I. Proposal Cover Page

Proposal Title: *The Effect of Microgravity on Bacterial Growth and its Resistance to Antibiotics.*

Submitting Grade: 10th and 11th

Submitting School: Valley Center High School

Submitting School District: USD 262 Valley Center

Submitting Teacher Facilitator:

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Proposal Summary:

We know that bacteria grown in space are more virulent, but does it make them resistant to antibiotics? The essential purpose of our experiment is to monitor the effect of microgravity on the growth of bacteria and how it resists an antibiotic originally used to eliminate the bacteria grown in the effect of gravity. Using FME Type 2 we will be able to control when each "ingredient" is added to the experiment. This should allow for precise results. Following its return the FME will be sent to Valley Center High School for analysis. Our bacterium, *staphylococcus epidermidis* is a very common bacterium that is quite harmless. The antibiotics of our choice are penicillin, erythromycin, and vancomycin which have been used to treat infections of this bacterium.

II. Student team members page

Co-Principal Investigators

Name: Chandler, Garrett
Grade level: Grade 11

Name: Crow, Wesley
Grade level: Grade 10

Name: Klinkhammer, Cole
Grade level: Grade 10

Co-Investigators

Name: Burks, Logan
Grad level: Grade 10

Name: Sheahan, Samuel
Grade level: Grade 10

Collaborators

None

III. Experimental Materials and Handling Requirements

1. Fluid Mixing Enclosure Type

FME Type 2

2. List of Proposed Experiment Samples:

FME Main Volume: pH of Contents 7.1-7.5

5.0 mL of Nutrient Broth

Long Ampoule: pH of Contents 7.1-7.5

0.5 mL of MicroKwik® culture of *staphylococcus epidermidis*

IMPORTANT: Are any of the proposed samples human in origin?

✓ No

3. Special Handling Requirements During Transportation

There are no special handling requirements.

4. Timeline for Experiment Aboard the International Space Station

1.) D-10 Days: Break long ampoule, shake FME for 20 seconds

IV. The Question to be Addressed by the Experiment

Description of the basic question:

Antibiotics are used to kill many bacteria. Our question is: Will bacteria be less susceptible to antibiotics after it is grown under the influences of microgravity? Results from previous experiments have shown differences in the growth and reproduction while influenced by microgravity. Previous experiments were not found on the susceptibility of those bacteria to antibiotics.

Current scientific understanding:

A study done by Mennigmann and Lange (Kacena, Merrell and Manfredi) showed that similar bacteria, *Bacillus subtilis* grown in space had an increased growth rate and total biomass yield. Another study done by Klaus (Kacena, Merrell and Manfredi) found that *Escherichia coli* grown under the same conditions had a cell population of 88% more than the control. These two examples prove that bacteria growth in microgravity is different than here on earth. Unfortunately there are very few experiments that have been conducted on the exact bacteria we are using, *staphylococcus epidermidis*. This is one of the main reasons we have created this experiment. We know that the antibiotic is effective by the circle that it forms, preventing the bacteria from growth within the circle on the petri dish. With this understanding we will be able to recognize the difference between the bacterial reaction grown on Earth and the sample grown on the ISS.

Description of the insight that will be gained from the experiment:

This experiment can be beneficial in multiple ways. If the results vary from our control it has the potential to show that the antibiotics we currently have, may not be enough. Groundbreaking information like this has the possibility to give us knowledge about microbiology that has been previously unknown. Through each step of our experiment we will be learning how to grow and analyze bacteria. We have already gained a better understanding of the way bacteria can grow. In conducting the experiment our knowledge will expand in the field of microbiology and life at the molecular level.

V. Experiment Design

The experiment rationale:

Our experiment tests the effect of microgravity on the growth of *staphylococcus epidermidis* and its resistance to the antibiotics: vancomycin, penicillin, and erythromycin. By using dormant bacteria we can figure out exactly how the bacteria grows differently when under those specific effects of microgravity. We have considered using growth inhibitors to allow for even more precise results but the chance of killing the bacteria and ruining the experiment is not worth it. Also *S. epidermidis* is a stable enough bacterium to where the same cell characteristics should be the same through many generations.

