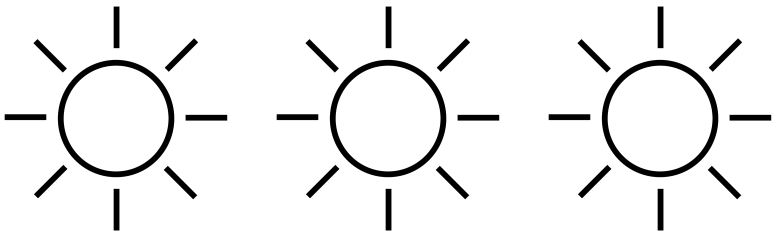

THE EFFECTS OF UV RADIATION ON ANTIBIOTIC RESISTANCE AND THE DNA OF E. COLI

Colorado State University

SPACE RAMS

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NASA Colorado Space Grant Consortium – DemoSat B
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Table of Contents

Biological

1.0 Introduction

E. coli is a very well known gram negative bacterium that is used in many research labs for general testing. The E. coli strain used most commonly and what was used for our testing is called E. coli Top 10 strain, this strain is known to not have any severe effects on the environment and to people. In this experiment, the effects of radiation, specifically UV-C, on E. coli were being tested to see if this would cause a spontaneous rifampicin resistant mutation in E. coli. The antibiotic rifampicin was used because E. coli is known to have a spontaneous mutation in the RNA polymerase gene (rpoB) from this mutation E. coli is known to grow on this antibiotic (Reynolds, 2000). From past literature using Mycobacterium tuberculosis, it was seen that there was a spontaneous rifampicin frequency of about ~1.1 × 10 ^-8, and this gave rise to the fact that this E. coli Top 10 Strain could be used in a similar context to find spontaneous rifampicin mutations.

2.0 Methods/Materials

Preliminary Biology Design

Overnight cultures of E. coli Top 10 strain were grown in culture tubes containing 3mL of LB, Miller broth. An OD600 measurement of the overnight cultures were taken in order to ensure there was enough E. coli that had been grown up for further testing, to ensure that enough E. coli was present the OD 600 needed to be in the range of 0.1-1. After confirming E. coli growth,

serial dilutions were conducted on LB agar plates. The serial dilutions consisted of using a 96 well plate and pipetting 180microlitters of water into 8 rows and 8 columns of the well plate. Then, 20microliters of the overnight culture was added to the first 8 wells located in the first column, after, using a multichannel pipette each sample was mixed 7 times by gently pipetting up and down. 20microlitters of the sample was then transferred from the first column to the second, and the previous steps were repeated until all 8 columns were diluted, thus resulting in 8 dilutions of: 10^-1, 10^-2,10^-3,10^-4,10^-5,10^-6,10^-7,10^-8. Following, the dilutions were then plated onto the LB agar plates, one plate contained the dilutions of 10^-1,10^-3,10^-5,10^-7, while the other plate contained the dilutions 10^-2,10^-4,10^-6,10^-8. Once the plates were made they were put into a 37degC incubator for overnight growth. The colony growth for each dilution was then counted and converted into a CFU/mL, which gave information about how much E. coli should be plated onto the rifampicin plates.

In order to ensure spontaneous rifampicin mutations of the E. coli on the rifampicin plates, dilutions of 10^-1,10^-2,and 10^-3 were used. Following the same procedure as used for the LB agar plates, but using 80microliters of the overnight culture of E. coli, the three dilutions were plated on rifampicin plates. Using the dilution that yielded the most mutations on the rifampicin plates, testing with the UV-C light of 254nm was ensued. Using blotting paper whole punches, the paper was dipped into the overnight culture of E. coli and exposed to 45 minutes of UV-C radiation. The whole punches were then frozen in a -80degC to ensure the E. coli could withstand extreme cold, and then the whole punches were placed in LB media overnight cultures. Once enough E. coli had been grown in the overnight cultures, the E. coli was diluted 10^-1

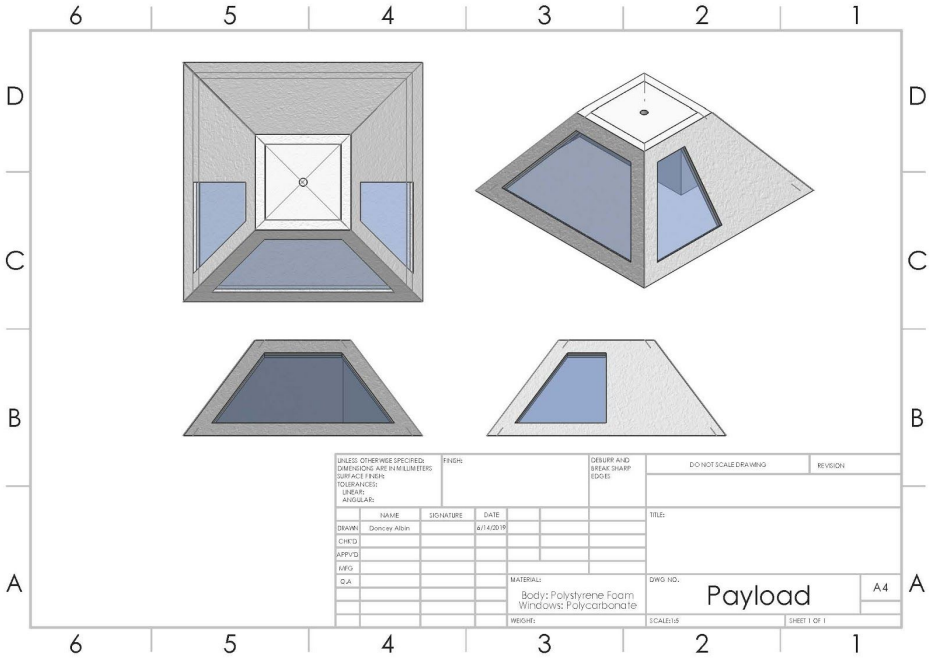
using the same method as previously described and aliquoted onto rifampicin plates. The plates were then put into the 37degC incubator for overnight growth.

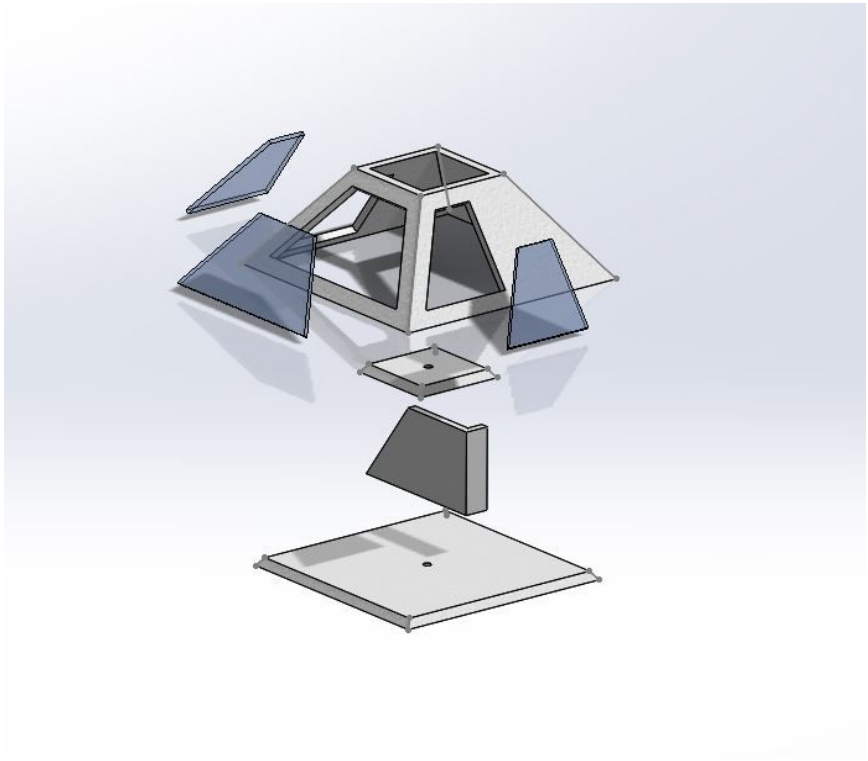
Actual Biology Design

Overnight cultures of E. coli were grown in LB media Thursday night before the Launch Day, 50 whole punches of blotting paper were created and dipped into the cultures and placed on the 8 quartz glass pieces. Quartz glass was used to ensure that the UV-C radiation would make contact with the E.coli on the paper. After placing the hole punches of E. coli onto the glass with no adhesives to ensure sterilization, the lids of the petri dishes were used to compress the papers onto the glass and create a seal. The readymade slides were then kept in a 4degC refrigerator to ensure the E. coli would not have too much growth on the plates until they were ready to be placed onto the Payload. Through the course of handling the slides, some of the E. coli hole punches had fallen out, leaving 40 E. coli hole punches to be sent up on the payload. Once the payload reached a max altitude of 107203ft at a UV-C wavelength range of 200-280nm and a temperature of 49degC, the payload then made its descent and returned to Earth. Unfortunately, from inadequate adhesion of the slides of E. coli to the payload, small openings on the slides created a way for ants to enter the slides, and contaminate the E. coli hole punches. After ridding the ants from the slides, the E. coli was kept in cool conditions until they could be grown in overnight cultures of LB media. After overnight growth 13 out of the 31 cultures were plated onto LB agar plates in order to check for any cross contamination, and then using the same 13 samples the E. coli was plated onto rifampicin antibiotic plates. All plates were placed in the 37degC incubator for overnight growth, and collected in the morning for data analysis.

