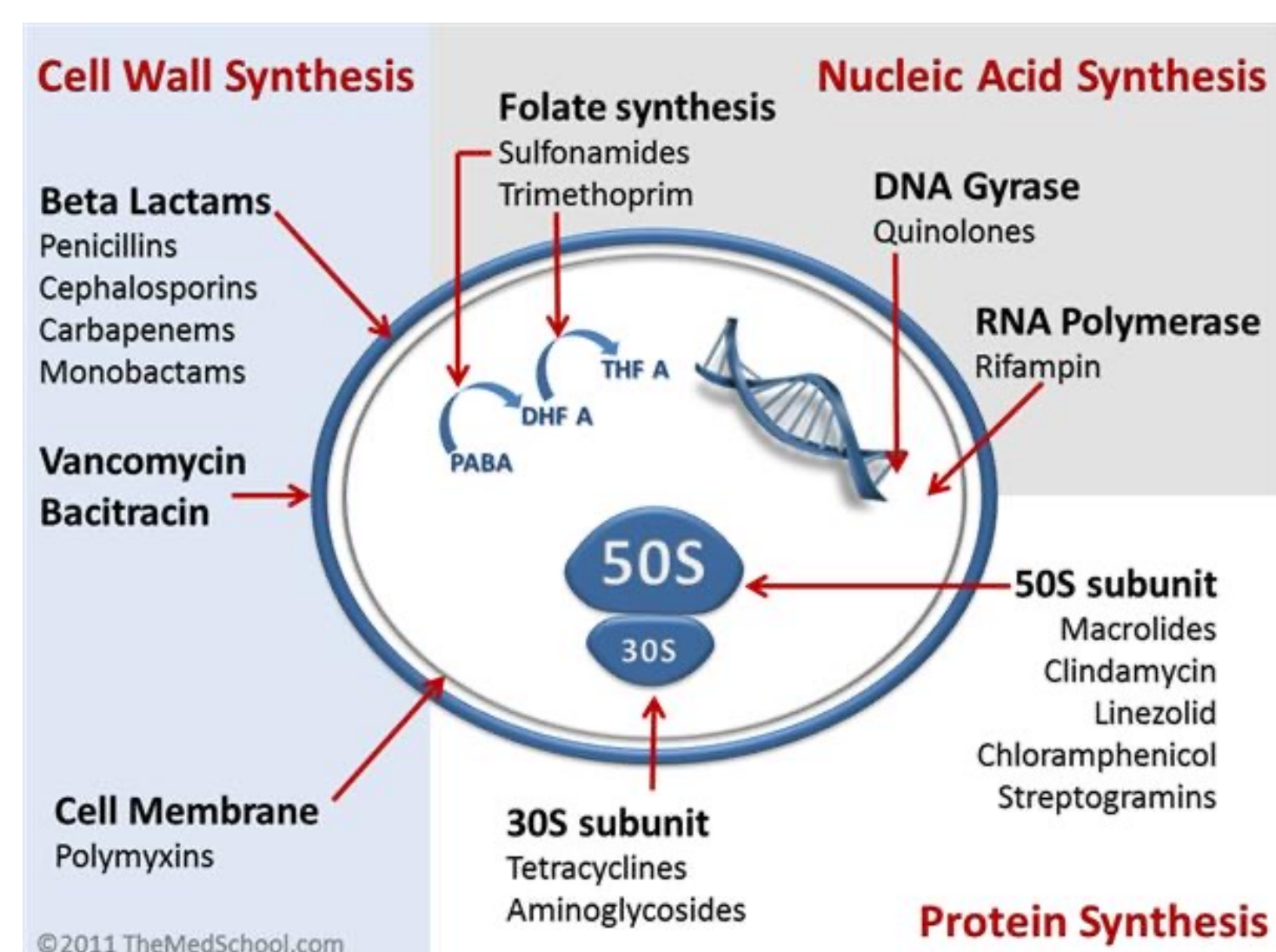


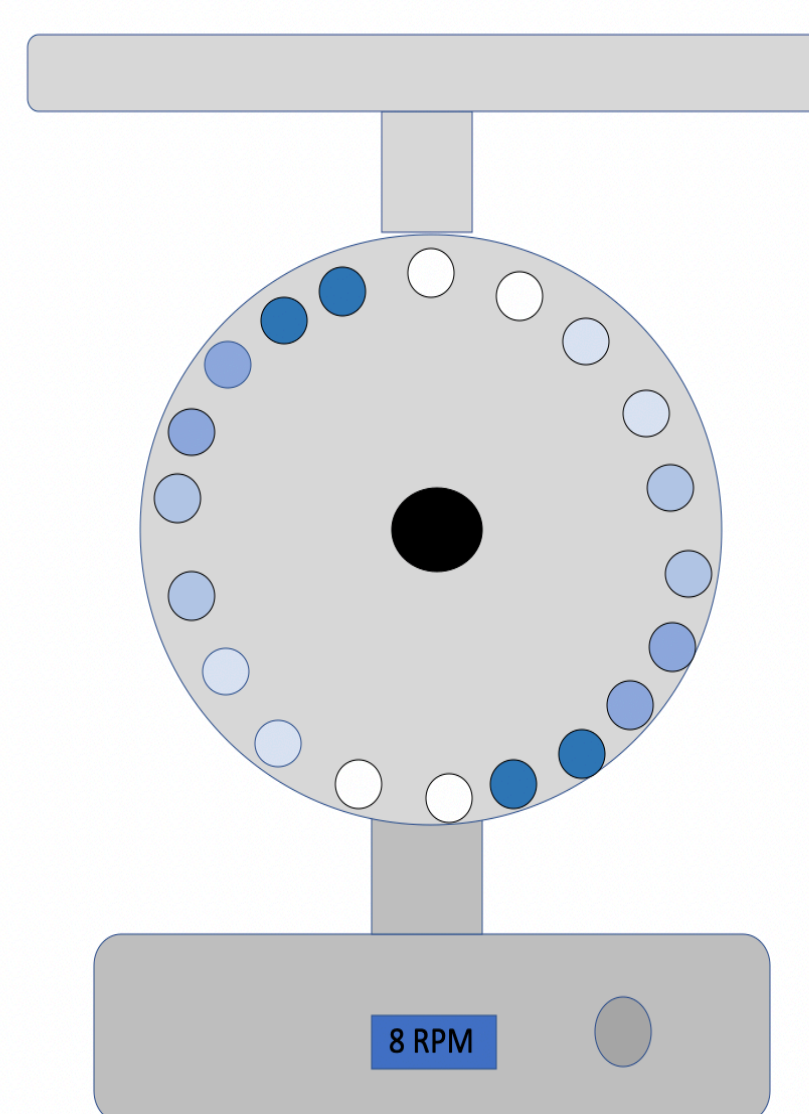
Introduction

- One of the ways researchers are investigating ways to maintain optimal health in astronauts is through antibiotic resistance.
- An astronaut's immune system functions differently in microgravity than it does on Earth due to metabolic changes.
- This study aims to determine the MIC (minimum inhibitory concentration) of a certain antibiotic and measure its effect on *E. coli* under simulated microgravity.



