Research On Measuring the Capacity of A UV-Disinfectant Machine by Disinfecting Masks Used by Covid-19 Infected Patients

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
Brac University
October 2022

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled “Research on measuring the capacity of a UV-Disinfectant machine by disinfecting masks used by covid-19 infected patients” submitted by Sanjida Akter Suchona (ID: 18346045) of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on November, 2022.

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

The present study aims to measure an Ultraviolet type C compartment's disinfection capacity of mask used by covid-19 patients through biochemical testing. The sample was taken from covid-19 positive patients and stored in TSB contain VTM. After conducting proper calculation, the sample was diluted and prepared freshly for biochemical testing. All the reagents were measured and autoclaved accordingly and plating was done under biosafety cabinet and kept in 24 hours incubation. After one day incubation we compare the plates of before and after UVC-espoused samples microorganism's growth. The results obtained from the biochemical study suggest that the ultraviolet type C chamber could be a promising approach for developing a proper disinfection machine with a broad spectrum of usage.

Keywords: SARS COV-19; UVC; TSB; incubation; biochemical testing

Dedication

To my Ammu & Abbu

Acknowledgement

Firstly, I would like to begin by thanking Almighty Allah for keeping me in good health and blessing me with the capability, strength and assistance needed to complete this project. However, this research paper would not have been completed without the support of several individuals and I would like to express my gratitude to all of them.

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Lastly, I want to thank my parents and my family for their unconditional love, blessings and prayers that has given me the strength to pursue my dreams and helped me become the person I am today.

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List of Acronyms

SARS	Severe acute respiratory syndrome
BSL	Biosafety Level
VTM	Viral Transport Medium
Rt-PCR	Reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
S1	Sample number 1
MRSA	Methicillin-resistant Staphylococcus aureus
UVC	Ultraviolet type C
TSB	Trypticase Soy Broth

Chapter 1 Introduction

1.1 Background

In May, 2021 BBC reported that scientists found dyes, toxic chemicals as well as bioaccumulative heavy metals while testing disposed masks. Bangladesh disposed of a total of 13099 million face masks in the environment from March 8-November 18, 2020, which is the equivalent of 392970 tons of waste with a rate of 1535 tons/day. According to daily star, 25 lakhs of face masks sold daily, worth more than 1 core taka are sold across the country every day. Developing countries like Bangladesh are already polluted and it became a concern to find ways of making less pollution and saving money. This is the point where we focus and our sole purpose is to recycle the disposal mask, reuse, save money, and reduce pollution. In these circumstances, we are using an Ultraviolet Disinfection machine (EEE department, Brac University) to disinfect the used mask from covid-positive patients volunteered across Dhaka city.

1.2 Purpose of the Study

In the latest COVID-19 pandemic, UV air and surface disinfection have attracted tremendous attention, and many products became available on the market (Shining a Light on COVID-19, 2020). Along with hospitals, public places like restaurants, shopping malls, amusement parks etc. are also establishing UV disinfection systems to protect against contamination. Widespread use of UVC disinfectors is also advised to limit virus spread after the reopening of public places. (Garcia de Abajo et al., 2020). Before pandemic various UV products were available in research or other purposes like mercury –UV lamps (UVC- LEDs, far UVC radiating excimer and micro plasma lamp). Now raise the question of whether a UV disinfectant is capable of disinfecting a covid infected mask or not. Our research is based on finding the capacity of a built-in UV disinfectant machine. We are culturing a certain portion of infected masks and culture in TSA. We are measuring the Optical density of the diluted samples to plot a standard curve before and

after UV exposure. We will also use rt-PCR of both before and after UV exposure samples to detect the presence of the covid-19 virus. Furthermore, to identify the presence of certain microorganisms we will run biochemical testing. As we will perform our test based on mas used by covid-positive patient. As well as covid-virus we also focus on other microbes which commonly circulates in the air. Our motive is to culture both UVC espoused samples in agar medium, where only specific microbe can grow and reveal identity. We will select most common agar medium for common microbes found in air of our surroundings as well as hospitals. Then we will culture and observes each media of before UVC espoused sample to identify the presence. After identifying the presence of each group, our target is to compare the growth in terms of after UVC espoused samples. If we consider this as a parameter analyzing the presence and growth rate of certain species of microbes we can predict and judge the performance quality of our UVC disinfectant chamber.

Chapter 2 Methodology

2.1 Sampling

The samples were collected from covid-positive patients across Dhaka city with their full consent. Double gloves and double masks were used while collecting samples from the patient. The masks were placed in zip-locked bags and carried in ice-contained iceboxes and later stored in the freezer (4°C).

Inclusion criteria: We only included the surgical or disposable from the collected sample. Doctors and health workers were the first priority for the inclusion of the samples. 3 layered mask, easily available and much cheaper than K95 was our main concern. Moreover, we ensured that the mask was used for a long duration of time by the patients.

Exclusion criteria: mask which contains any sort of cotton or other material rather than disposable 3 layered mask was not collected. Although, mask was not collected after a long period of time or from a patient who is no longer covid positive.

2.2 Analytical methods

To identify and count the presence of different types of bacteria, fungus. In our before UV exposed sample and after exposed sample, as well as compare the result between them we approached for biochemical test. This cauterization test is done frequently in research to identify and asses the bacterial growth. Nowadays modern kits are also available to make those biochemical tests easier (Hussain, 2013). However, we followed traditional approach for biochemical testing here.

2.3 Method

2.3.1 Preview

Our research method is quantitative as we use numbers and statistics to test a hypothesis through data collection and analysis. We divided our experiment into before and after UV exposure segments. 2 masks are collected from 2different covid-positive patients, those masks were cut 1cm/1cm on the left and right sides under BSL. 1 portion of 1cm/1cm of each mask was stored in a 5ml TSB containing test tube for bacterial culture and kept in an incubator (37 °C) for 1-day incubation at Brac University. Another portion was added to viral transport media (VTM) and stored in Gonoshasthaya (-80 °C) for before *UV optical density measurement*, *bacterial culture*, *Spore Strip inoculum preparation*, *Biochemical Assay and rt-PCR.* All the masks were then exposed to the UV disinfectant machine for 5 minutes duration in the EEE laboratory. After UV exposure, same procedure followed after completing UV treatment of the samples.

(i)UV Treatment: Masks were exposed to UV disinfectant machine at EEE Laboratory for minutes and hanged separately to pass the UV light well.

(ii)Optical Density Measurement: Incubated samples, both before and after UV were diluted to 1 Lakh folds by maintaining the dilution factor ratio of 1:10, 5 test tubes from the stock solution for each sample by maintaining dilution factor. Photometric method (600nm) and Bio method UV DNA Quantification method (280nm) used to measure the optical

density. All the generated data were compiled in excel sheet and plotted for standard curve.

(iii)Plate Culture: Plate culture was done to observe the presence of bacteria in before and after UV treatment samples. We prepared one plate for each dilution by using lawn culture under laminar airflow machine and incubated for 1 day at incubator (37°C). Plates were counted that had a satisfactory bacterial growth which was counted and plotted against optical density in the excel sheet.

(iv)Biochemical Assay: Biochemical assay was done to identify the types of bacteria. We prepared before UV and after UV plates for each sample in different culture media. Prepared plates were incubated for 1 day in incubator at 37°C. Different culture media showed the presence of different types of bacteria.

(v)Spore Strip inoculum preparation*:* Three medium tubes were collected and labeled for the inoculum preparation. One positive and two UV-C exposed were taken inside the laminar air flow hood, one intact sample spore strip and the post UV-C exposed spore strips were transferred to the respected labeled medium tubes with the help of sterilized tweezers and transferred into the incubator for bacterial growth (52°C) for seven days.

(vi) RNA Extraction and rt-PCR: Before and After UV samples ran for rt-PCR after RNA extraction at BSL 2+ Lab. RNA extraction was done by using the reagents and protocol of FavorPrepTM Viral Nucleic Acid Extraction Kit.

2.3.2 Biochemical testing

All the agar base required for per plates calculated and weighed using a balance machine. Each agar base prepared in Duren bottle, distilled water used as vehicle. Along with those freshly prepared agars medium and 60 cleaned plates and other equipment autoclaved at 121degree Celsius. Few agars medium required hot water bath at 100 degrees Celsius. After autoclaving all the reagents, equipment was taken into laminar air flow to avoid contamination. Samples of both before and after UV treatment stored in freezer, takeout out before and using a vortex machine mixed the sample property before plating. In the meantime, the reagents cooled down, plates dried out. We labelled and started pouring the agar media in the plates accordingly. When plates become hard, samples had drawn out from test tube and poured in the plates in drops. Then speeded with the glass rod. Kept in incubator for 24 hours at 37 degrees Celsius. After 24

hours we checked the bacterial growth in both before and after UV treated samples containing plates and verified the presence of differ bacteria, fungi as well.

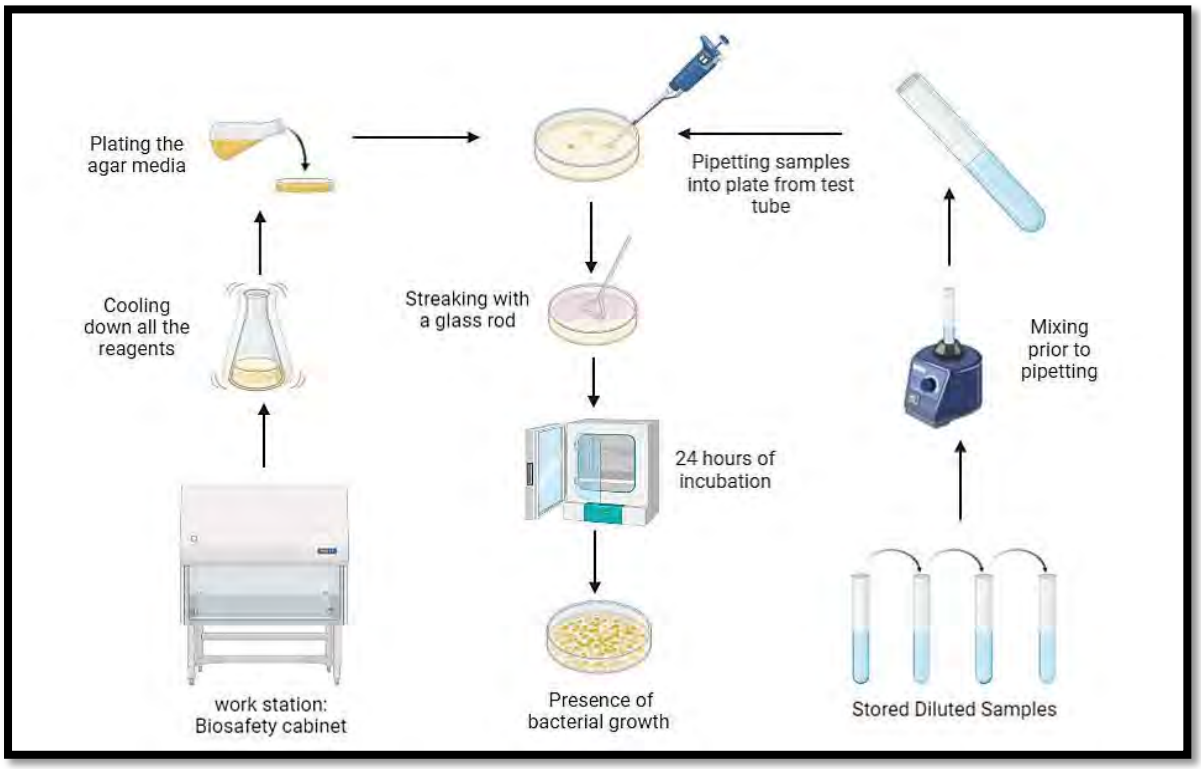

Figure 1: A flowchart of the procedure of performing biochemical testing

2.4 Obstacles

Few plates were not possible to analyze due to the overgrowth of bacteria, but majority of the plates had shown the desired results. One possible reason for the error might be using samples that require more than enough or poor spread ability. There is a slight chance of contamination during plating and storage. We used 1 spirit lamp under laminar air flow and maintained all the personal hygiene to avoid human contamination and cross contamination. Laminar air flow of upper grader using of 2 spirit lamps instead of 1 during plating might enhance the quality of the experiment as well the results.