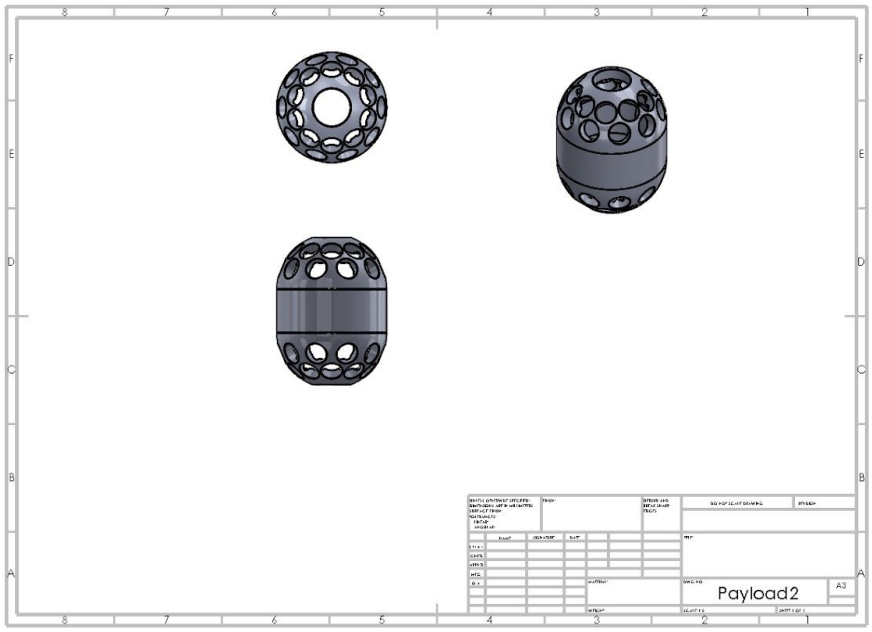
3.0 Design

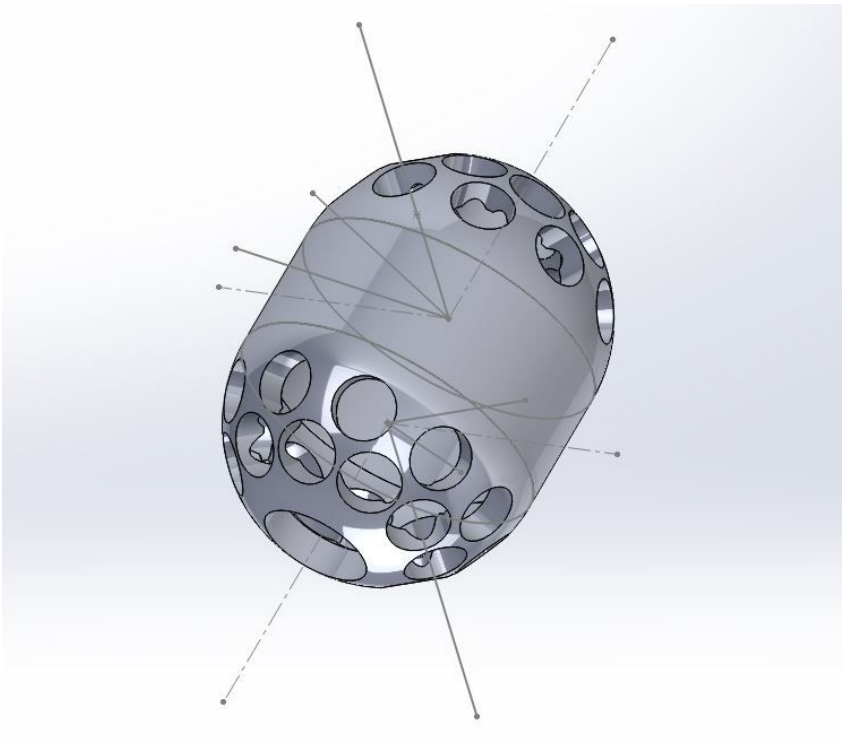
Payload Design 1



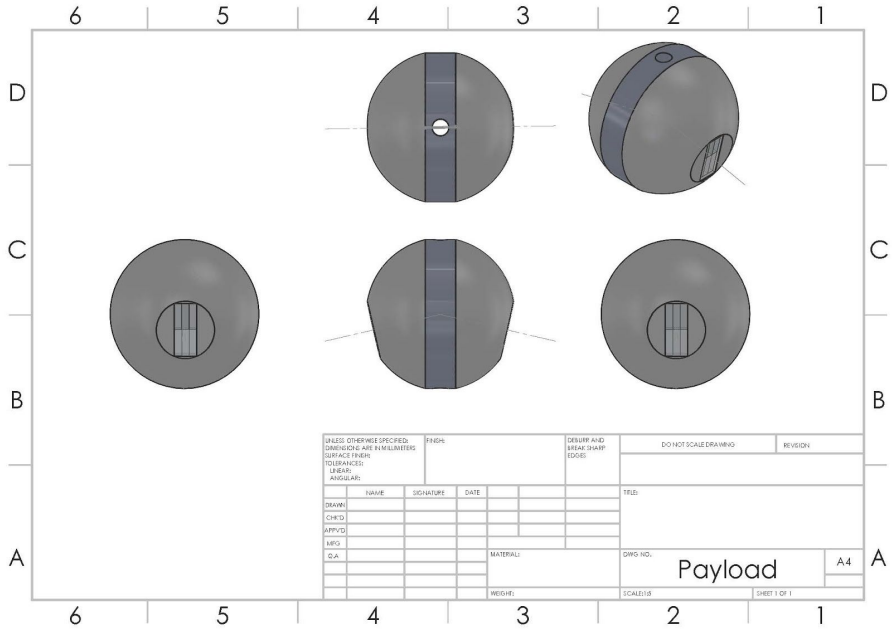


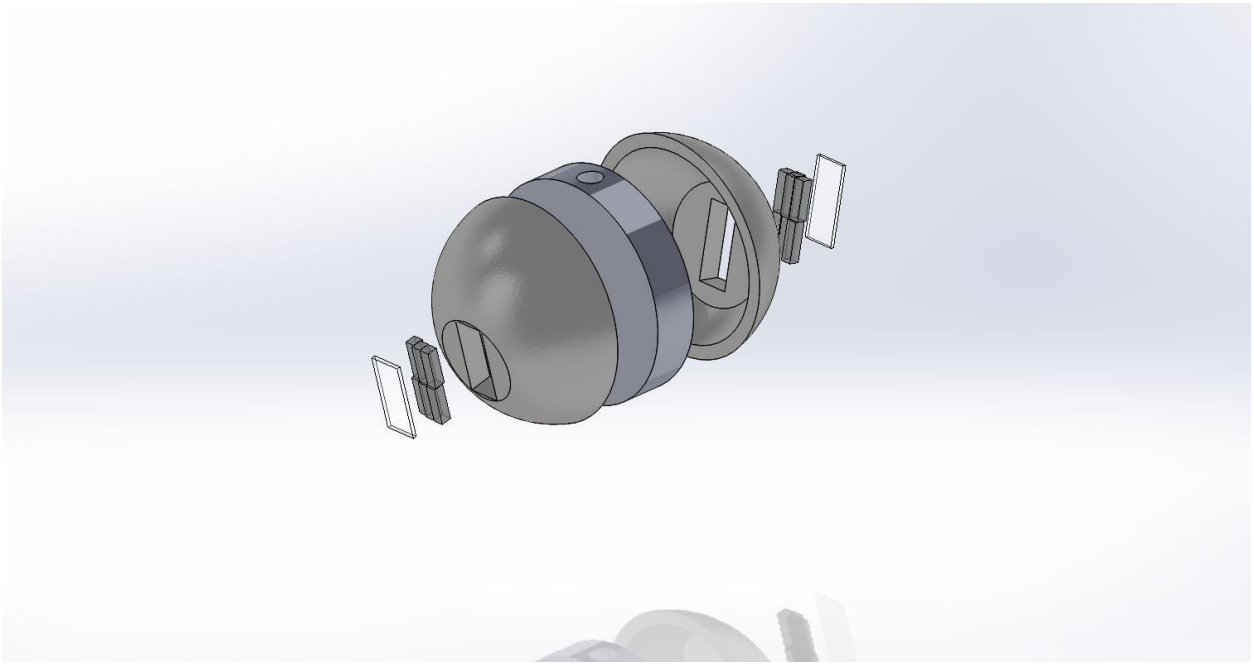
Payload Design 2



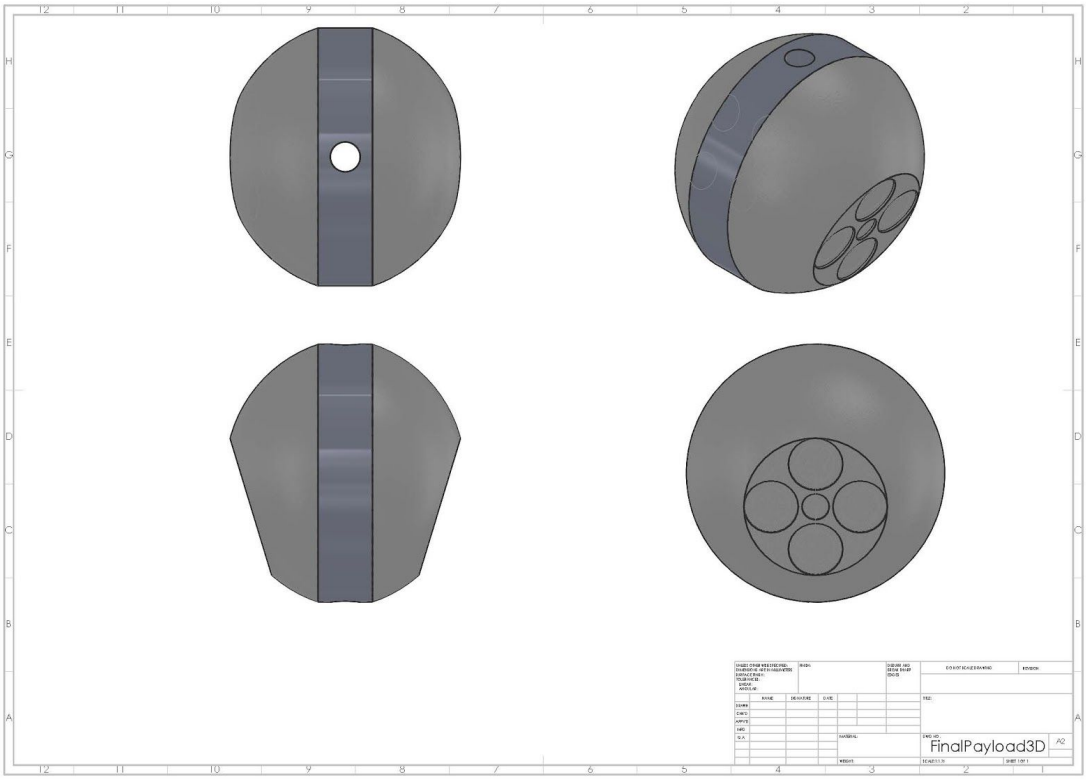


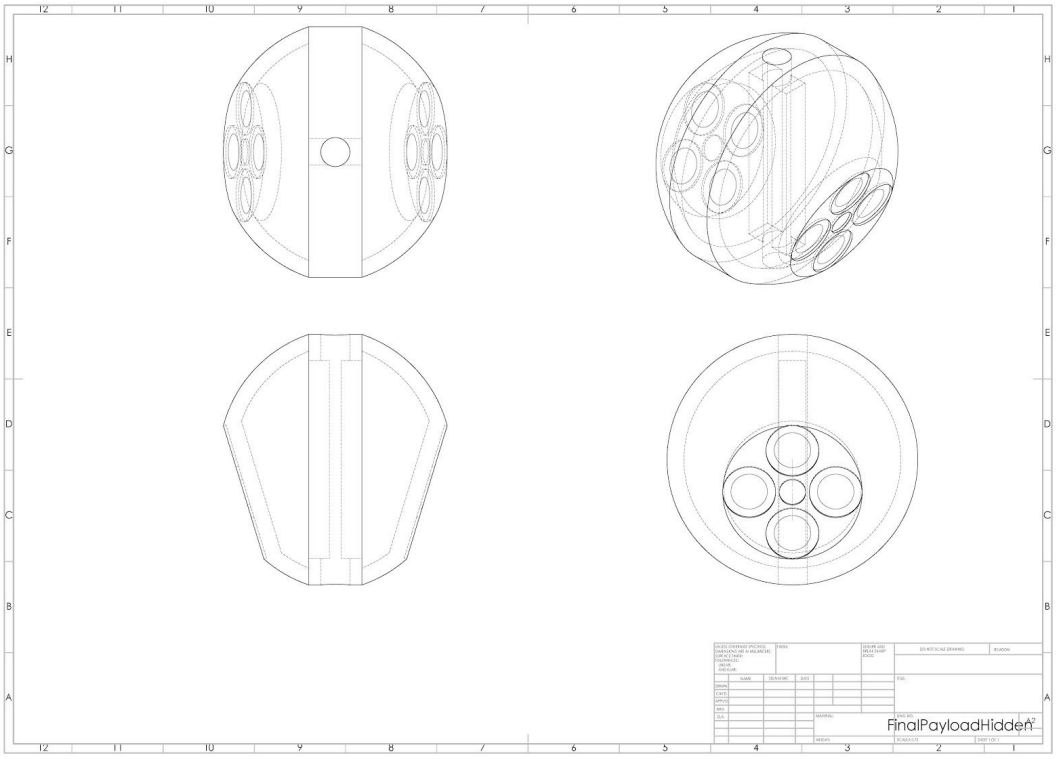
Payload Design 3





Payload Design 4 (Final)




FinalPayloadHidden