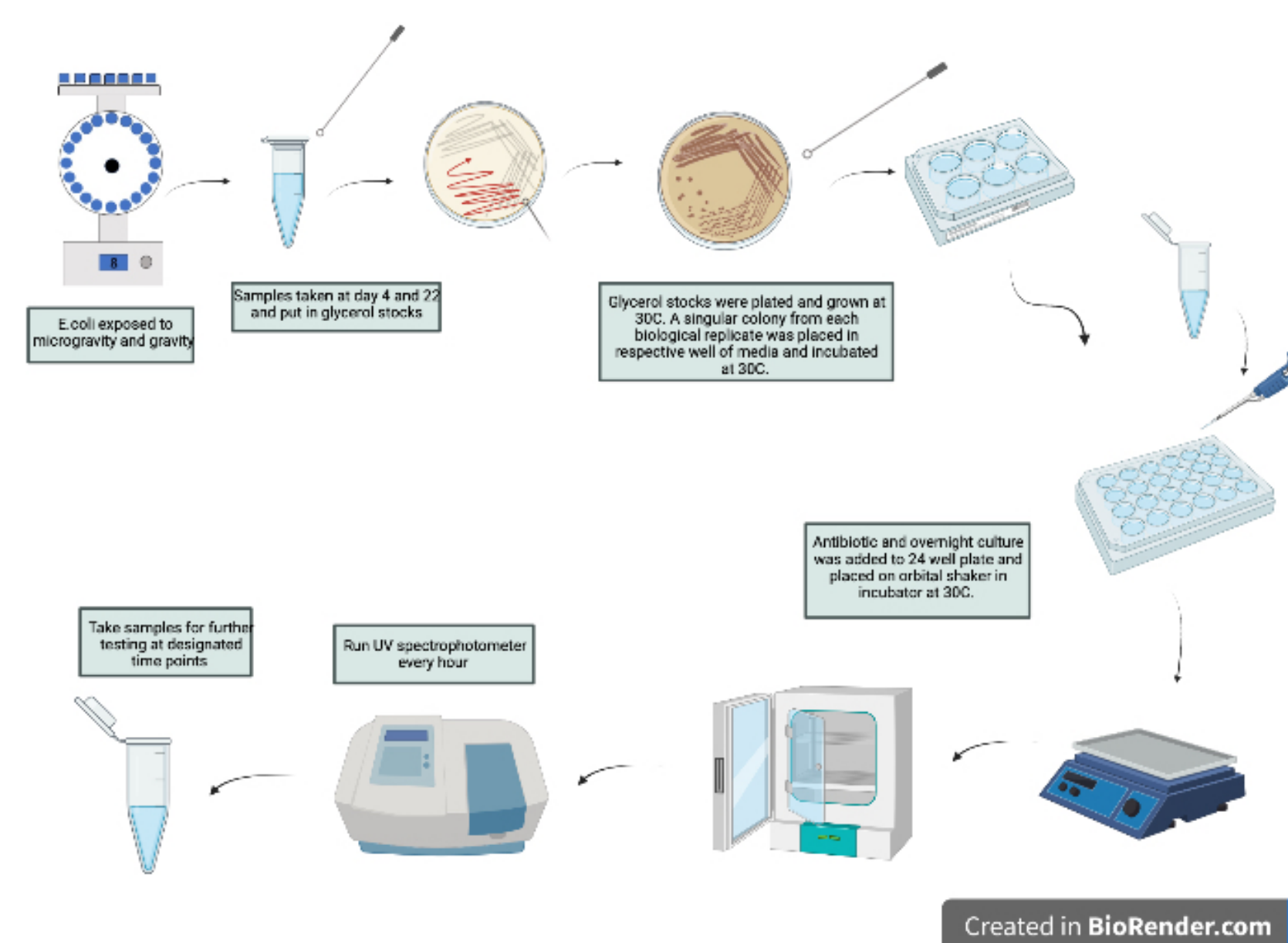
Experimental Design

- E. coli* was cultured from glycerol stocks for short term exposure experiments.
- E. coli* that was exposed to microgravity previously were used for Day 4 and 22 experiments.
- E. coli* was cultured in Luria Broth (LB) media.
- For 4 and 22 day experiments, cultures were grown in 24-well plates.
- 24-hour cultures were grown in epitubes on the 2D clinostat EAGLESTAT.
- Absorbance was read with a spectrophotometer every hour for 12-15 hours and again at 24 hours.