Chapter 3

Result:

Before UV **After UV**

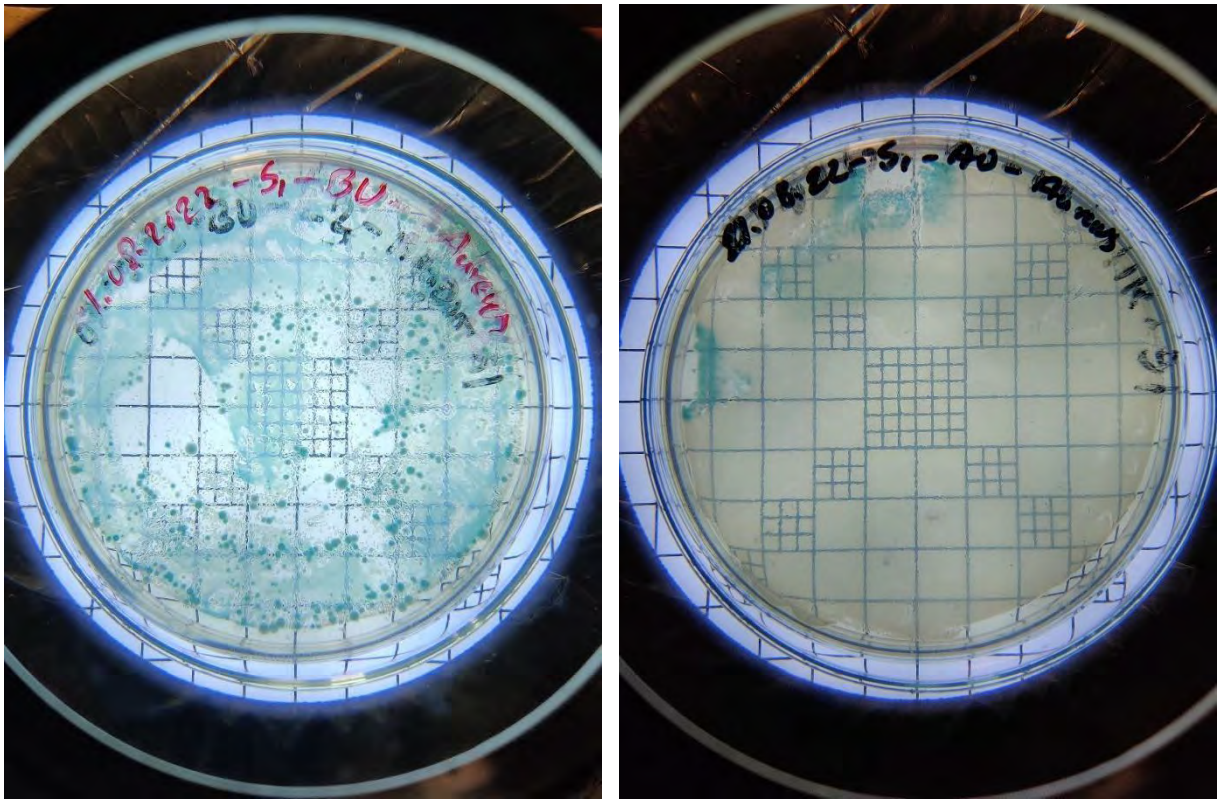

Figure 2: Bacterial growth differences in Chromatic Staph Aureus culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

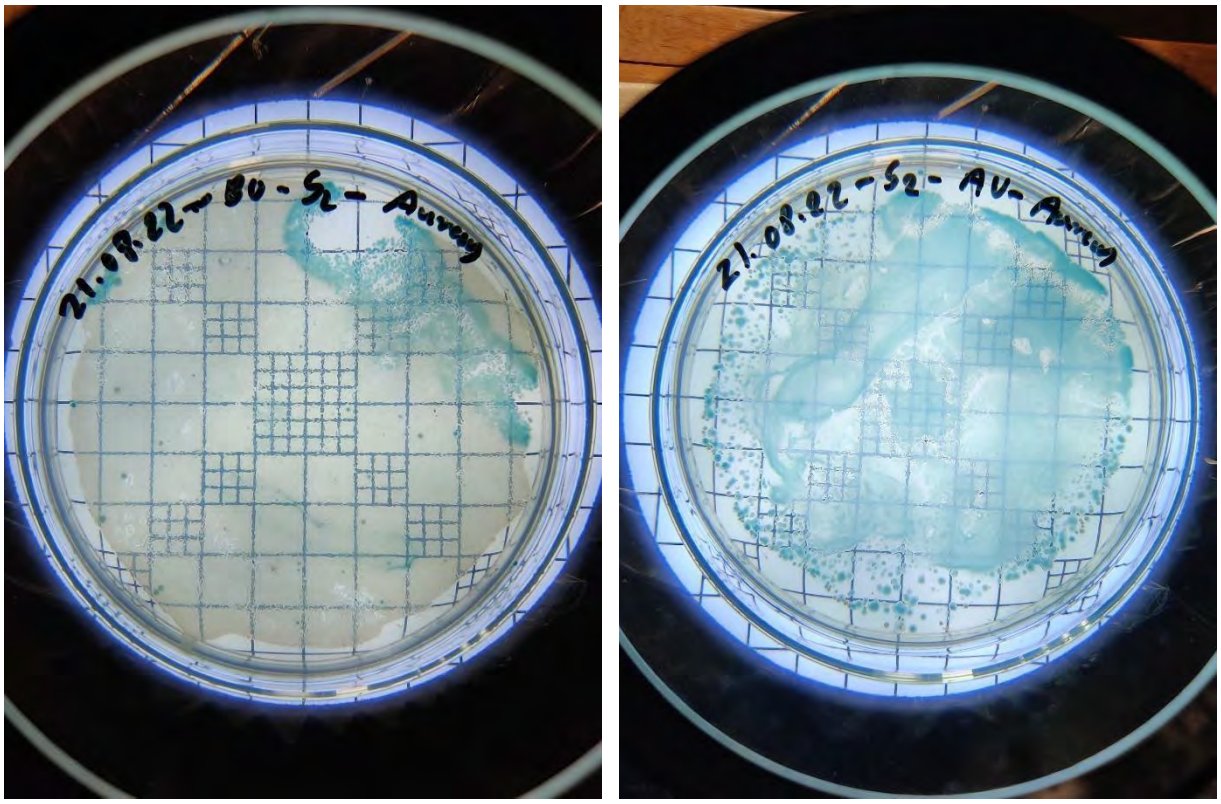

Figure 3: Bacterial growth differences in Chromatic Staph Aureus culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

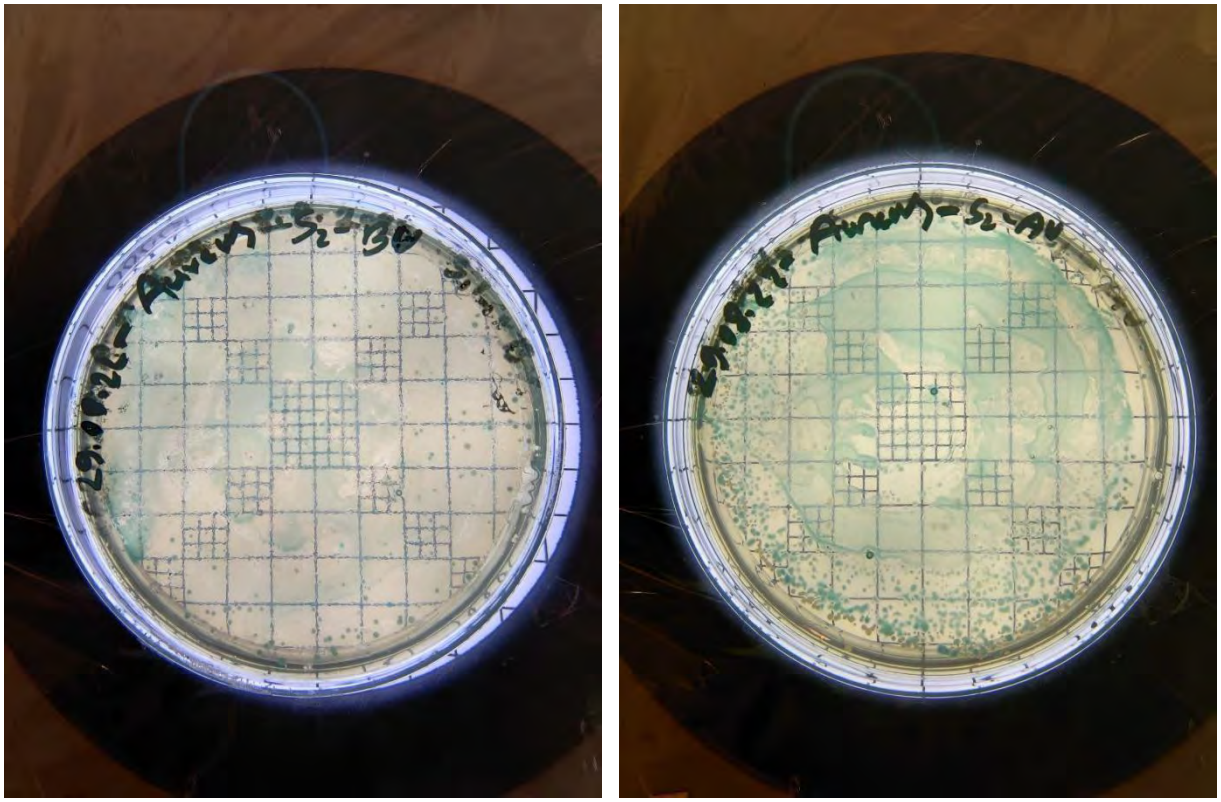

Figure 4: Bacterial growth differences in Chromatic Staph Aureus culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