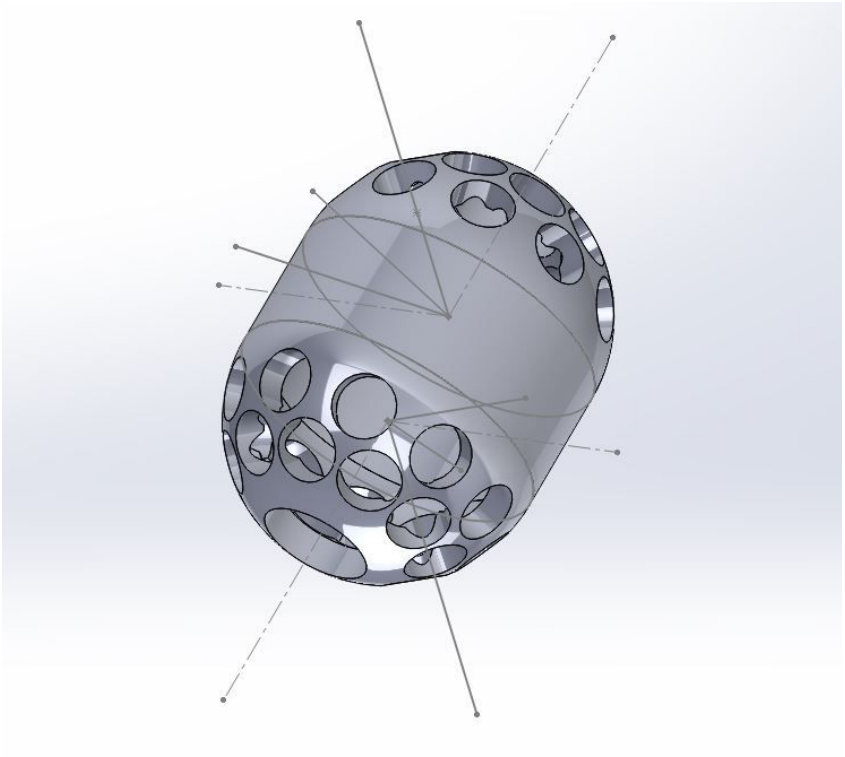
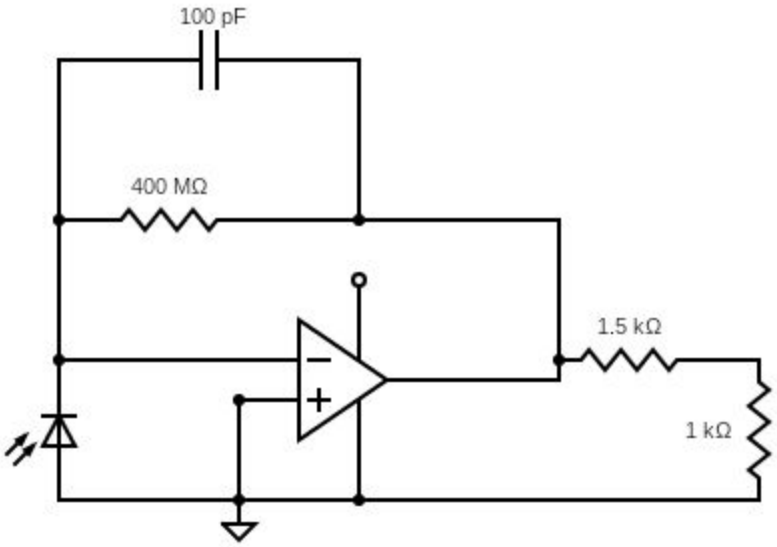


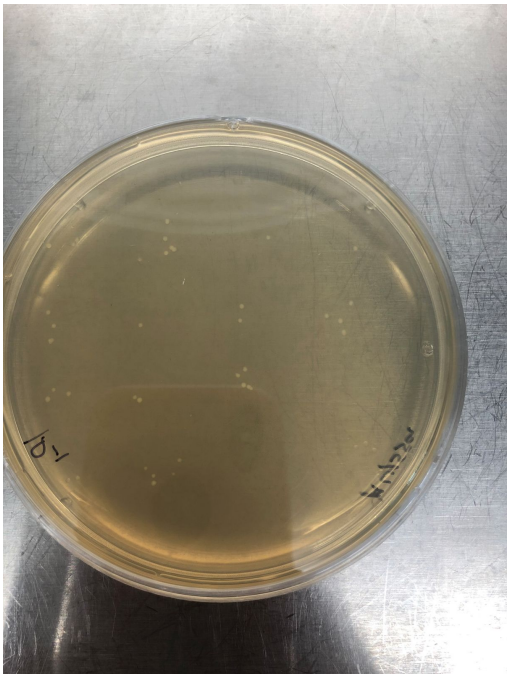
Figure 9: UV Sensor Design



3.0

Results

Preliminary Results



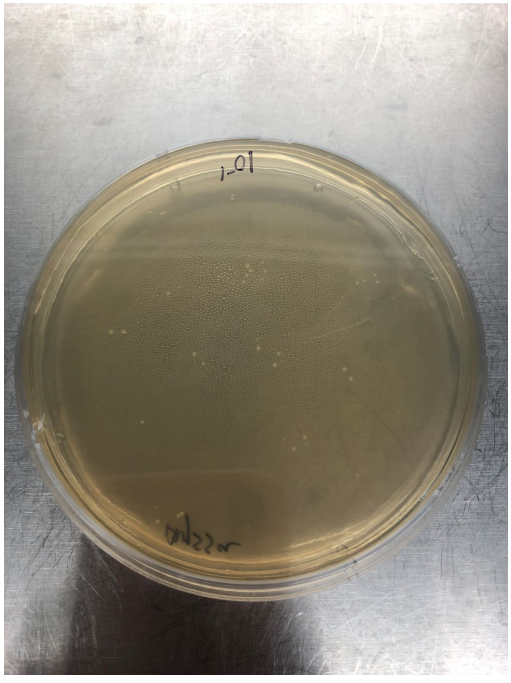


Figure 1: 10^-1 dilutions of E.coli on rifampicin antibiotic plates, with no treatment given to the E. coli in order to retrieve a baseline for spontaneous E. coli growth.