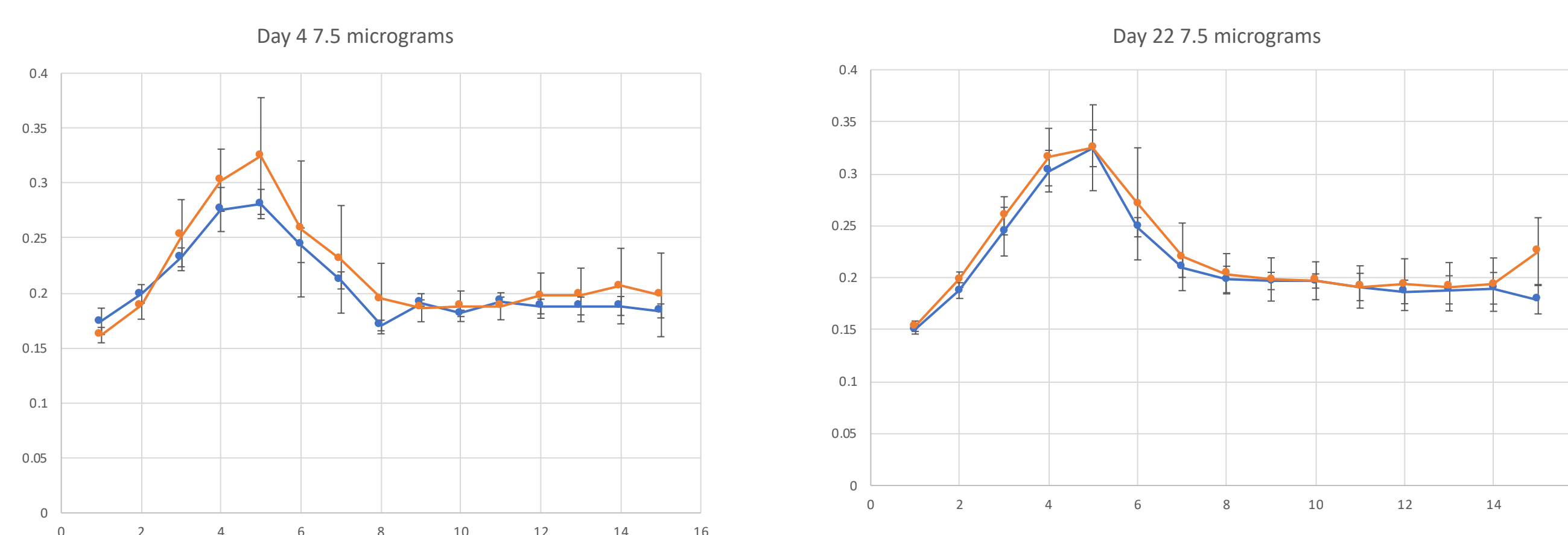


4- and 22-Day Exposure Response

- E. coli* was grown in 24 well plate with concentrations of Nalidixic Acid ranging from 1.875µg/ml to 30µg/ml.
- Plate was placed on a shaker and incubated at 30°C.
- Absorbance was read on a spectrophotometer to observe the changes in biomass.