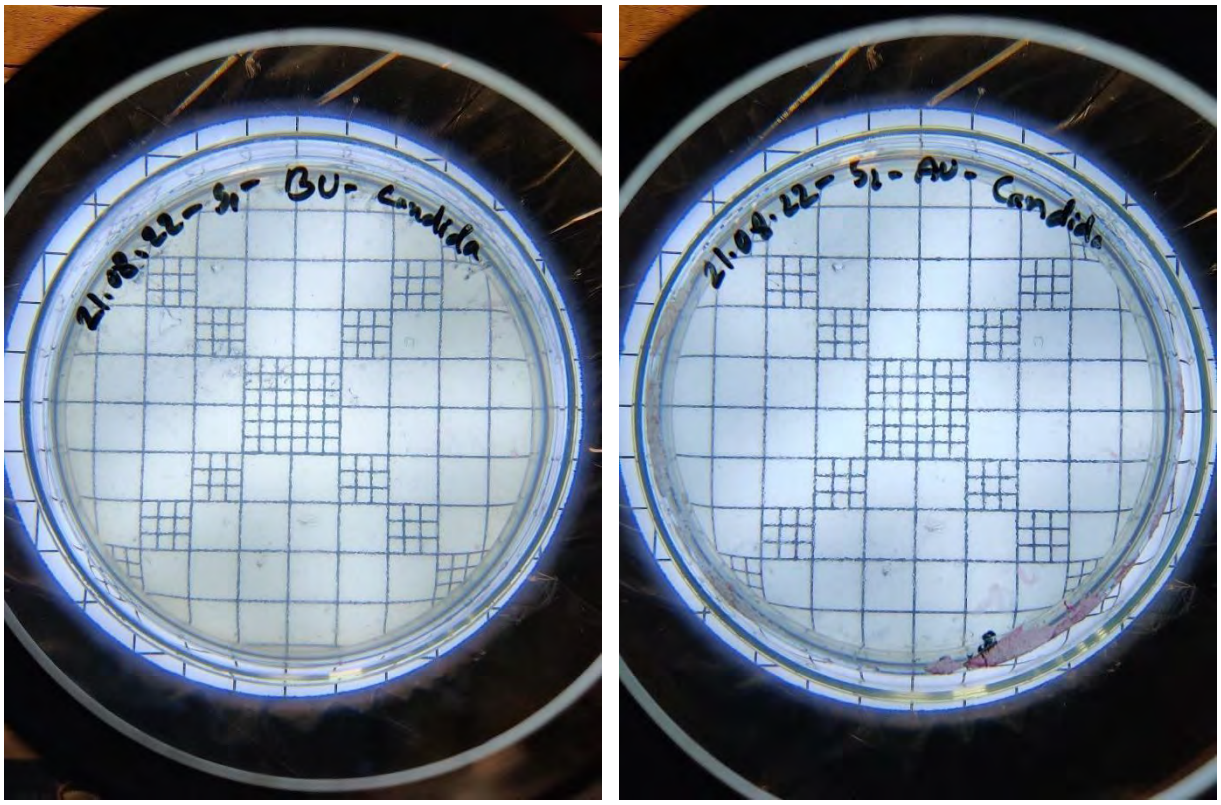

Figure 5: Bacterial growth differences in Chromatic Candida culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

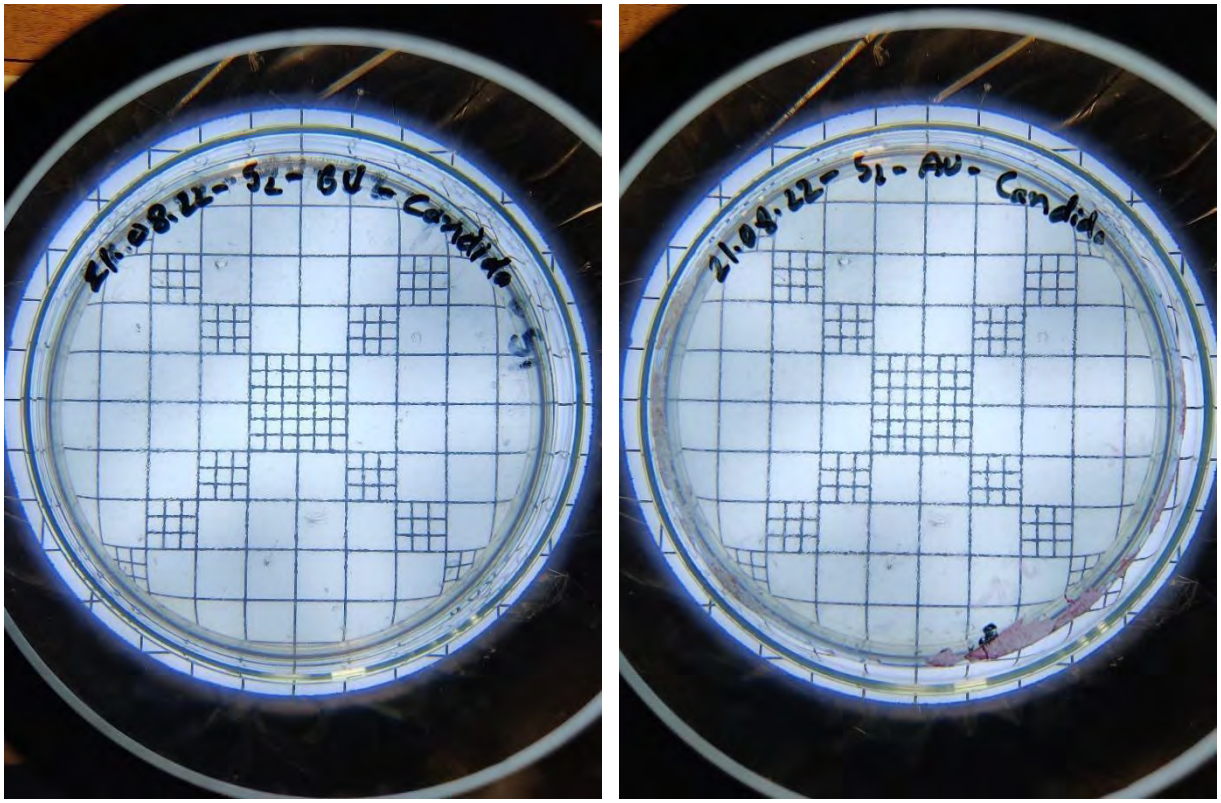

Figure 6: Bacterial growth differences in Chromatic Candida culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

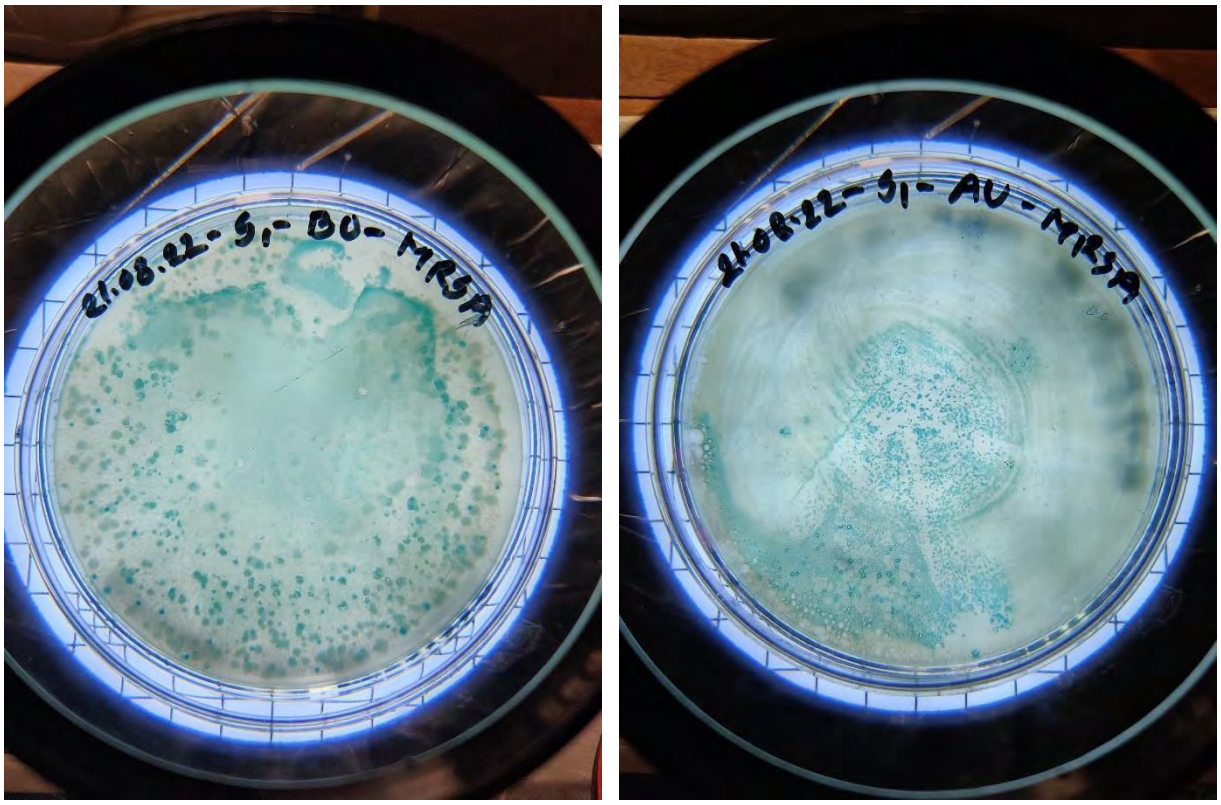

Figure 7: Bacterial growth differences in Chromatic MRSA Agar Base culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

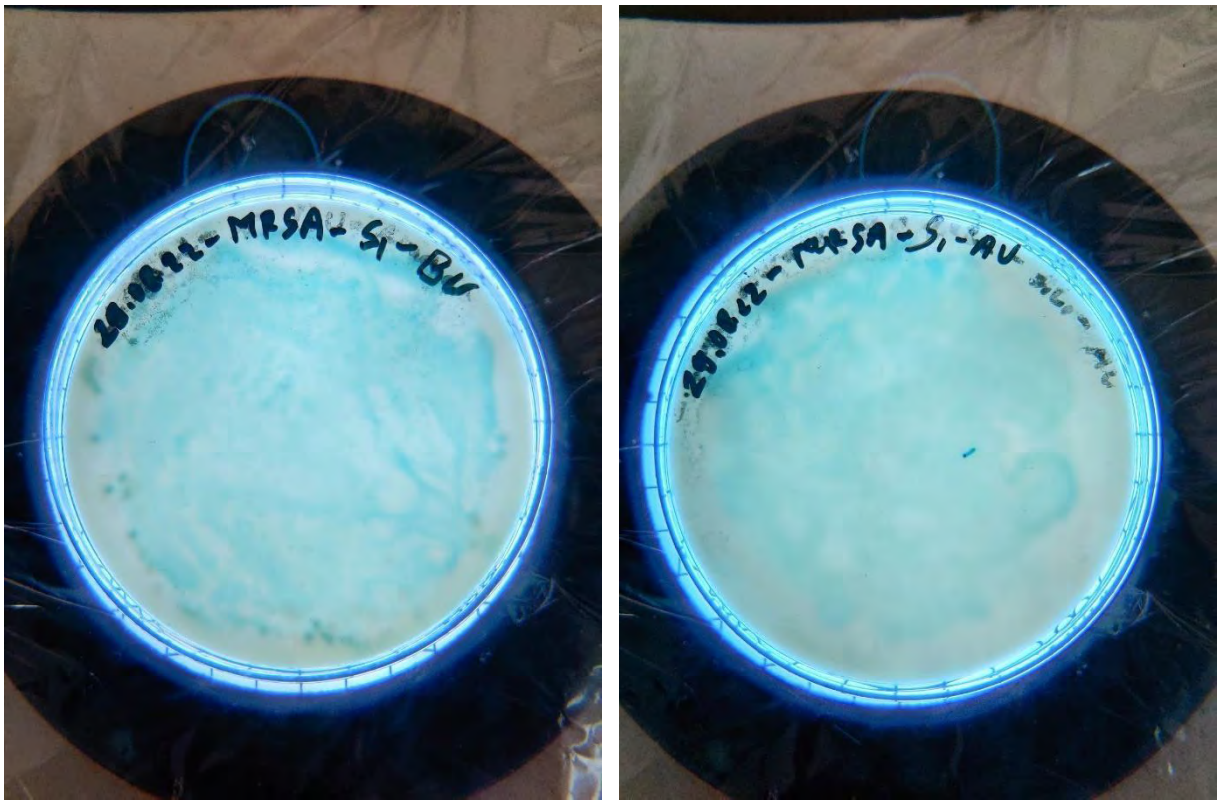

Figure 8: Bacterial growth differences in Chromatic MRSA Agar Base culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