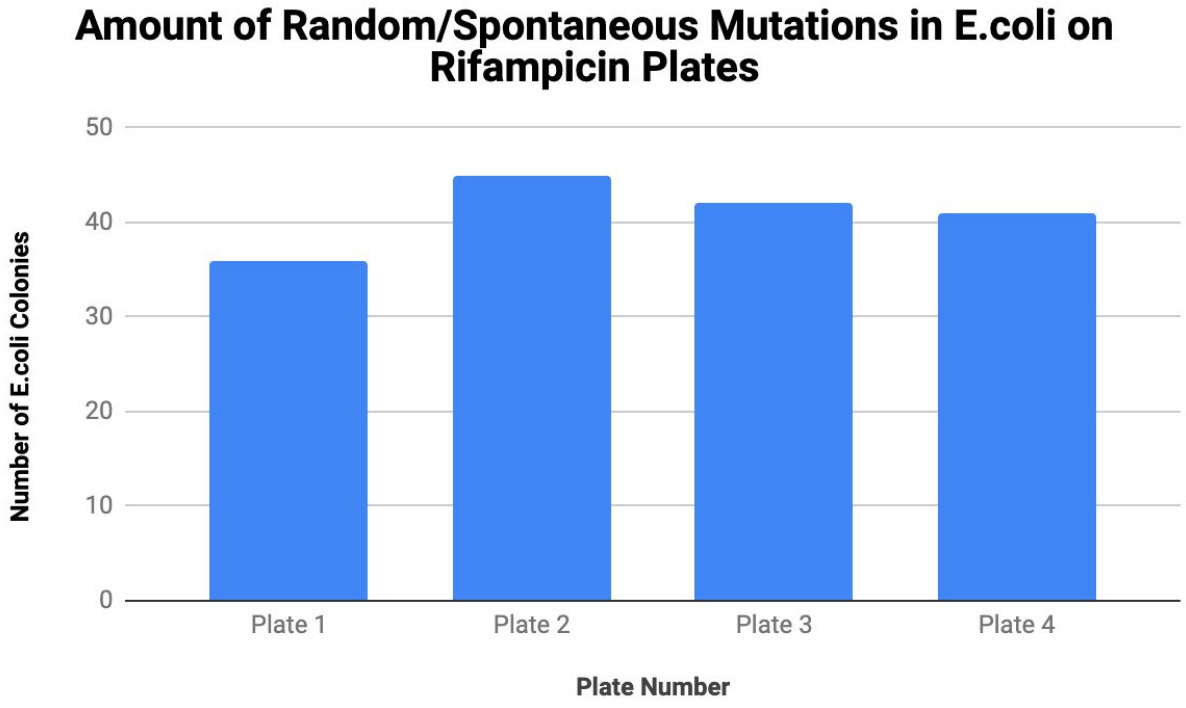


Figure 2: The amount of random/spontaneous mutations that arose from the E.coli when exposed to nothing, with a dilution of 10^-1.

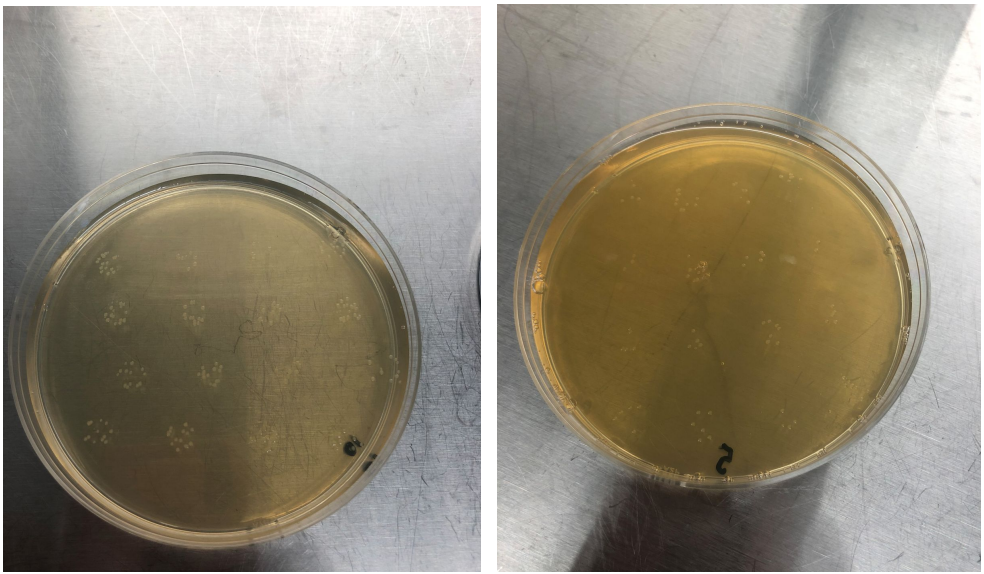


Figure 3: Rifampicin antibiotic plates with cultured E.coli exposed to 45 minutes of UV-C 254nm radiation collected at 10am, with a dilution of 10^-1.

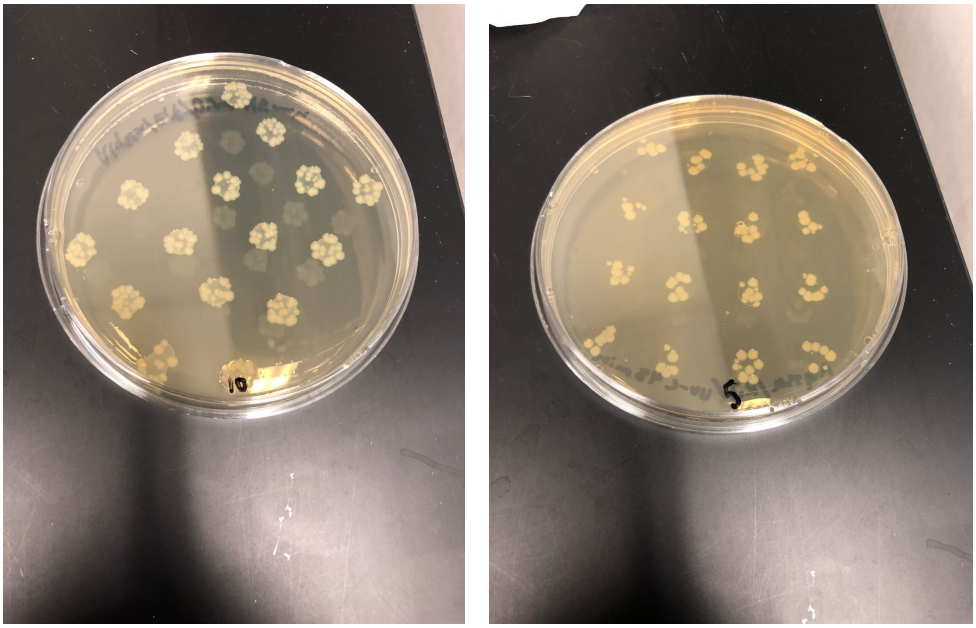


Figure 4: Rifampicin antibiotic plates with cultured E.coli exposed to 45 minutes of UV-C 254nm radiation collected at 4pm. A lot more bacterial growth has occurred as the E. coli has spread into bigger opaque clumps.

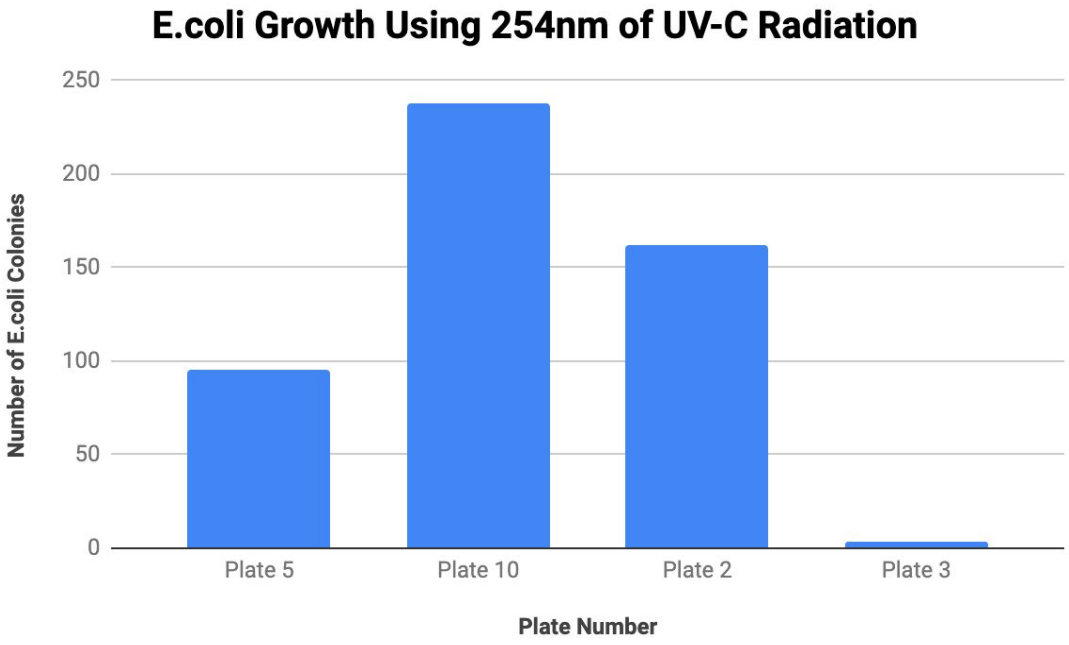


Figure 5: 3/6 plates that were sampled had optimal growth over close to or over 100 colonies, however the other 3/6 colonies only provided 3 or less colonies, which can be seen from plate 3.