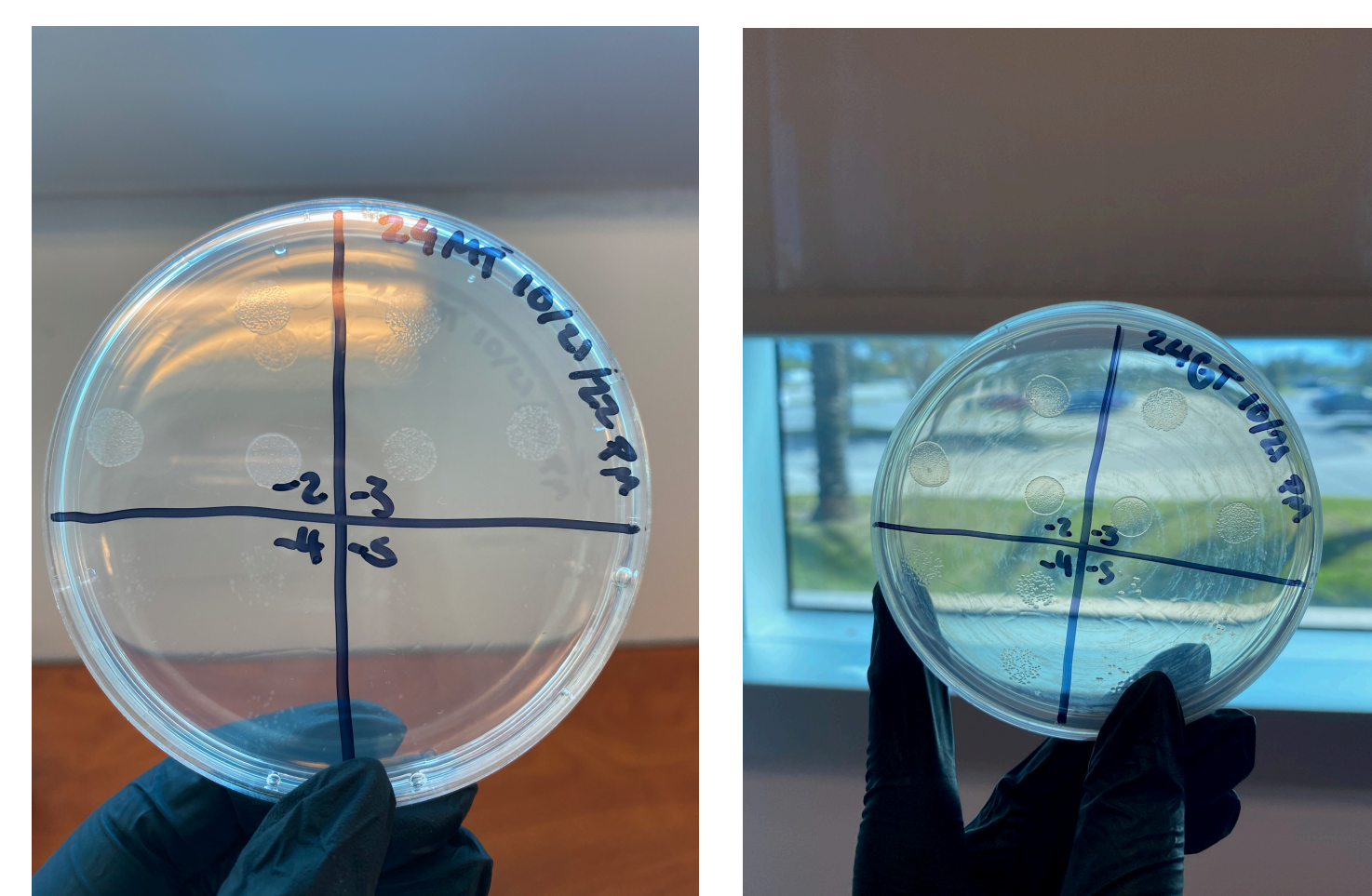


- Both days 4 and 22 showed a significant difference between the microgravity and gravity (control) samples.
- Samples showed an initial growth and then a decrease in biomass, and then a regrowth in microgravity.



Nalidixic acid long-term exposure experiments

- Dilutions were done to measure numbers of colonies that were able to grow after being exposed to the antibiotic and microgravity.
- Colony-forming units were measured by counting the number of colonies that were grown on the plates.