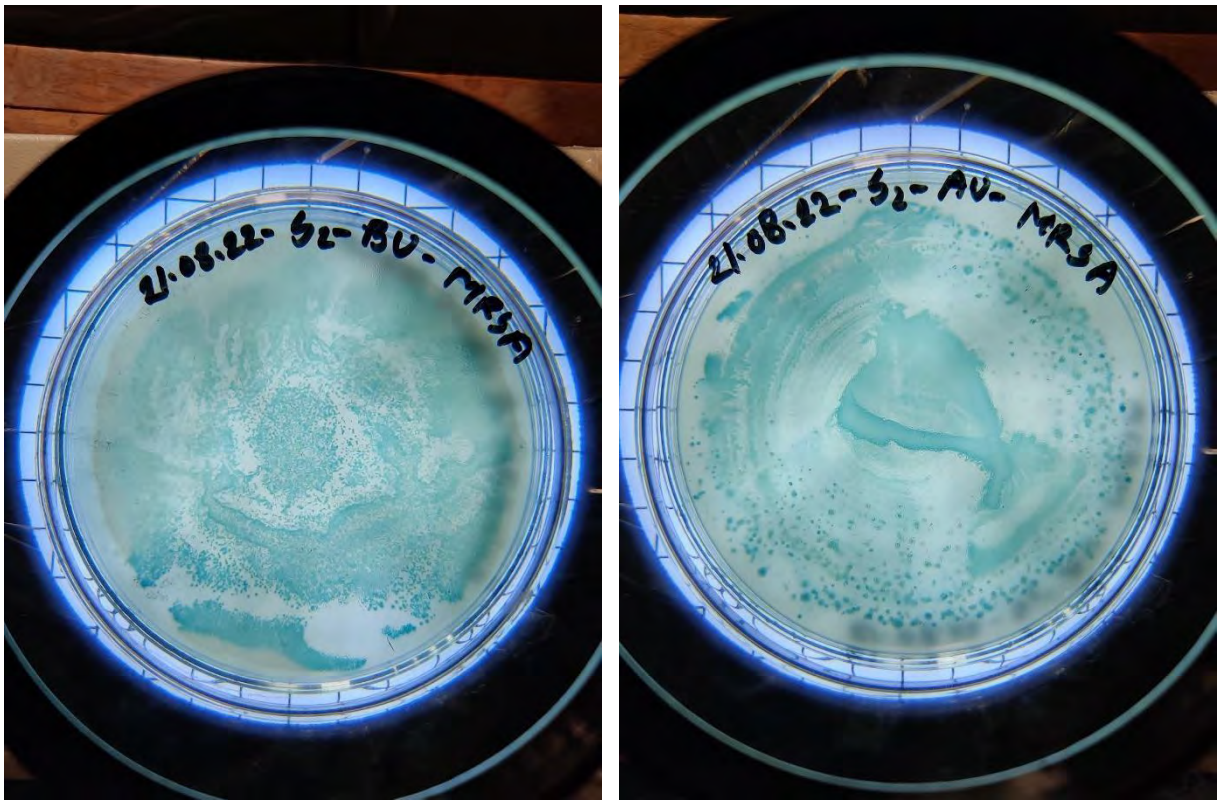

Figure 9: Bacterial growth differences in Chromatic MRSA Agar Base culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

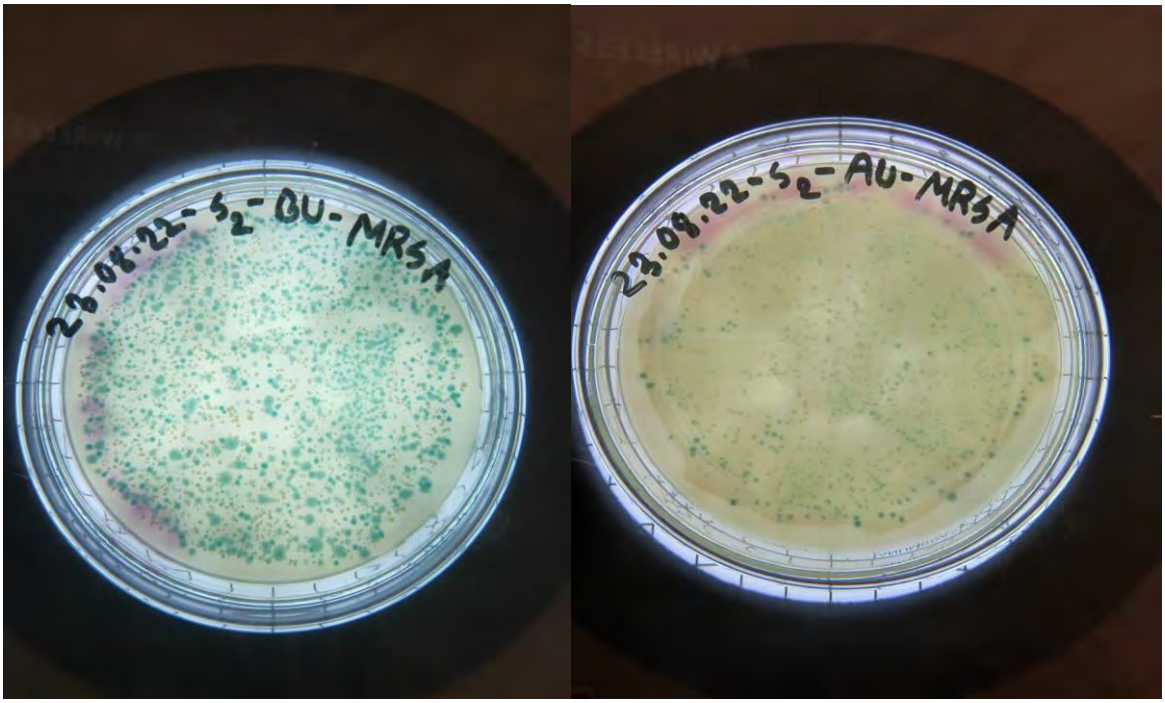

Figure 10: Bacterial growth differences in Chromatic MRSA Agar Base culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

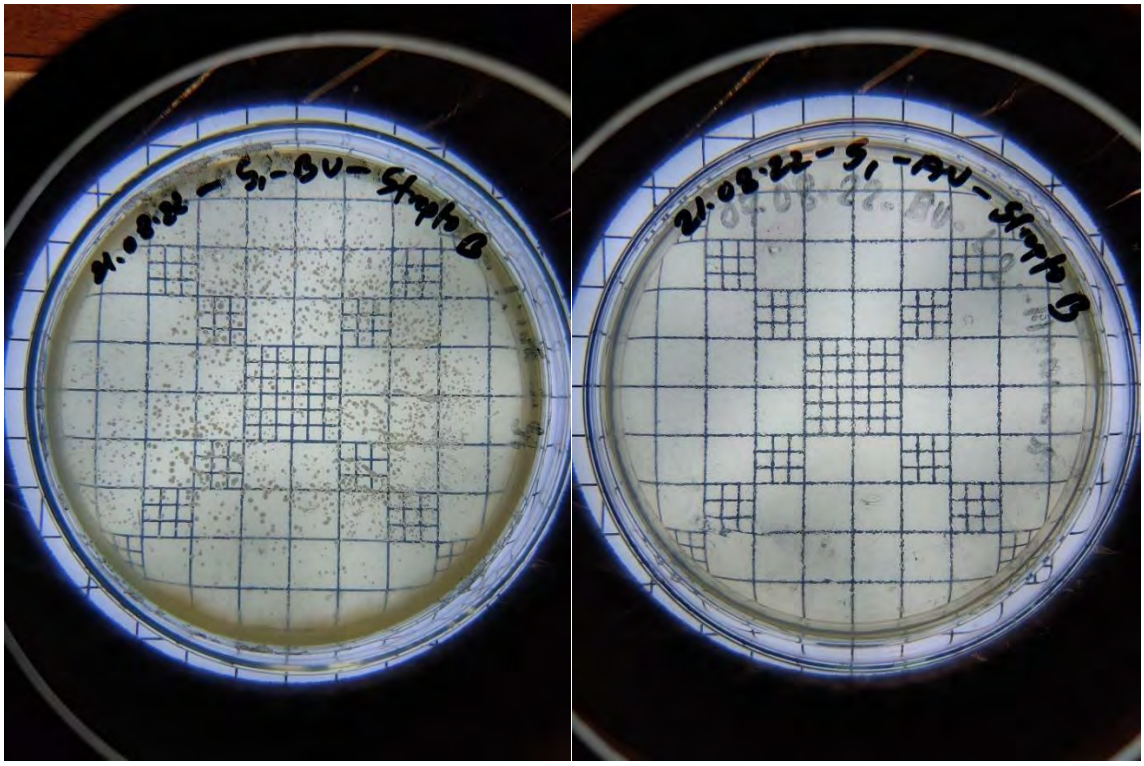

Figure 11: Bacterial growth differences in Chromatic Strepto B culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