Actual Results

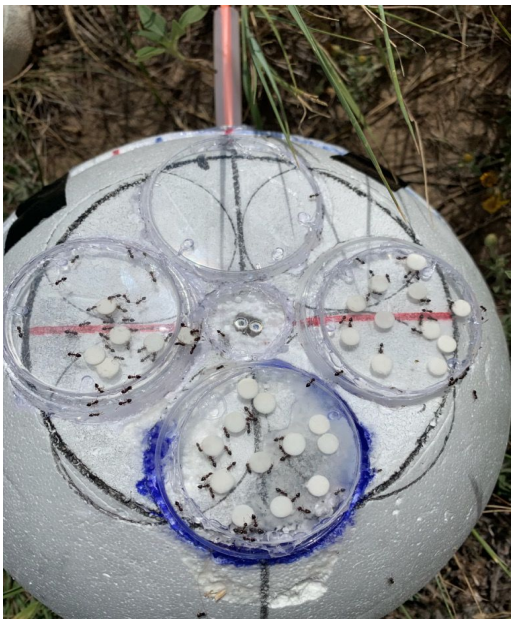
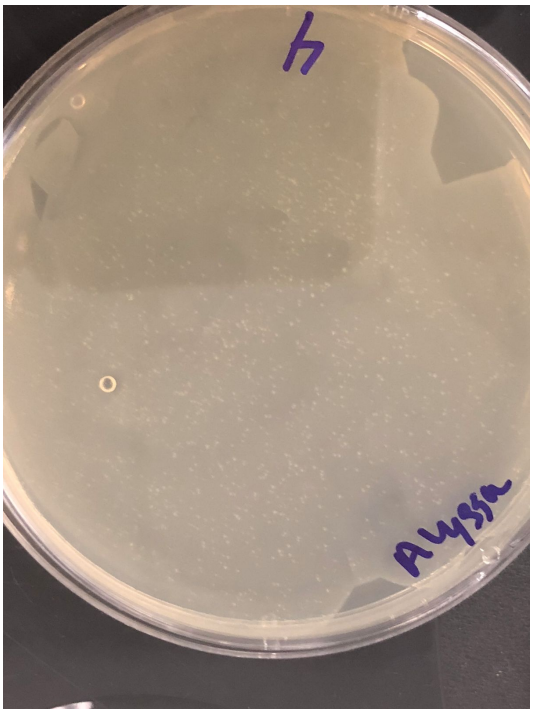


Figure 6: Payload infested with ants due to lack of hot glue surrounding the glass slides, E.coli, and the lids of the Petri dishes used to form a secure adhesive.



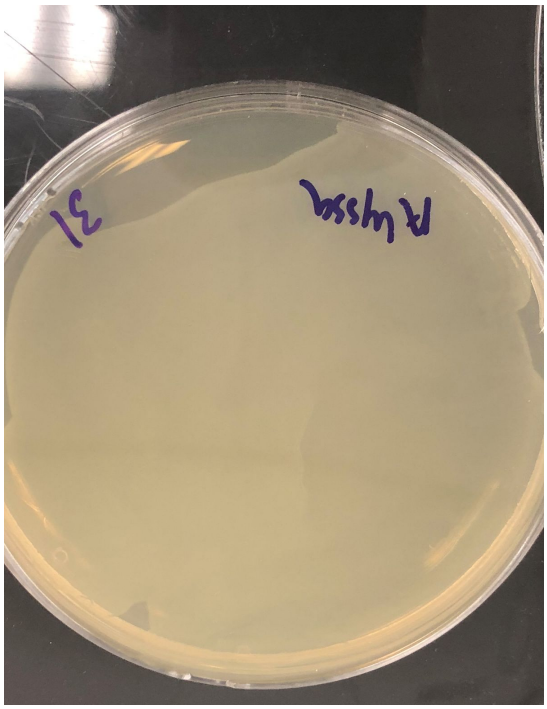


Figure 7: Cultured payload E.coli grown on LB agar plates, plate 4 shows noticeable white spotting within the growing E. coli, while plate 31 shows a clear lawn fashion of the E. coli.

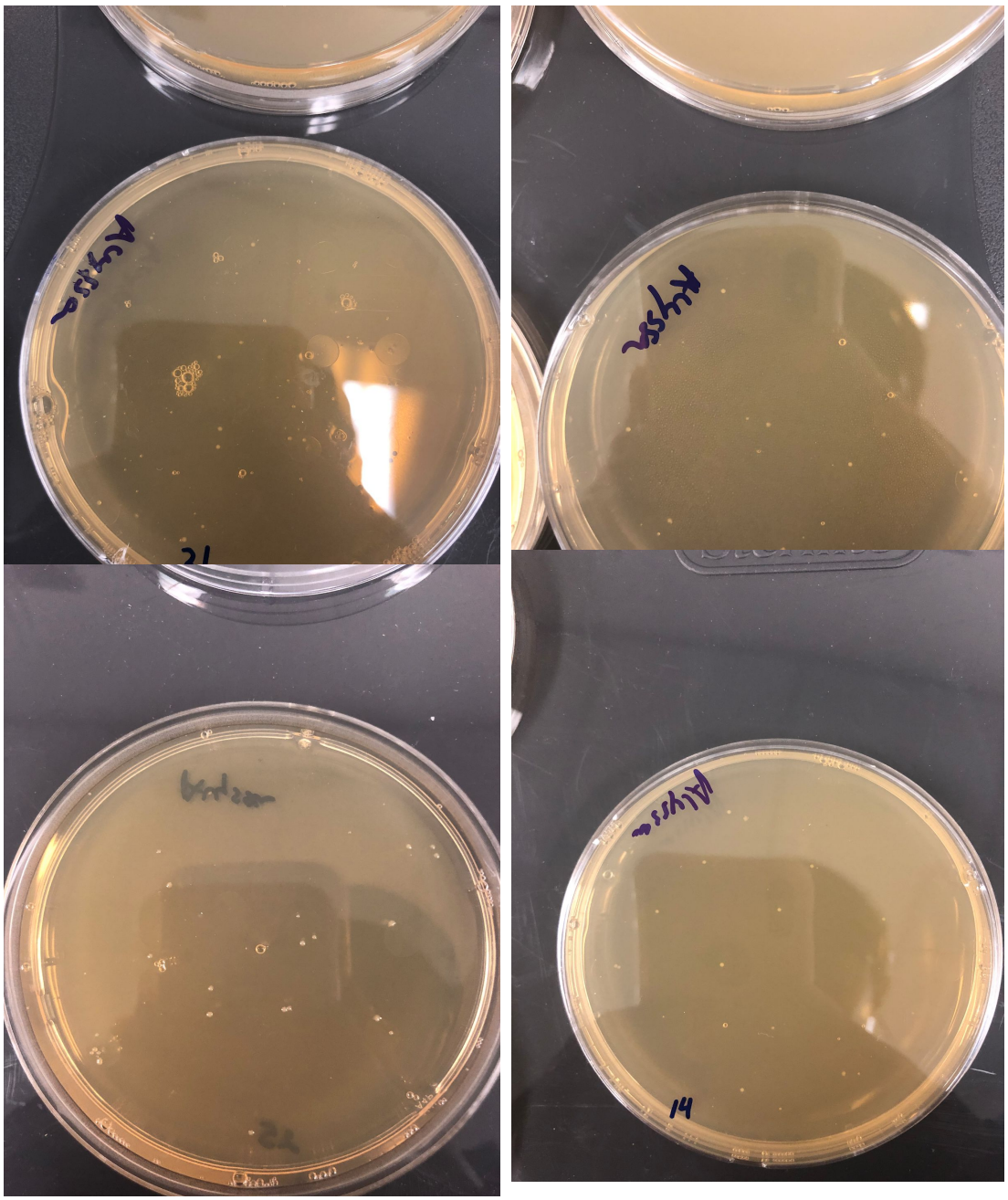


Figure 8: Rifampicin antibiotic plates after 24hrs, with cultured payload E coli. 4/13 plates that were cultured show very small E. coli growth, the E.coli can be seen as small opaque circles on the plates. Constructed at a dilution of 10^-1.

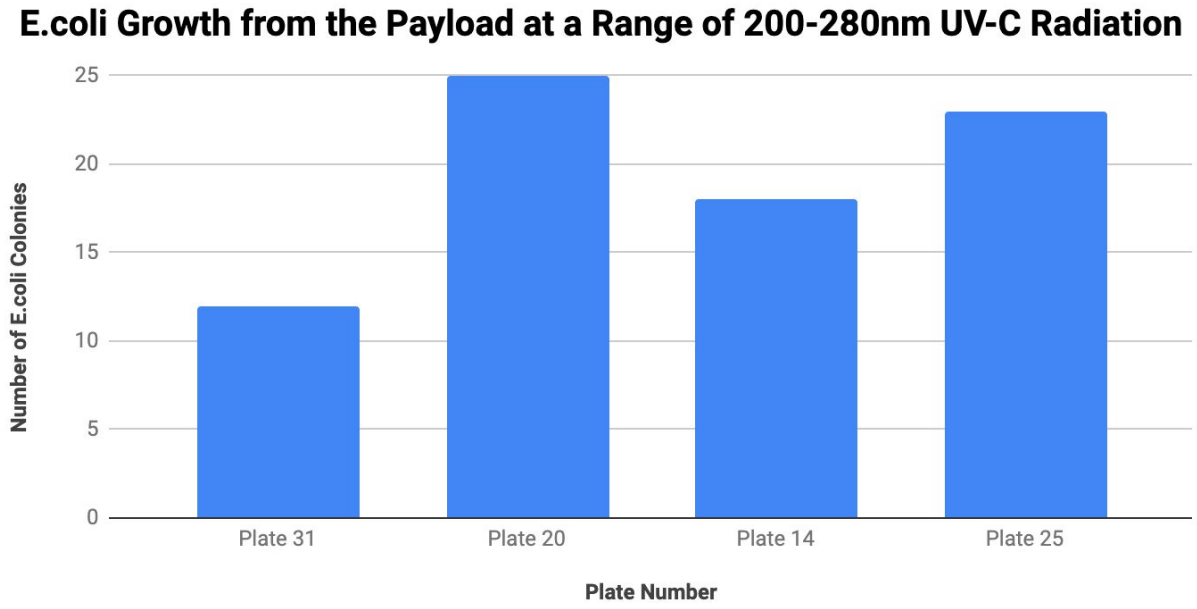


Figure 9: 4/13 plates with E. coli growth from the cultured E.coli retrieved from the payload, this E. coli is believed to have been exposed to UV-C radiation ranging from 200-280nm.

Figure 10: The culture sample on the left shows a clear rust coloration than the two samples located on the right, which shows the normal coloration for E. coli cultures.