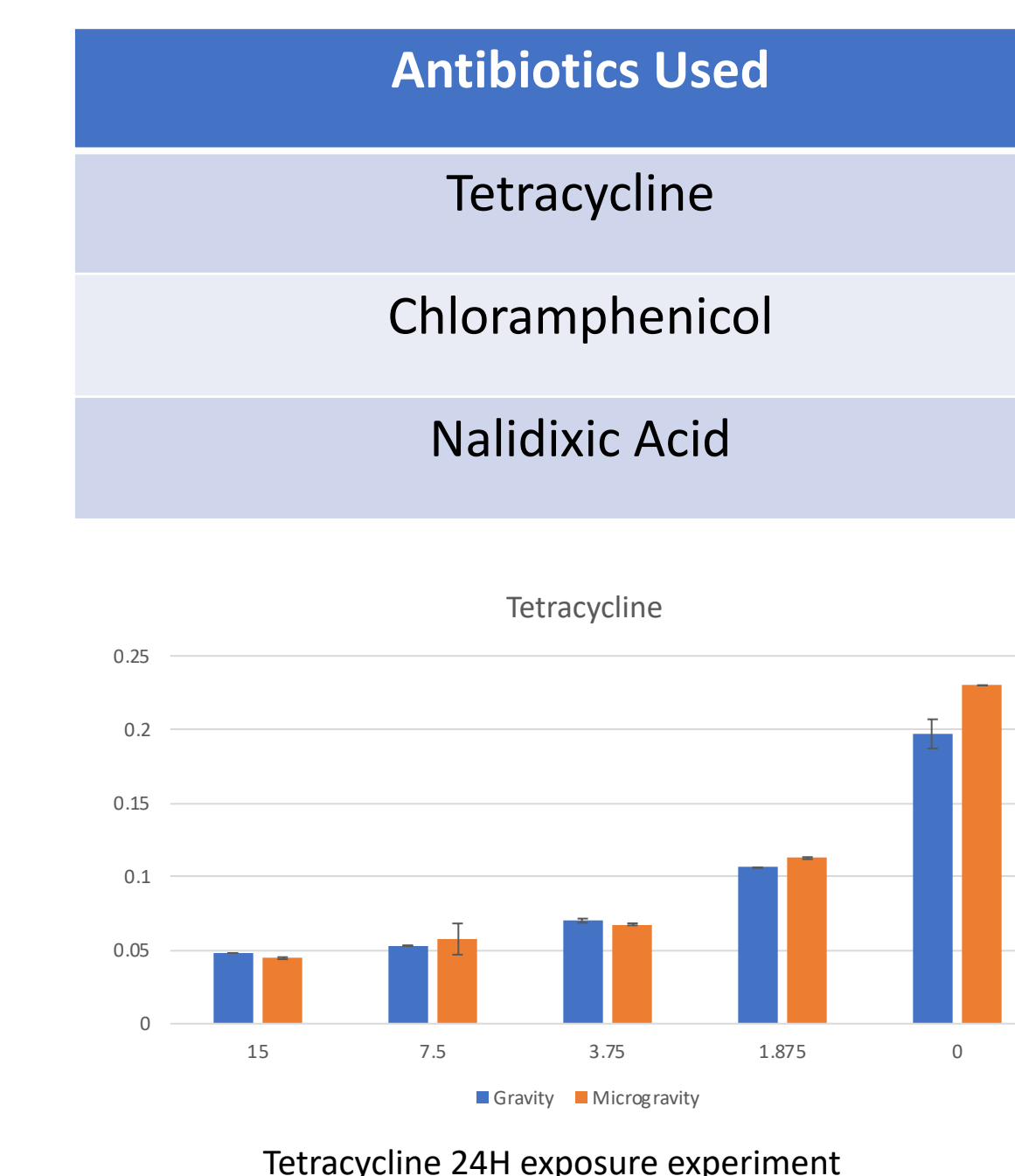


CFU plate count, 7.5 microgram/ml NA 24H MG

