597.36

Table 8. Data of the average amount of the A and B groups over the two-week trial of the less concentrated group.

Less								
Average	0 hours	2 days	4 days	6 days	8 days	10 days	12 days	14 days
A	8.45E+08	5.73E+08	8.45E+08	5.86E+08	2.66E+08	1.75E+08	3.35E+08	1.18E+08
B	9.90E+08	4.05E+08	5.13E+08	7.13E+08	1.88E+08	1.36E+08	3.47E+08	1.09E+08
Stdev								
A	81853527.72	100457287.8	270862818.9	299803268.8	66615813.94	65815398.4	81318304.62	13549292.72
B	109239797.4	85440037.45	218231528.4	435340479.8	59269863.06	26032031.55	71008802.27	7889866.919

Table 9. Data of the average amount of bacteria every two days for two weeks of the more concentrated group.

More Concentrated								
Day	0	2	4	6	8	10	12	14
Average	1.37E+09	1.04E+09	5.55E+08	5.61E+08	9.83E+07	9.50E+07	1.24E+08	1.52E+08
Stdev	522328235.6	212333968.8	54772255.75	271958636.6	35672948.93	29393876.91	28515346.75	48127620.52

Table 10. Data of the average amount of bacteria every two days for two weeks of the less concentrated group.

Less Concentrated								
Day	0	2	4	6	8	10	12	14
Average	9.18E+08	4.89E+08	6.79E+08	6.50E+08	2.27E+08	1.55E+08	3.41E+08	1.14E+08
Stdev	118291407.7	124377019	288861682.2	352636599.7	71655799.29	50807760.94	70953581.41	11335784.05