4.0 Discussion

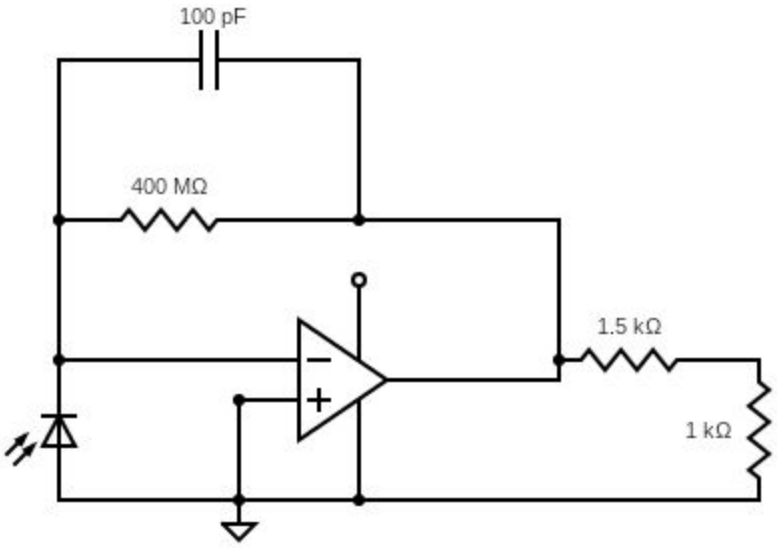
From the preliminary tests, it was concluded that the stated hypothesis had sufficient evidence to be accepted. It was found through the preliminary trials that using a UV-C light at 25W, 110V, and at a wavelength of 254nm, contributed to an increased amount of rifampicin antibiotic resistant E. coli, as demonstrated in Figures 2 and 5. In comparison, Figure 2 represents how much E.coli had spontaneous mutations without any UV-C treatment on the rifampicin antibiotic plates, whereas Figure 5 represents how much E. coli had grown on the rifampicin plates with 45 minutes of UV-C irradiation. From the figures it can be seen that the irradiated E.coli produced more colonies than the non-irradiated E.coli. Thus, giving sufficient evidence that our hypothesis was true.

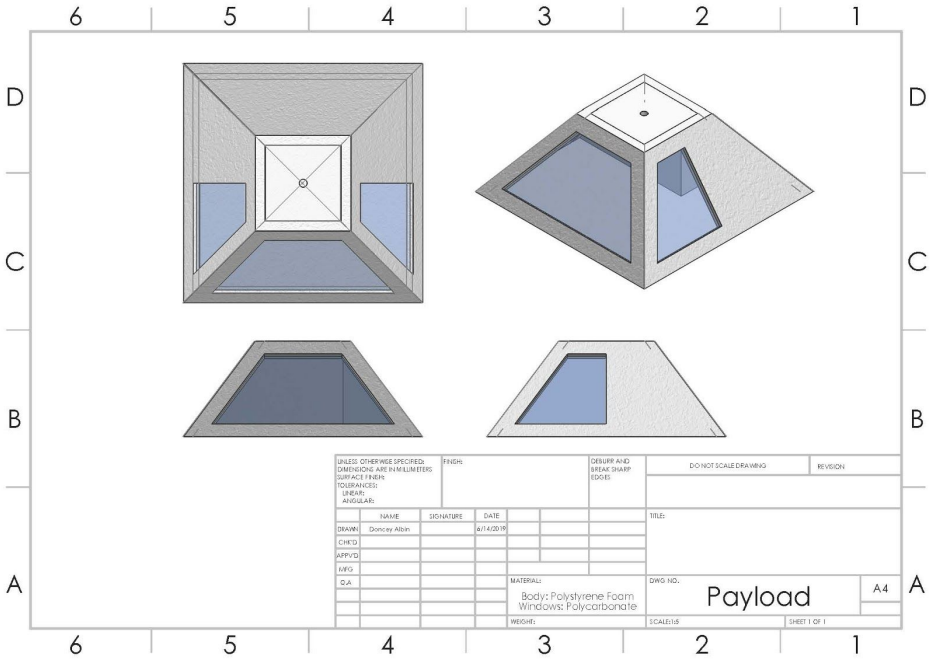
From the actual payload testing, the E. coli was essentially contaminated from the ants that had entered the E.coli slides after the landing of the payload. This contamination could be seen in Figure 10, which shows clear discoloration in the cultured sample that was retrieved from the payload. However, testing was conducted to see if any conclusive results could be obtained from Figures 8 and 9 it can be seen that there was some E. coli growth that had taken place on the rifampicin antibiotic plates, but not as much as seen in Figure 5. Thus, it is believed that due to the many errors that occurred over the course of pre and post-payload launch, or due to the fact that the E. coli was exposed to too much radiation this caused for a decrease in the amount of growth than what was seen in Figure 5. From previous studies, it was indicated that UV-C is usually used as a germicidal light fixture for purifying air, and will usually kill the bacteria

(American Ultraviolet). Providing sufficient evidence to believe that one of the leading causes as to why there was less bacterial growth was due to the over exposure to the UV-C, furthermore since the UV sensors that were used only measured a certain range of wavelengths, the full spectrum of how much and which wavelengths were mostly absorbed by the E. coli is unknown. On the other hand, because ants were able to enter the slides that contained the E.coli, thus causing a loss in sterility, there is no way of knowing the full extent of the damage that was done due to ant exposure. Overall, a clear amount of E.coli was able to be recovered on the rifampicin plates, but did not support our stated hypothesis.