CFU plate count, 7.5 microgram/ml NA 24H G

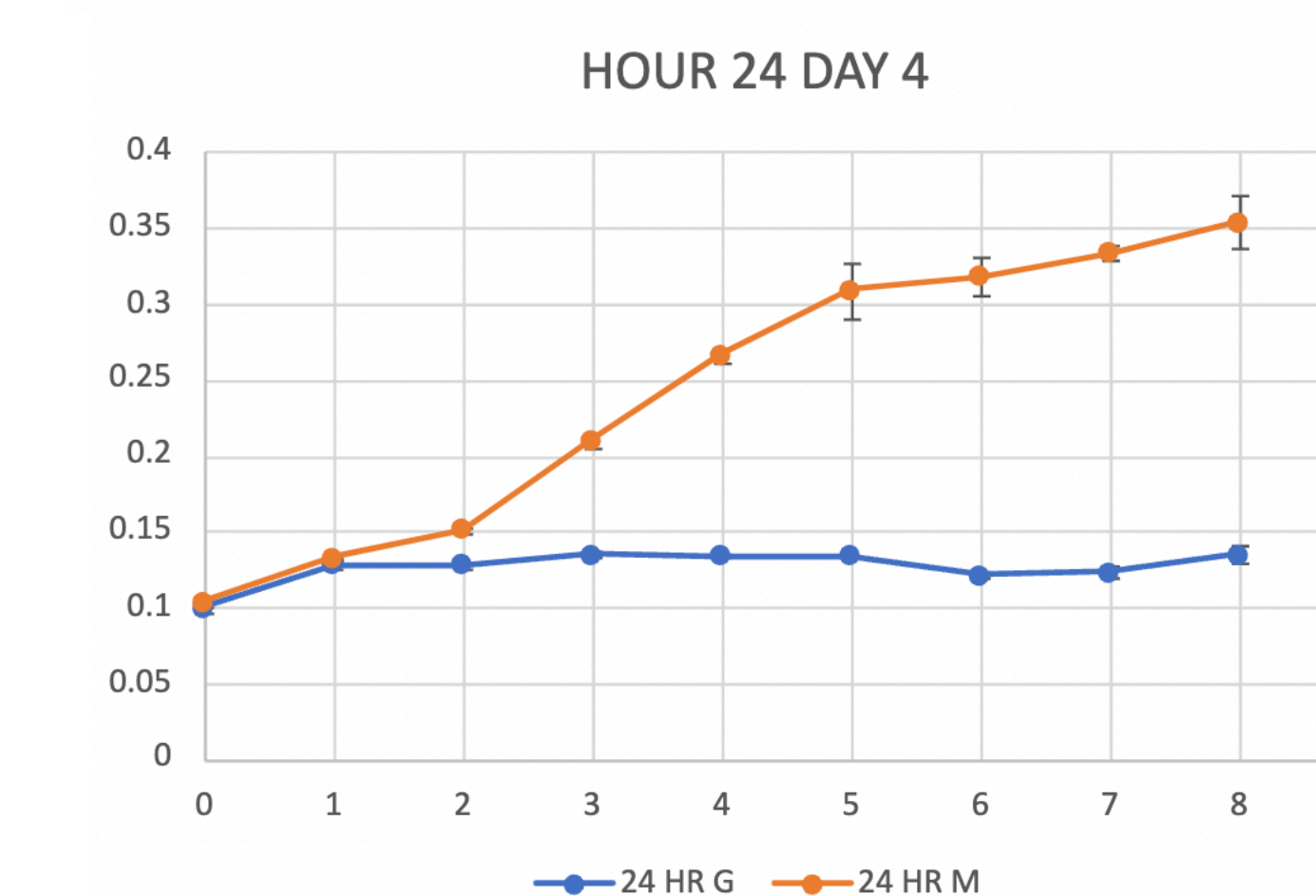