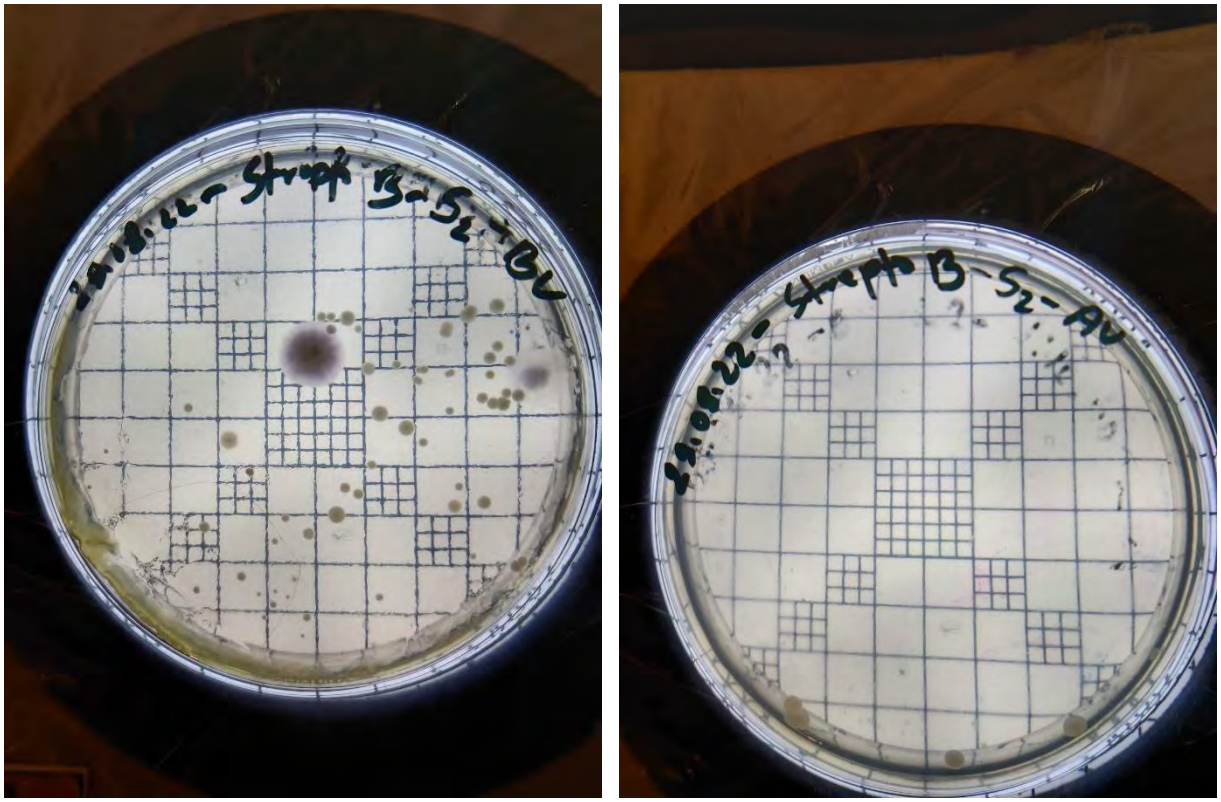

Figure 12: Bacterial growth differences in Chromatic Strepto B culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

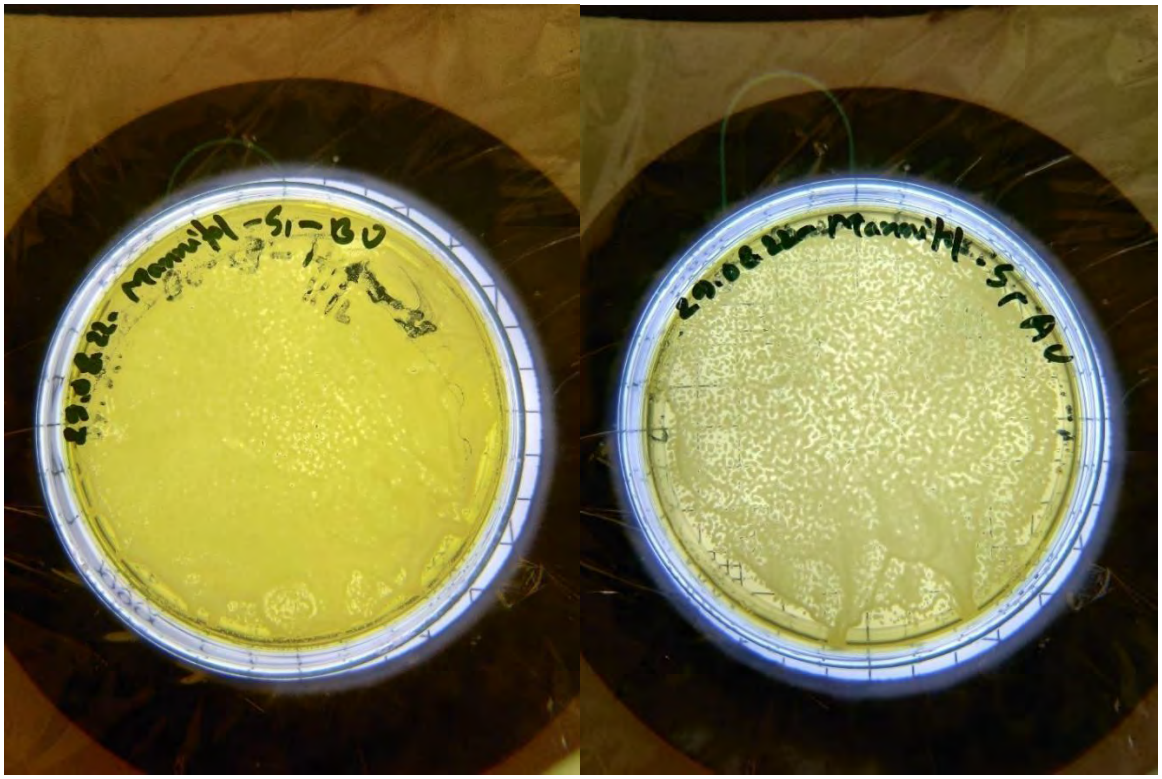

Figure 13: Bacterial growth differences in Mannitol Salt Agar culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

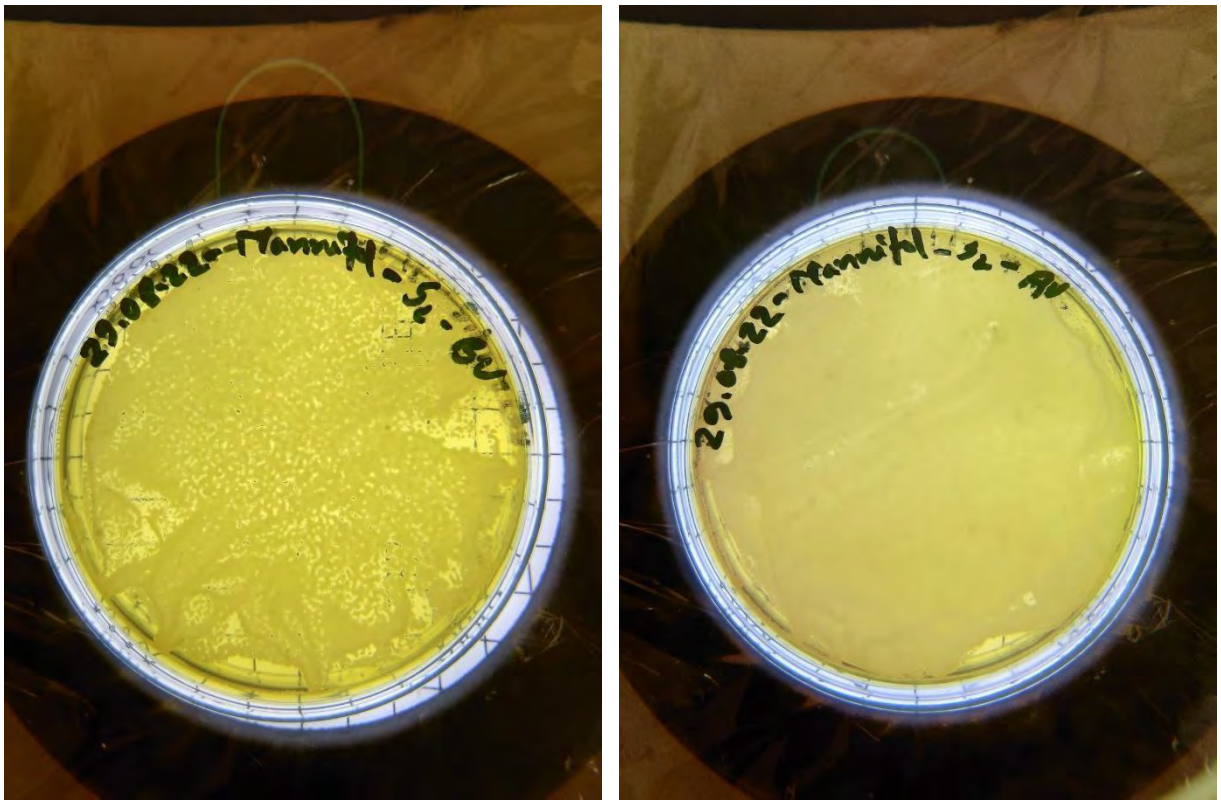

Figure 14: Bacterial growth differences in Mannitol Salt Agar culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

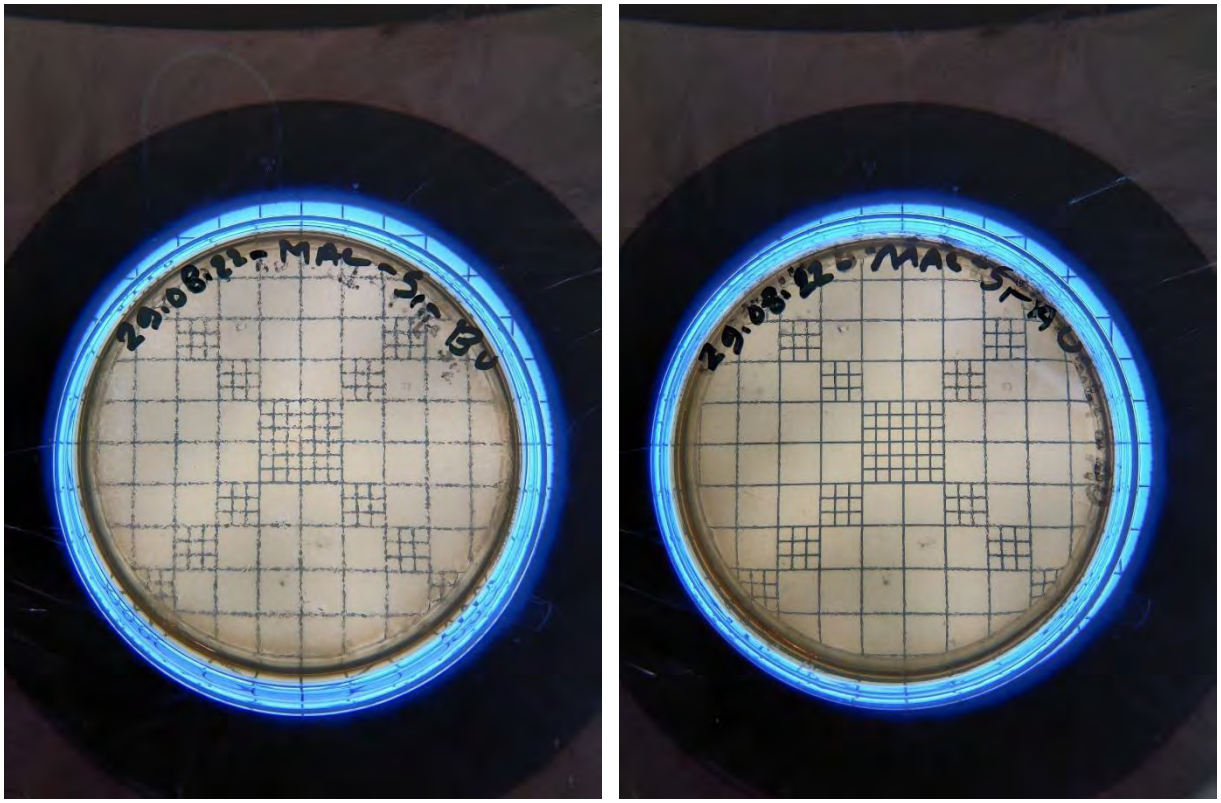

Figure 15: Bacterial growth differences in MAC CONKEY culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