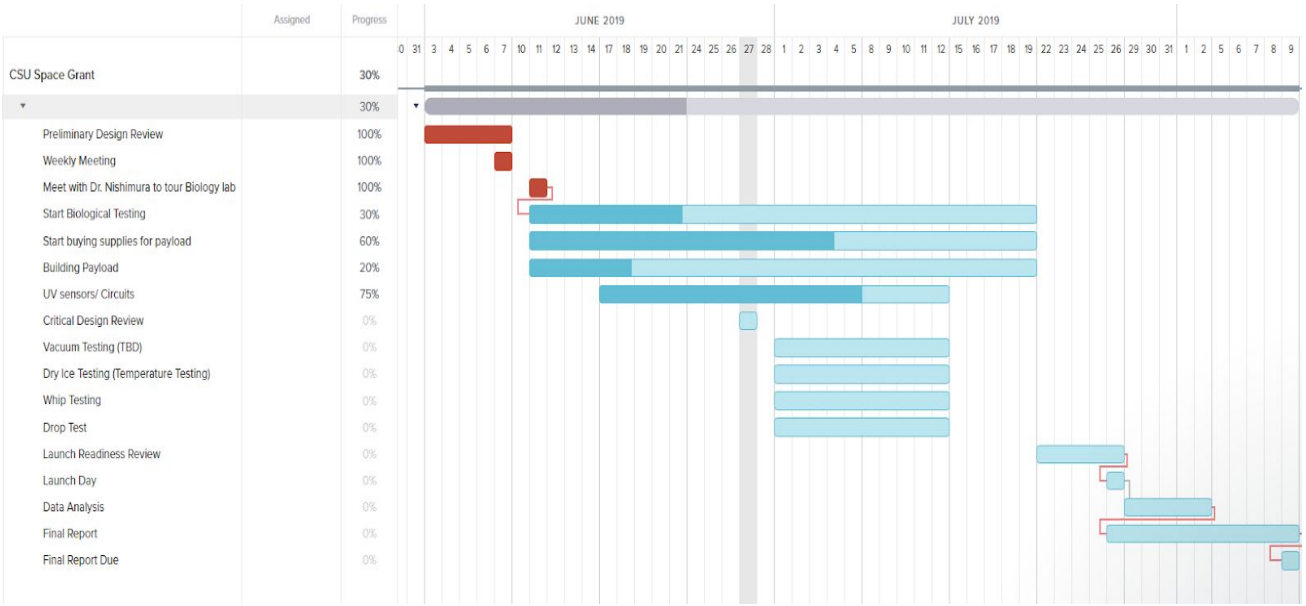
6.0 Design

Figure 9: UV Sensor Design





7.0 Management



Budget

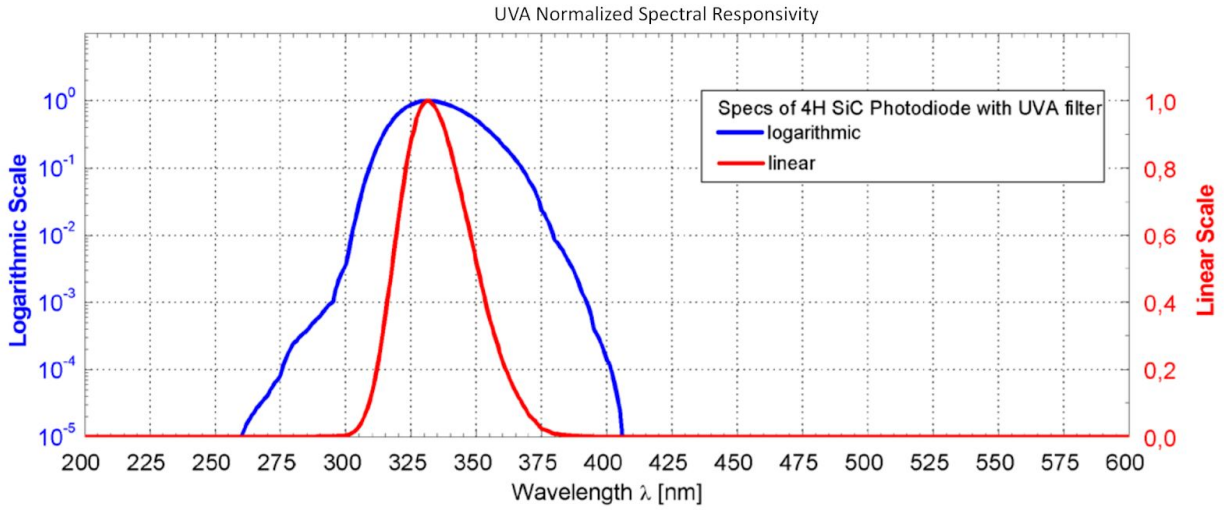
Bill of Materials

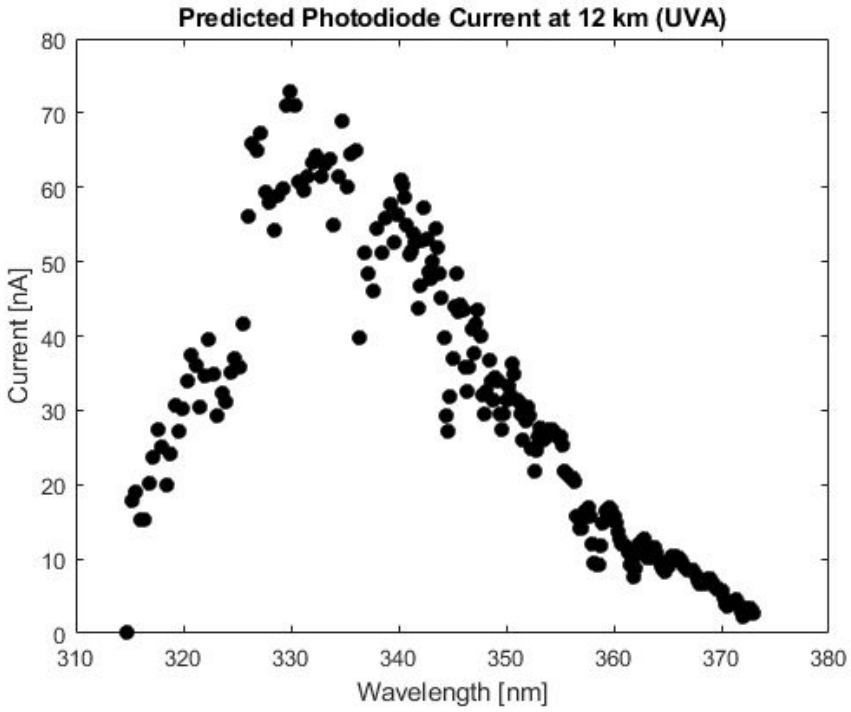
	Title	Unit Cost	Amount	Total Cost	Supplier	URL
1	Exo Terra Intense Basking Reptile Spot Lamp		1	Donated	Chewy	https://www.amazon.com/Germicidal-C
2	UV Germicidal Lamp	$17.90	1	$17.90	Amazon	https://www.amazon.com/Zoo-Med-Re
3	(2 Pack) 10.0 Uvb Fluorescent	$20.23	1	$20.23	Amazon	https://www.sigmaaldrich.com/catalog/
4	Rifampicin	$47.40	1	$70.40	Sigma Al	
5	2xyt media	$44.00	1	$67.00	Sigma Al	https://www.sigmaaldrich.com/catalog/
6	3-Pack Handing Lantern Cord	$12.68	1	$12.68	Amazon	https://www.amazon.com/dp/B01M8Q
7	UVT Acrylic Sheets	$104.00	1	$142.89	EMCO Plastics	https://www.emcoplastics.com/uv-trans
8	Smoothfoam™ Styrofoam® Hollow Half Ball, 10"	$12.00	4	$48.00	Michaels	https://www.michaels.com/smoothfoam
9	FloraCraft® Cleankut™ Foam Cutter	$20.00	1	$20.00	Michaels	https://www.michaels.com/floracraft-cl
10	Smoothfoam™ Styrofoam® Sheet, 2"	$15.00	1	$15.00	Michaels	https://www.michaels.com/smoothfoam
11	WATERPROOF SILICONE FLEXIBLE HEATING PAD	$7.99	4	$31.96	Amazon	https://www.amazon.com/Waterproof-S
12	Thermocouple Module	$3.55	2	$13.00	Ebay	https://www.ebay.com/i/123320201247
13	20 Mini petri Dishes 35mm	$4.95	1	$20.67	Amazon	
14	Solderable PC BreadBoard, 1 Sided PCB	$5.90	6	$35.40	Amazon	https://www.amazon.com/Solderable-Bre
15	2"X0.125" Quartz Disk	$16.10	8	$128.80	TGP	https://technicalglass.com/2x-125-fuse
16	1"X0.125" Quartz Disk	$5.40	2	$10.80	TGP	https://technicalglass.com/1x-125-fuse
17	Smoothfoam™ Styrofoam® Hollow Half Ball, 10"	$12	3	$35.97	Michaels	https://www.michaels.com/smoothfoam
18	Smoothfoam™ Styrofoam® Sheet, 2"	$15	1	$14.99	Michaels	https://www.michaels.com/smoothfoam
19	Arduino Uno Circuit Board	$11.86	2	$23.48	Amazon	https://www.amazon.com/Elegoo-EL-C
20	Tenergy Universal RC Battery Charger for NiMH/NiCd	$21	1	$21	Amazon	https://www.amazon.com/Tenergy-Univer
21	Tenergy NiMH Battery Pack 12V 2000mAh	$22	2	$44	Amazon	https://www.amazon.com/gp/offer-listin
22	10 pack 9v Battery DC Connectors	$5.80	1	$5.80	Amazon	https://www.amazon.com/AKOAK-Batt
23	SparkFun Pressure Sensor Breakout - MS5803-14BA	$59.95	1	$59.95	SparkFun	https://www.amazon.com/SparkFun-Pres
24	Insulfoam Faced Polystyrene Foam Board Insulation	$10	1	$10	Lowes	https://www.lowes.com/pd/Insulfoam-C
25	Chemical-Resistant Slippery PTFE Tube, 5/8" OD x 3/8	$6.73	1	$6.37	McMaster Carr	https://www.mcmaster.com/standard-p
26	Thermo Couple Arduino Connector	$6	1	$6		
27	Aluminum Tape	$15	1	$15		
28	Rubber Insulation	$30	1	$30		
29	Flight Tube	$5	1	$5		
30	Batteries	$20	1	$20		
31	Identification Sticker		1	Given		
32	American Flag Sticker		1	Given		
33	Metal washers		2	Given		
34	Incubator		1	Borrowed		
35	Wavelength detector		1	Borrowed		
36	Pipettes		1	Donated		
37	E. coli		1	Donated		
38	Petri Dishes		1	Donated		
39	Luria Bertani Broth		1	Donated		
		TOTAL:		**952.29**		
		REMAINING:		**47.71**		

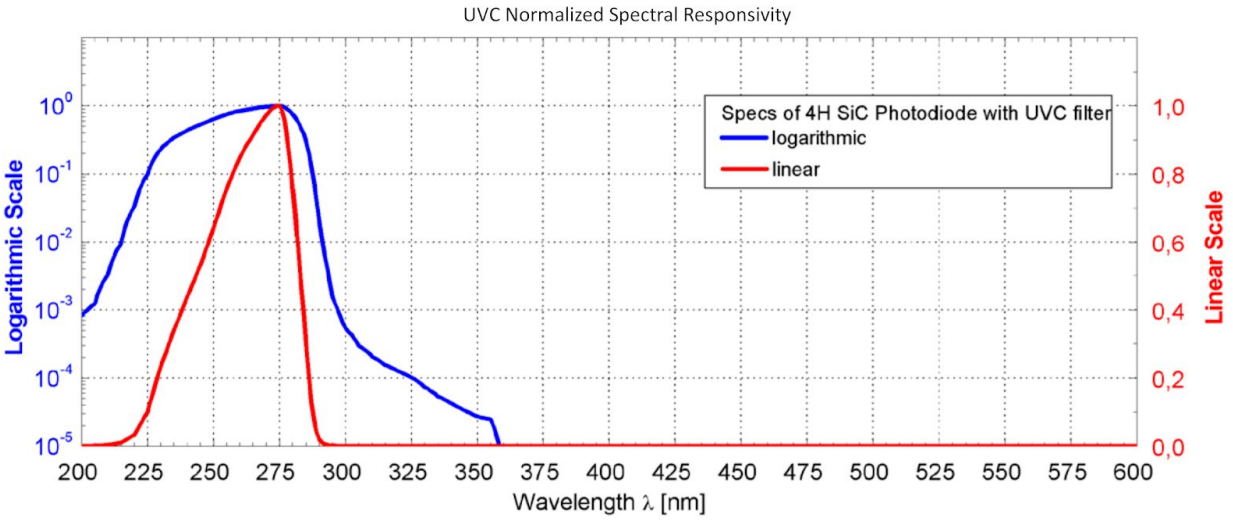
Part	Q	Mass (Kg)
Arduino Uno	1	0.02634
UV Sensor& wires	1	0.03137
SD Card Reader	2	0.00403
Payload Body	1	0.145
Heaters	2	0.0303
~Wires~	1	-
Switch and wires	1	0.01981
Arduino Mega	1	0.035
Quartz Glass (All)	1	0.12432
Petri Dishes	12	0.0012
9V Battery	0	0.04477
Circuit structure	2	0.02775
Thermocouple	0	0.01461
Relay Switch	1	0.01438
Insulation	1	0.07949
12V Battery	1	0.223
Altimeter	1	0.006
Current Mass:		**82.737%**
Remaining Mass:		**17.263%**

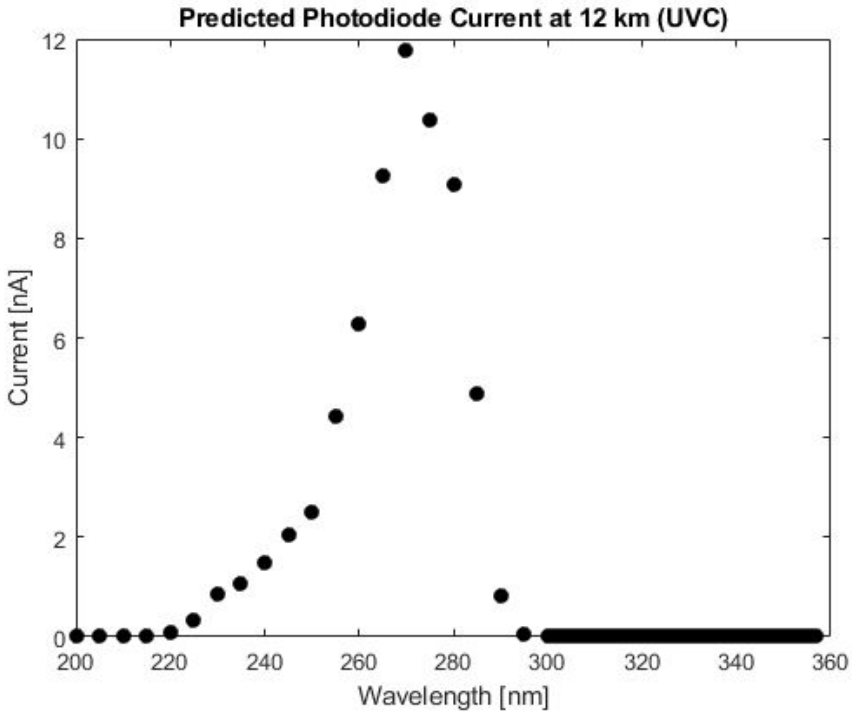
9.0 Expected Results

We connected the diodes to a power supply and an oscilloscope to measure voltage change. When we shined the 3 lights with different wavelengths on the diodes, we were able to measure how much resistance we needed for each based on voltage increases. We used data from NOAA displaying how much UVA and UVC was collected relative to altitude and the Normalized Spectral Responsivity graphs from the suppliers of the Diodes. Then, we combined them to get values of current that we then used to determine the amount of gain that was needed. For UVA, the max current value we got was 7.2992e-8 and the max resistance for UVA is 1.0206e9. For UVC, the max current value was 1.1758e-8 and the max resistance is 1.0206e9. Therefore, for UVA we used a resistance of 40 M ohms,but for UVB and UVC, the resistance was 400 M ohms for each.