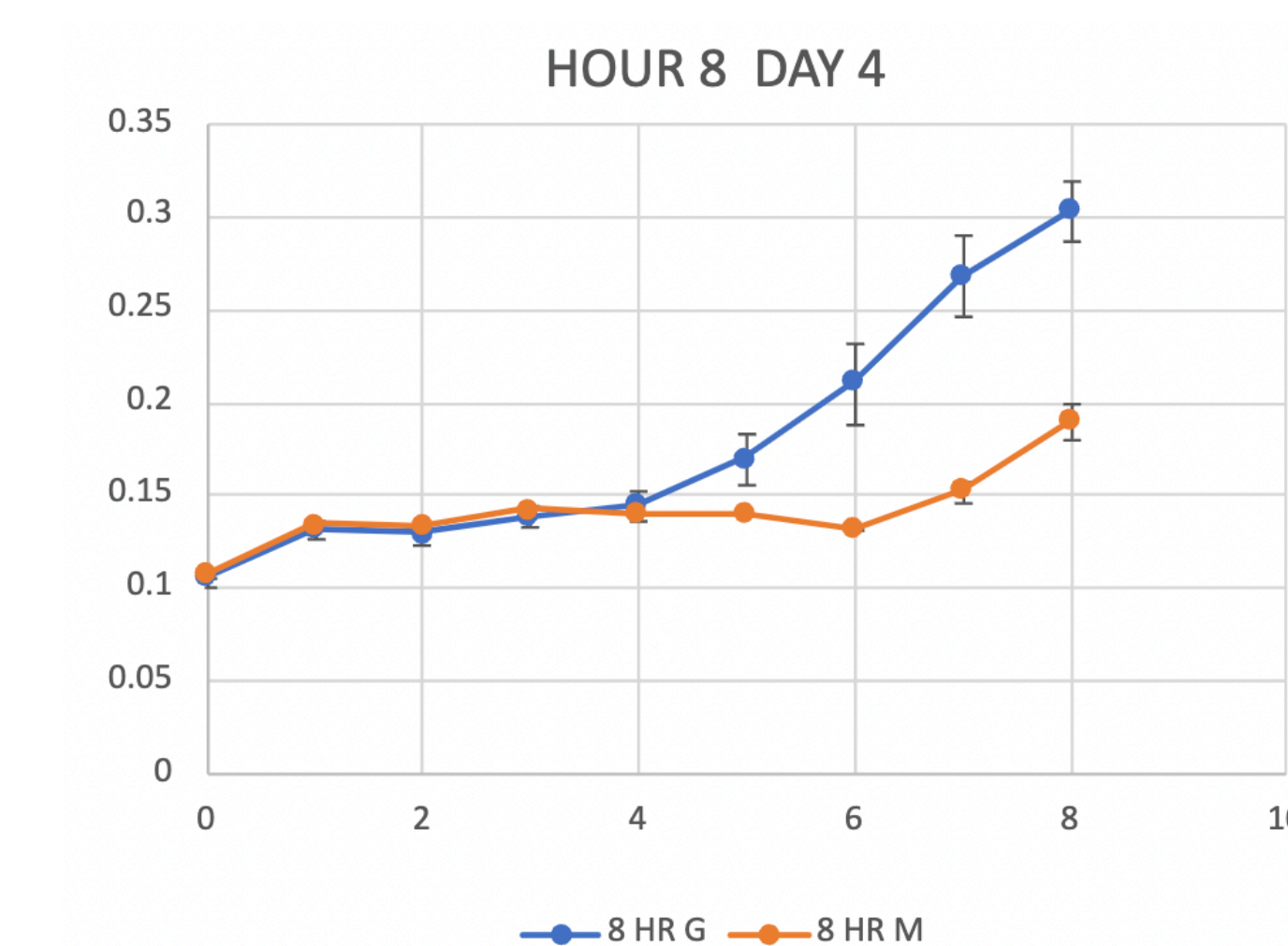
24 Hour Exposure