The experiment materials:

There are four samples in our experiment. *Staphylococcus epidermidis* was chosen as our bacteria because it can be easily obtained by ordering it from the Carolina Biological Supply Company, along with all the other materials needed for our experiment. When we obtain the bacteria it will already be freeze dried, which will allow it to be dormant until activation on board the ISS. *Staphylococcus epidermidis* is a normal part of the human skin flora, also found in mucous membranes of humans and animals. This shows that this is a safe and prevalent bacterium. We will use nutrient broth for the bacteria to feed on and grow. The last samples are the three antibiotics. Vancomycin we used because it is used most often to kill this bacteria. Penicillin was chosen because *S. epidermidis* has grown a resistance to it and we would like to observe if that resistance is consistent. Lastly we chose erythromycin because it is often used to treat urinary tract infections, which can be caused by the bacteria contaminating urinary catheters.

The experimental procedure:

In pre-flight stages, before ingredients are added to the FME, we must sterilize the FME tubes using a UV cabinet. While on board the ISS the experiment will sit idle until 10 days prior to departure, when the astronauts will break the long ampoule mixing it with the nutrient broth for it to grow. Allowing 10 days for the freeze dried bacteria to grow and reproduce in the broth. The typical incubation temperature for *staph epidermiduis* is 37°C and the incubation period is 24-48 hours. Due to the fact that our sample is freeze dried and being maintained at room temperature, extra time has been provided for the sample to grow and develop. Following the return of our project it will be transported back to the high school where we will continue with the next step of our experiment. The bacteria will be swabbed on 3 separate culture plates containing nutrient agar. Four disks will be applied to each plate, one is the control (containing no antibiotic), and the remaining three contain one of each antibiotic previously mentioned. The plates will be incubated for 48 hours at 37°C prior to being analyzed.

The ground elements:

Our ground experiment will be conducted simultaneously to the experiment that is sent to the ISS, and the only difference in the two will be the absence of gravity. There are a few constants in both experiments. Each will be using the Type 2 FME along with equivalent amounts and types of nutrient broth. In our analysis we will be using the same types of antibiotics. Keeping this consistency will reveal any difference between the two samples.

The experimental analysis:

Our analysis will consist of three parts. Part 1 includes moving the bacteria from the FME into three separate culture plate. Next we will apply our antibiotic disks to multiple parts of the culture plates. Following incubation we will analyze the effectiveness of the antibiotics on the bacteria. Using a control from our ground experiments we will be able to compare if the bacteria has still been killed as easily, or if it has grown in a way to make it more or less susceptible to antibiotics. We will determine this by measuring the radius of the kill zone the disk creates. The larger the kill zone the more susceptible the bacteria is.

Works Cited:

Klaus, M. A. Kacena, G. A. Merrell, B. Manfredi, E. E. Smith, D. M., and P. Todd. "Bacterial Growth in Space Flight: Logistic Growth Curve Parameters for Escherichia Coli and Bacillus Subtilis." *SpringerLink*. Springer Science Business Media, 3 Sept. 1998. Web. 16 Nov. 2012.<http://www.springerlink.com/content/u3v2u547lg2d9g7d/>.

Evans, Julie, DVM. Personal interview. 08 Oct. 2012.

Schleifer, Kloos W. E., K. H. "Staphylococcus Epidermidis." *Wikipedia*. Wikipedia, 17 Nov. 2012. Web. 5 Dec. 2012. <http://en.wikipedia.org/wiki/Staphylococcus_epidermidis>.

VI. REQUIRED Letter of Certification of a Student-Directed Effort

November 9, 2012

I certify that the student team designed the experiment described herein and authored this proposal, and not a teacher, parent, or other adult. I recognize that the purpose of this letter is to ensure that there was no adult serving to lead experiment definition and design, or write the proposal, and thereby provide content and/or professional expertise beyond that expected of a student-designed and student-proposed experiment.

I also understand that NCESSE recognizes that facilitation of thinking across the student team by the team's Teacher Facilitator, and other teachers, parents, and local area researchers, is not only to be encouraged but is absolutely vital if students are to receive the necessary guidance on the process of scientific inquiry, experimental design, how to do background research in relevant science disciplines, and on writing the proposal.

I also certify that the samples list and the special handling requests listed in this proposal are accurate and conform to the requirements for SSEP Mission 3 to ISS. I confirm that the team, after reviewing their procedure and budget for obtaining the samples for the experiment, is certain that they will be able to obtain the necessary samples for their experiment in time to meet the deadline for shipping the flight-ready FME to NanoRacks. If using human samples, the team is aware that these samples must be tested for prohibited viruses before the experiment can be selected for flight. Finally, the Teacher Facilitator certifies that the student team will have access to the proper facilities to prepare the Fluid Mixing Enclosure for flight and to analyze the samples after the flight.

Mr. Jeff Tracy
Teacher Facilitator