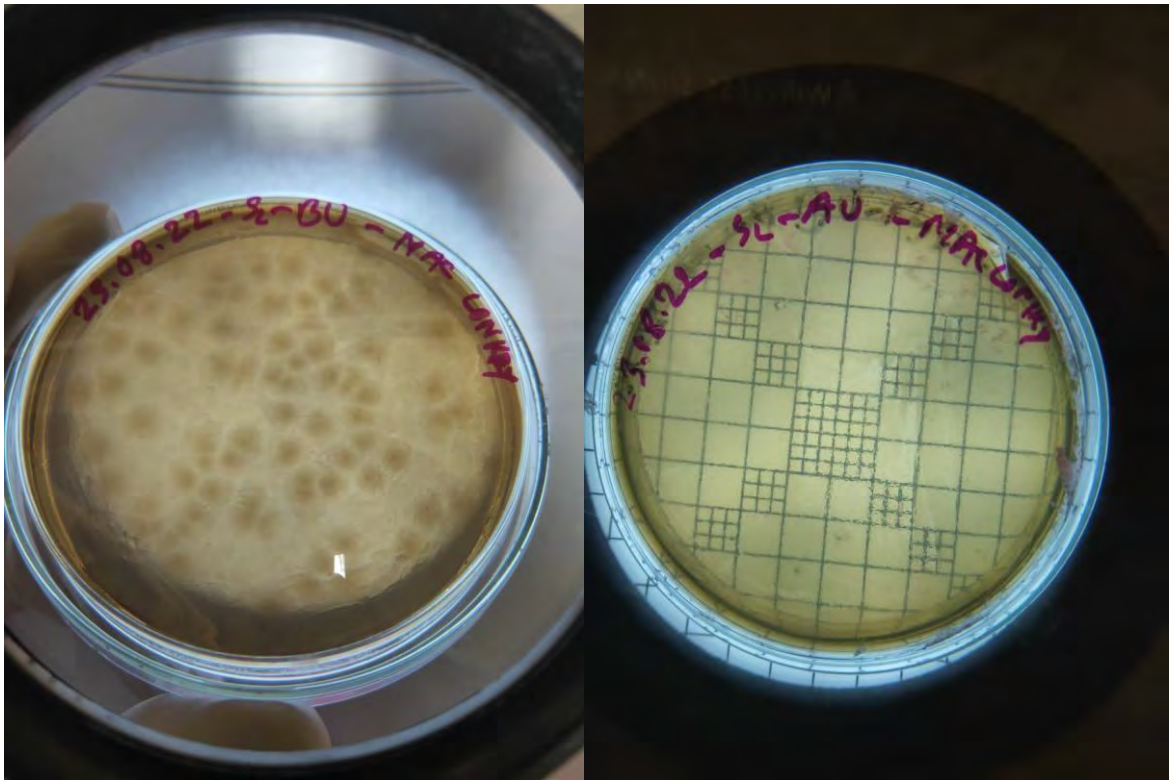

Figure 16: Bacterial growth differences in MAC CONKEY Culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

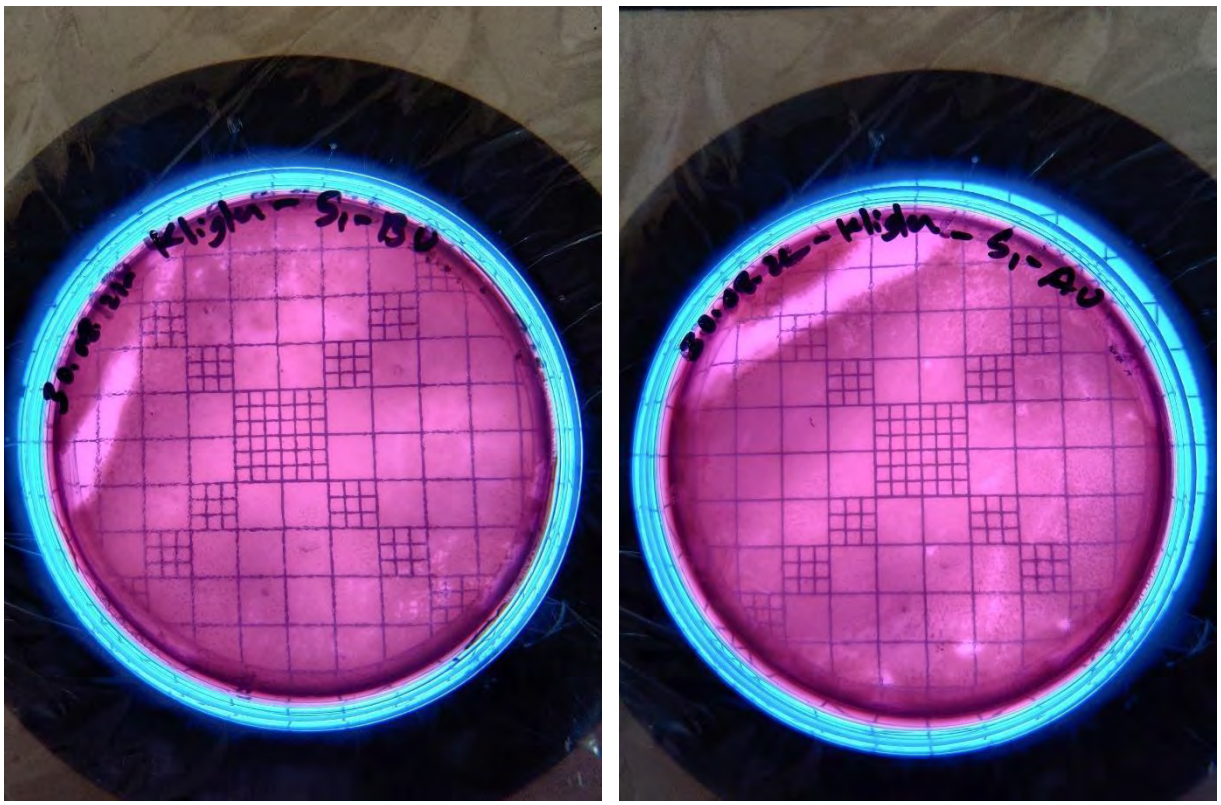

Figure 17: Bacterial growth differences in Kligler Iron Agar culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

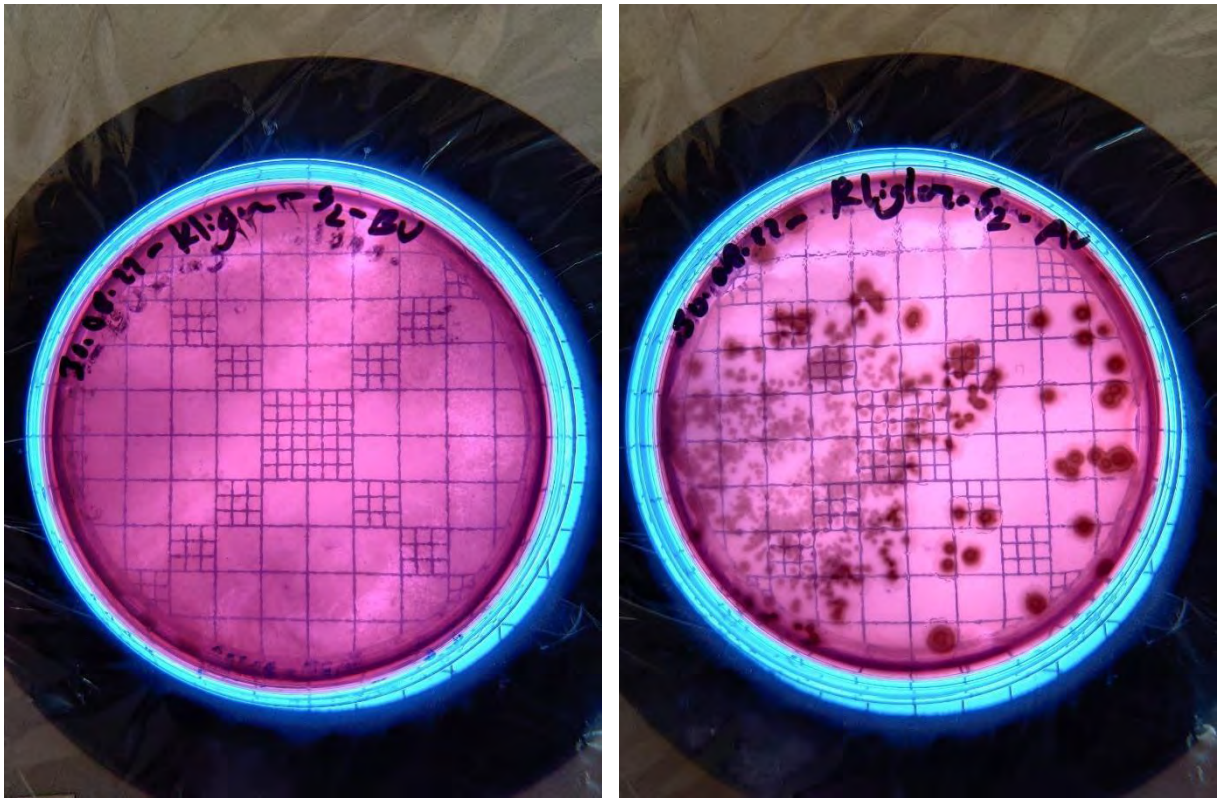

Figure 18: Bacterial growth differences in Kligler Iron Agar culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

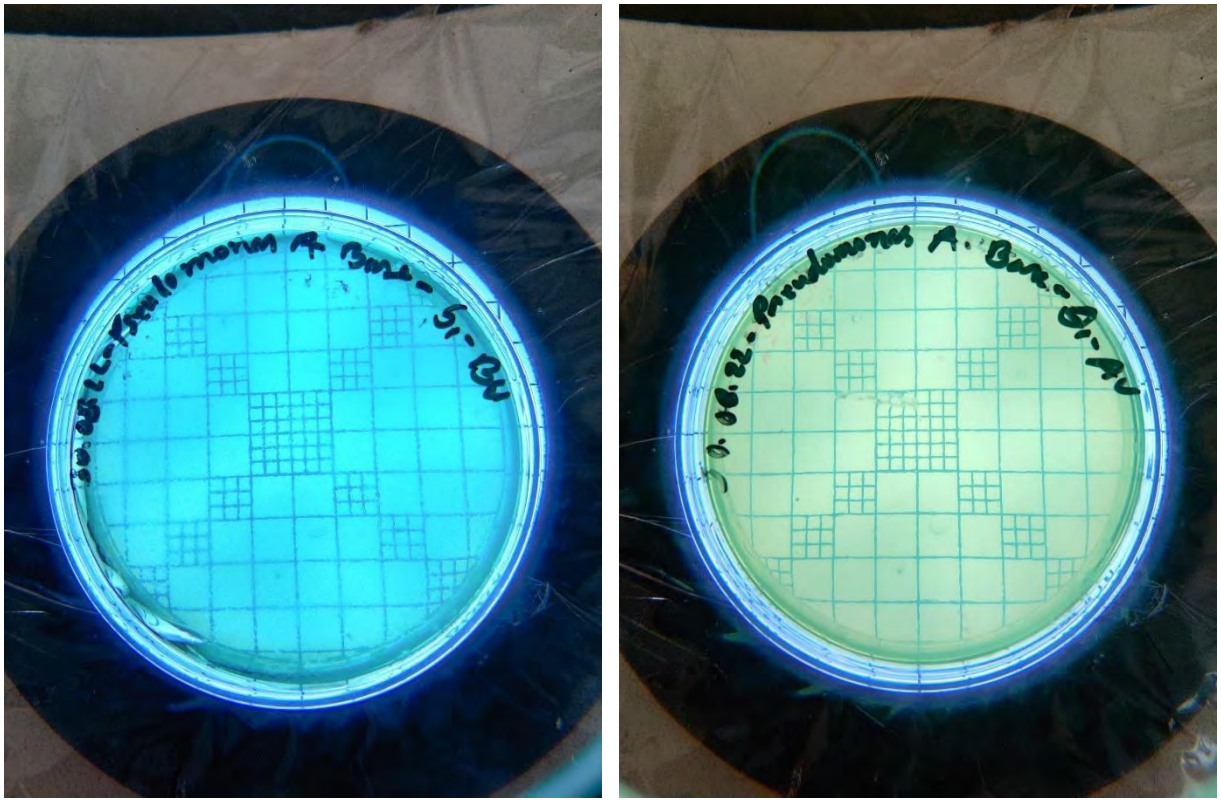

Figure 19: Bacterial growth differences in Pseudomonas Agar base culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