- E. coli* was grown in 2ml epitubes on the EAGLESTAT and incubated for 24 hours.
- Epitubes had range of antibiotic concentrations. The purpose of these experiments was to determine the MIC, so a large range was used.



Post-Antibiotic Exposure

- Samples from 8 and 24-hour antibiotic exposure were collected to test their viability at those time points.
- These samples were incubated for 8 hours and their absorbance was measured every hour.
- Results indicate a major difference between the two time points, with gravity being more viable at 8 hours and microgravity being more viable at 24 hours.



Future Research

- Expand screening of antibiotics.
- Observe antibiotic mechanisms of action.
- Expand in the usage of bacterial models including *S. epidermis*.
- Study changes in gene expression.

References:
 Tirumalai, M. R., Karouia, F., Tran, Q., Stepanov, V. G., Bruce, R. J., Ott, C. M., ... & Fox, G. E. (2019). Evaluation of acquired antibiotic resistance in *Escherichia coli* exposed to long-term low-shear modeled microgravity and background antibiotic exposure. *Mbio*, 10(1), e02637-18.
 Cira, N. J., Ho, J. Y., Dueck, M. E., & Weibel, D. B. (2012). A self-loading microfluidic device for determining the minimum inhibitory concentration of antibiotics. *Lab on a Chip*, 12(6), 1052-1059.