UVA Normalized Spectral Responsivity
Specs of 4H SiC Photodiode with UVA filter
logarithmic
linear
Logarithmic Scale
Linear Scale
Wavelength λ [nm]


Predicted Photodiode Current at 12 km (UVA)
Current [nA]
Wavelength [nm]

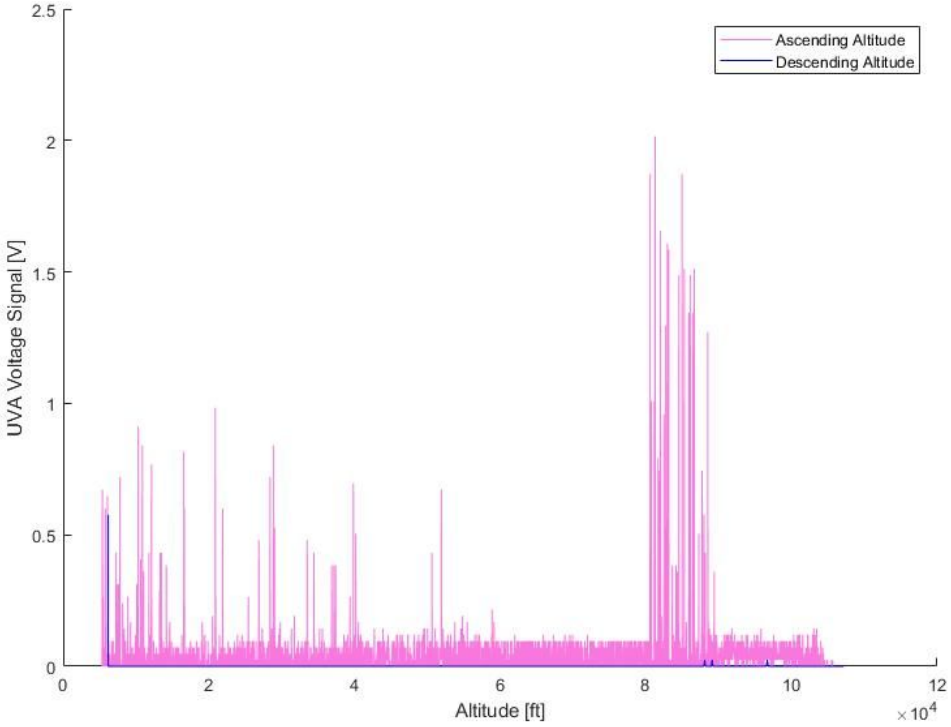


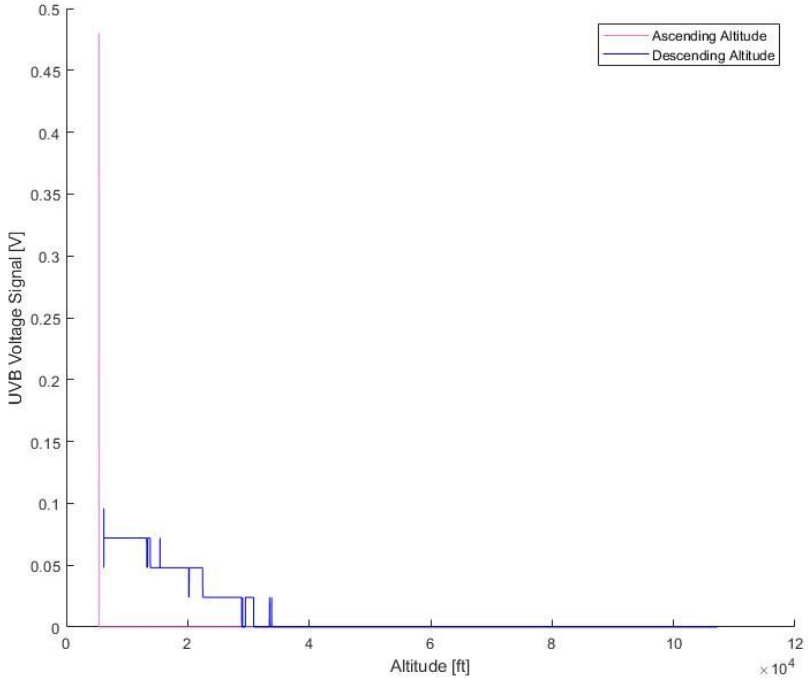


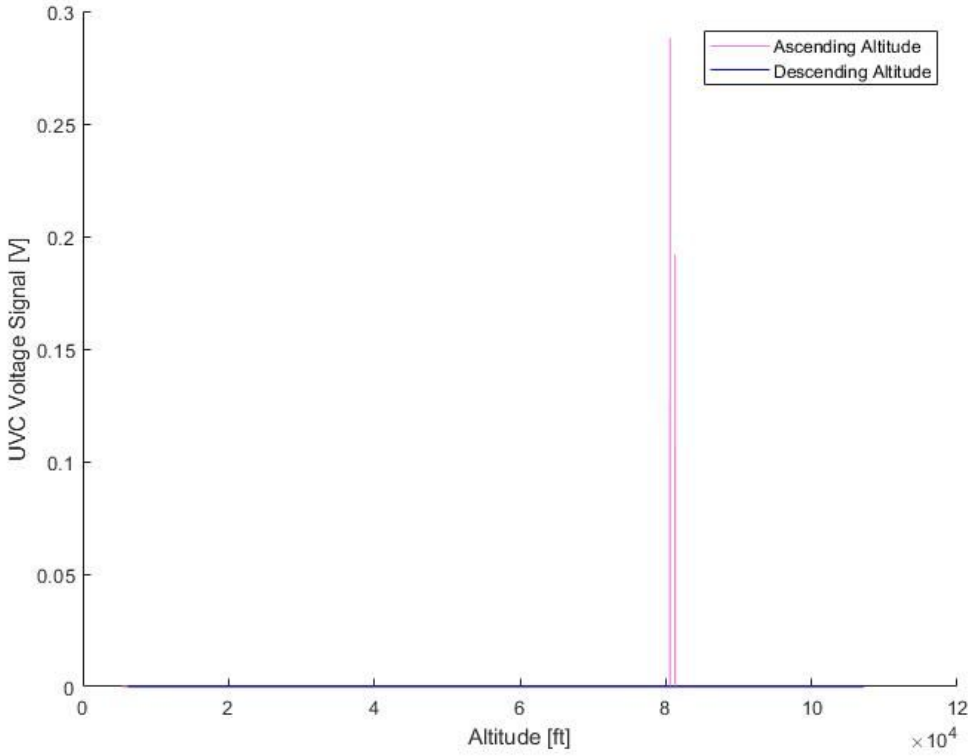
After thorough testing of the UVA, UVB, and UVC sensors; we were confident that there would be enough gain on the resistors for there to be a significant voltage change when the sensors collected light to register so we could measure how much UV we are receiving.

11.0 Results, Analysis, and Conclusions

UV light covers a wavelength spectrum from 100 to 380 nm and is subdivided into three regions by wavelength: UVA (320 to 400 nm), UVB (280 to 320 nm), and UVC (200 to 280 nm). Among them, UVC has the strongest germicidal effect. The photo diodes that we used to measure There is an acceptance range from 309-367 nanometers for UVA, 231-309 nm for UVB, and 225-287nm for UVC. Since the UVB and UVC sensors had very similar ranges, UVB was placed on one side of the payload and UVC was placed on the other. UVA was placed directly next to UVB. It worked out well that the wavelength ranges were very similar because the main concern was, “How much UVC are we receiving?”. Based on the UVA data, the sensors collected UVA all along the path from accent and decent. The UVC data shows that exposure only occurred once we got to 8,000 ft. Our data seems to be in line with reality for UVA and UVC. Most UVA is not absorbed through our atmosphere and reaches Earth. However, most of the UVC rays do not reach the Earth and are absorbed through our atmosphere. Once it was 8,000 ft from the ground we started getting UVC data. The highest voltage we received from UVC was 0.3. Unfortunately for the UVB sensor; it was broken when we retrieved it from launch. According to the data, we believe that the force from the launch is the cause for the UVB sensor to break.


Ascending Altitude
Descending Altitude
UVA Voltage Signal [V]
Altitude [ft]
×10^4


Ascending Altitude
Descending Altitude
UVB Voltage Signal [V]
Altitude [ft]
×10^4



14.0 Message to Next Year

Colorado Space Grant Demo Sat program is a unique opportunity to learn so many things from many different aspects of engineering. The advice we have is to make the most of the amount of time you are given. In the beginning, it seems like you have all the time in the world, but towards the end you have to cut some things out. Be prepared to completely scrap your ideas and start completely over many times. It is just part of the process. For example, we had about 7 different payload designs before we had a final design. Use the thickest possible wires that fit into the circuit board and the arduino. Find wires that fit perfectly with no extra room. There were many problems with wires breaking often. Plan ahead with what you think you are going to need so you avoid spending your budget on fast shipping. Don't

worry about getting everything finished. If you section things out, you can cut things out at the very end if needed. Relax and soak up every moment of growth and exploration you can!

Space Rams

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