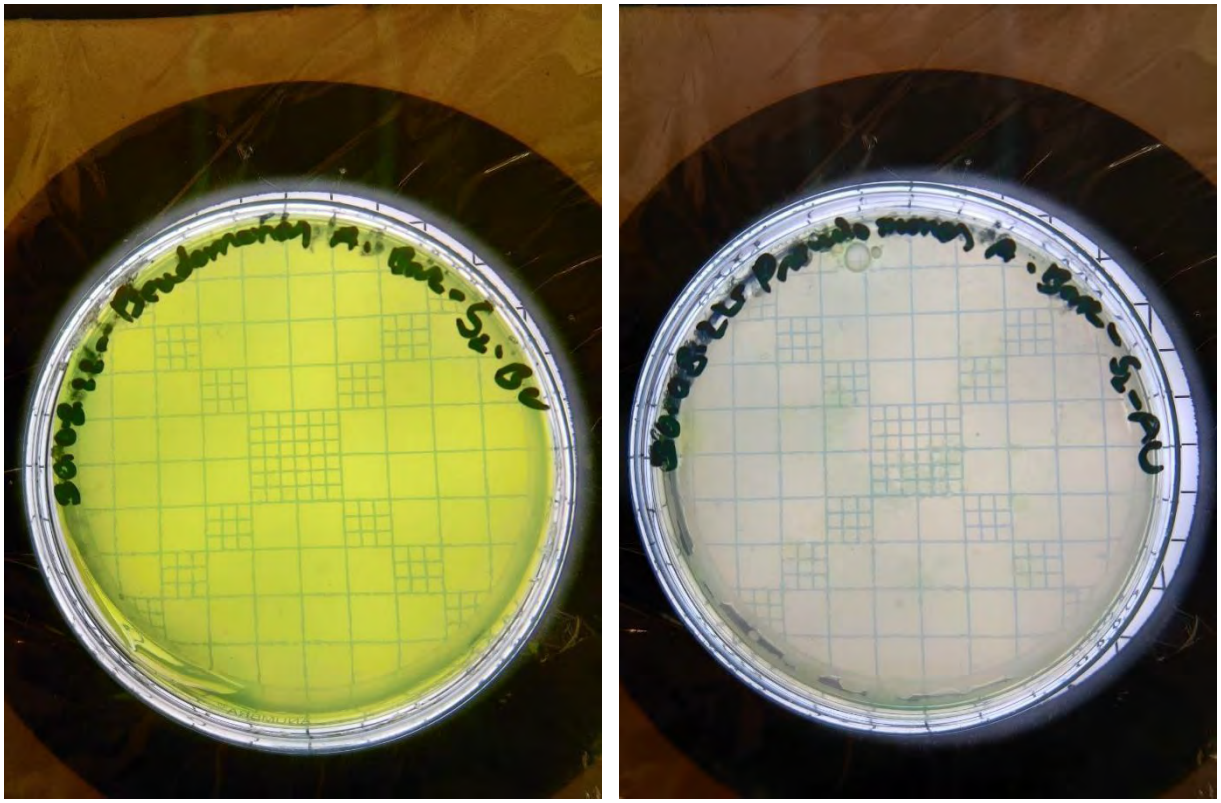

Figure 20: Bacterial growth differences in Pseudomonas Agar base culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

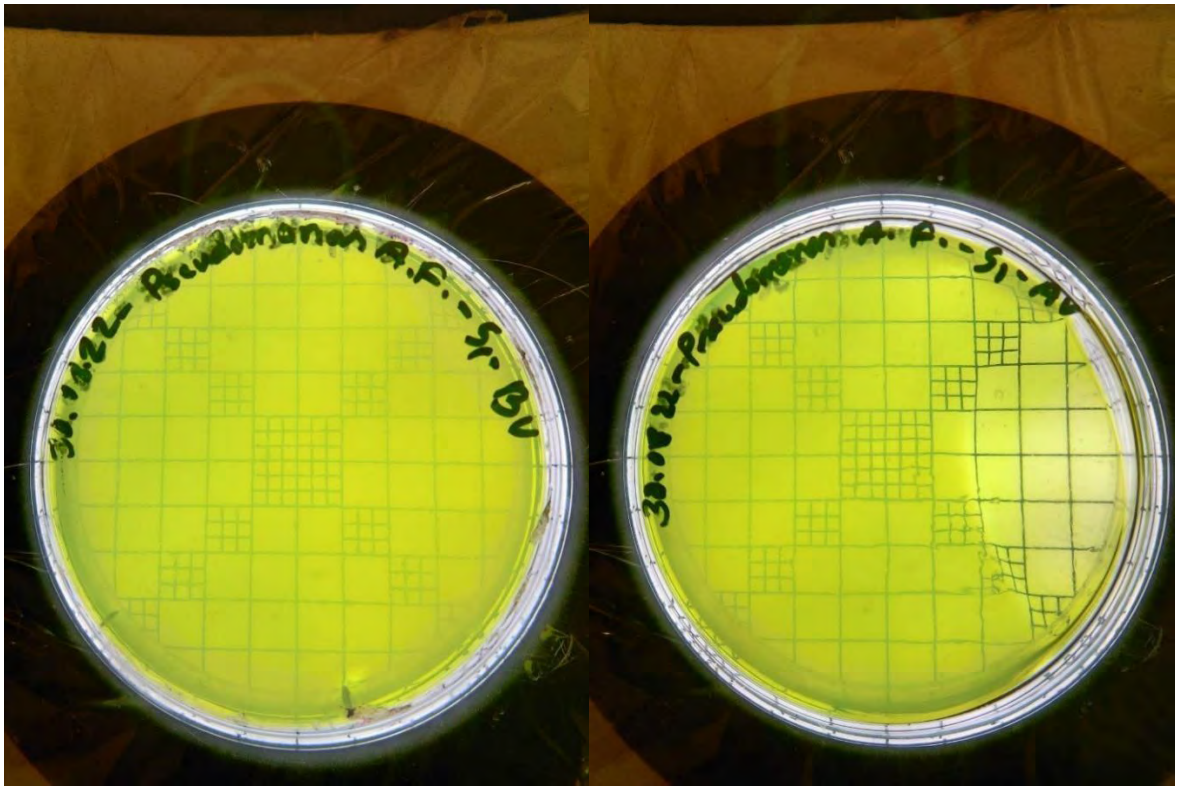

Figure 21: Bacterial growth differences in Pseudomonas Agar F culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

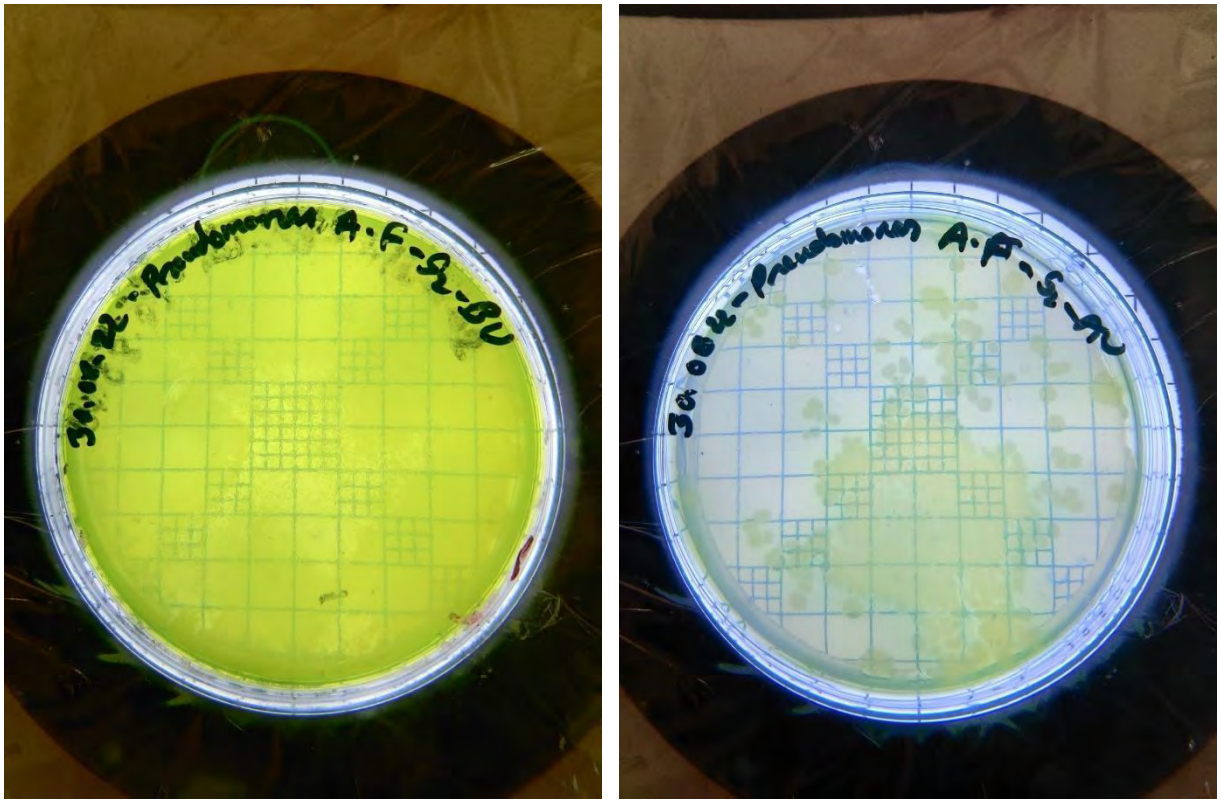

Figure 22: Bacterial growth differences in Pseudomonas Agar F culture media between Before UV and After UV. (Sample 2)

Before UV **After UV**

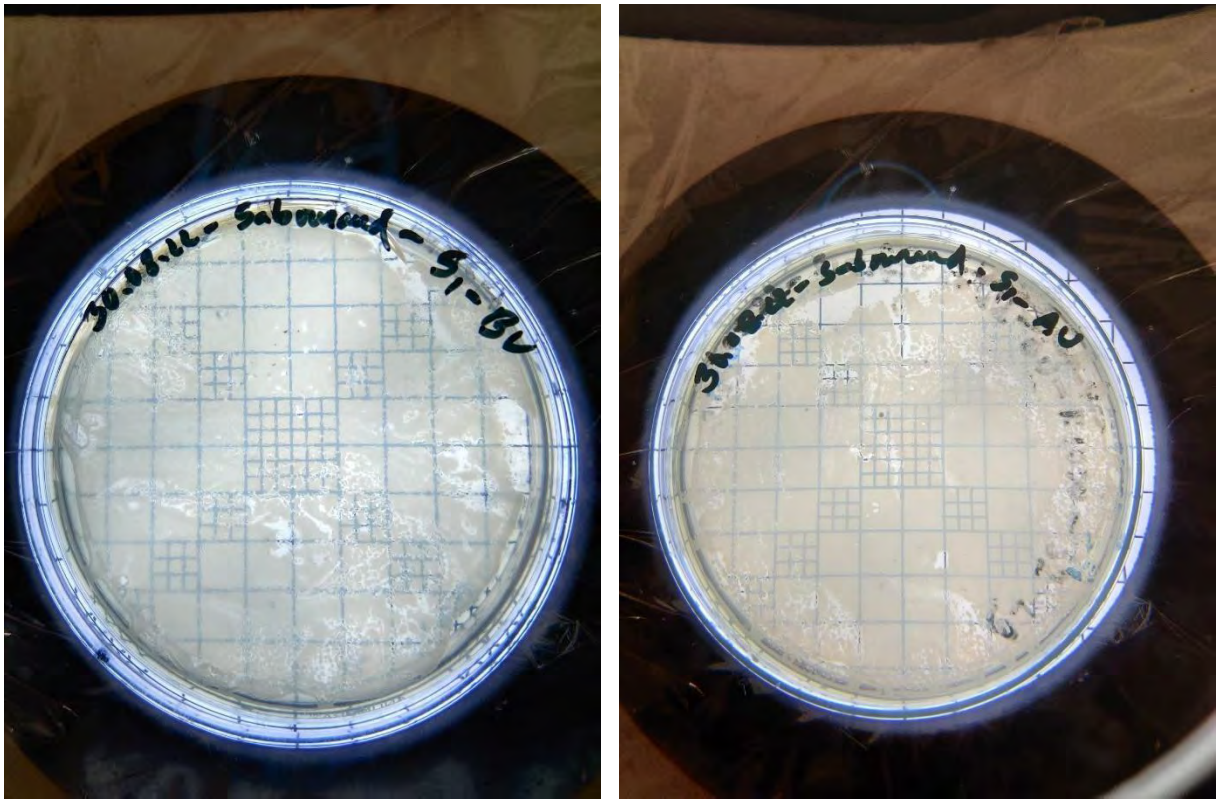

Figure 23: Bacterial growth differences in Sabouraud Dextrose Agar culture media between Before UV and After UV. (Sample 1)

Before UV **After UV**

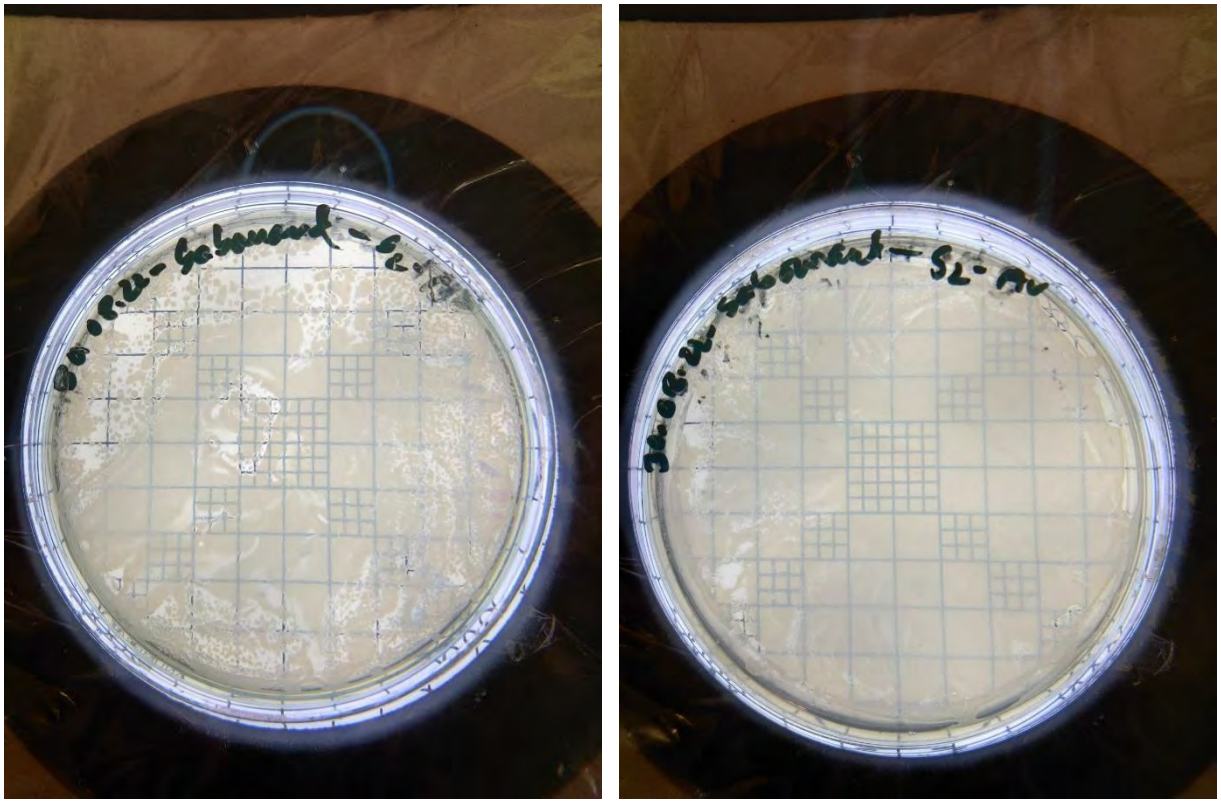

Figure 24: Bacterial growth differences in Sabouraud Dextrose Agar culture media between Before UV and After UV. (Sample 2)

Chapter 4

Discussion

Tryptic Soy Broth/TSB was our main transport media or storage media of our sample. By increasing or decreasing the TSB with the sample, we manipulate the dilution factor accordingly. Autoclave is the best way to kill microorganism by adjusting temperature and pressure avoiding any contact with the equipment. (Laboratory Animal Medicine, 2015). Vortex Mixer was used to mixed the sample with TSB thoroughly to maintain dilution factor equal in every area (Afanasenko, 2019). Incubator is famous in lab work to optimize desired bacterial growth (Cooper, 985). Laminar Air Flow Unit is required to maintain the atmosphere surrounding the area where we perform culturing to control cross contamination and other contamination. (McDade,1965). Microprocessor Colony Counter Instrument useful in analysing Bacterial or other microorganisms growing on a agar plate (Hogekamp, 2020)

Chromatic Staph Aureus Selective chromogenic medium for isolating most common Staphylococcus aureus (Merlino, 2000). Here, in Chromatic Staph Aureus agar, we found the presence of aureus bacteria in sample 1, before UV espoused. In after UV sample, the presence of species faded, the result is satisfactory. However, in case of sample 2, we observed that the presence of species found in after UV espoused sample, instead of before UV treated sample in 21.09.2022. We again performed the test in 29.09.22and got the same result again, which is misleading.

Chromatic Candida is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows (Jorjensen, 2015). For, Chromatic Candida agar, there is no presence of any microbes in both before and after UV espoused sample 1 and sample 2. So, we confirmed there is no presence of yeast.

Chromatic MRSA Agar Base used for the isolation of methicillin/oxacillin resistant Staphylococcus aureus (liofilchem, 2018). In case of, Chromatic MRSA Agar Base, we performed the test for sample 1 and sample 2 times (21.08.22 and 29.08.22). Although the results are same in each days but more visible and clear in 2nd days experiments that the 1st day. We found the presence of microbes more in before UV treated samples and low in after UV treated samples.

ChromaticTM Strepto B Selective chromogenic medium for the identification of all group B Streptococci (GBS) strains (liofilchem, 2013). Chromatic Strepto B, the result we got also desired here. After UV treatment the presence of microbe gone in both sample 1 and 2.

In case of Mannitol Salt Agar, Mannitol salt Agar This type of medium is both selective and differential. The MSA will select for organisms such as Staphylococcus species, capable of fermenting mannitol which will cause the pH indicator, phenol red, to turn yellow (Aryl, 2022). The presence of microbe increased after UV exposure in sample 1. However, in sample 2 the presence diminished satisfactorily after UV treatment.

MAC CONKEY II Agar is a selective and differential medium for the isolation and differentiation of Enterobacteriaceae and a variety of other Gram-negative rods from clinical specimens (BD, 2013). In MAC CONKEY II Agar base, there is no presence of enterobacteria in both before and after UV treated sample 1. But the presence of found in sample 2 which decreased after UV espoused.

Kingler Iron Agar used in identification of Enterobacteriaceae, determine H_2S produced by enteric gram-negative bacilli and H_2S produced by some strains of Pseudomonas.(Aryal, 2022) Kligler Iron Agar, sample 1 shows no presence of Pseudomonas in both case of before after UV treatment. Sample2 showed uniformity as the presence of microbe higher after UV treatment.

Pseudomonas Agar Base isolation of Pseudomonas species contains magnesium chloride and potassium sulphate to enhance pigment production (king, 1954). In Pseudomonas Agar base, bacterial growth decreased in both samples after UV exposure.

Pseudomonas Agar F with the help of glycerol acts as an activator for pigment production by Pseudomonas. (King, 1954). Pseudomonas Agar F, for sample 1, the bacterial growth did not observe in before and after UV treatment. But in sample 2, the growth dramatically decreases from before UV treatment to after UV treatment.

Sabouraud Dextrose Agar (SDA) is used for the isolation, cultivation, and maintenance of non-pathogenic and pathogenic species of fungi and yeasts (Aryl, 2022). Sabouraud Dextrose Agar, the presence of microbe's is gradually decreased in after UV treatment than before UV exposure for both sample 1 and sample 2.

Chapter 5

Conclusion

During biochemical testing we have able to ensure the presence of certain type of microorganisms in most of the cases. The results of those testing help us to compare the growths of microorganisms in terms of before UVC espoused sample to after UVC espoused samples. The comparison leads to a conclusion as how much effective the UVC exposures on those microorganism as well as paves path for future work. We can also confirm about the presence of certain microbes' presence on the surrounding air (hospital/home) of a patients affected in covid-19. Though this finding we take measure on air contamination prevention process. The findings also reveal some unusual pattern of decreasing or increasing microbes
in some cases. There might be various factors involved.

Chapter 6

Future work

In this experiment we followed traditional way of biochemical testing to find out the presences of certain microorganisms. Although we got our desired result comparing both before and After UV espoused samples, while performing the test we observed some difficulties like- our method is time consuming and lot of works need to done in a short time: while plating and pipetting samples there was a huge probability of contamination, any slight change in air or pressure may lead to error, sometimes sensitive microorganisms identification become difficult due to slight change in incubation period or spreading techniques (Tshikhudo, 2013). To avoid those circumstances and get more precise and accurate result faster we would like to use mod, new automated md to identify microorganism.

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