



## CHAPTER 10:

# DEVELOPMENT OF AN ULTRAVIOLET C IRRADIATION DEVICE: INITIAL FINDINGS FOR THE TREATMENT OF SPIKED WHOLE BLOOD

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## ABSTRACT

*Sepsis is a life-threatening condition that occurs when the body's response to a source of infection is damaging to its own tissues. Sepsis is responsible for substantial health loss worldwide, particularly in sub-Saharan Africa. Increasing rates of antibiotic-resistant bacteria severely reduce the ability of physicians to treat the underlying infection that causes sepsis, especially when the infection is multidrug-resistant. Ultraviolet irradiation of the blood was historically used to treat infections.*

*This project explores this therapy as a novel treatment modality for multidrug-resistant sepsis through the development of a device to perform ultraviolet irradiation of the blood. This study reports on the initial findings of the in-vitro irradiation of bacterial-spiked, expired, whole blood with ultraviolet light. Spiked whole blood was exposed to ultraviolet light under different experimental conditions using a custom-made testing rig. The initial findings have suggested that the inactivation of bacteria in whole blood may be dependent on a variety of conditions. Further work is being done within this project to identify and quantify these conditions. These findings will then be utilised to guide further testing and development of the device design proposed in this project.*

Keywords: sepsis, multidrug-resistant bacteria, whole blood, UVC inactivation, medical device

## **INTRODUCTION**

Sepsis is a life-threatening condition that occurs when a host's dysregulated response to an infectious organism causes organ dysfunction (Singer et al., 2016). No sepsis-specific treatments currently exist beyond the use of antibiotics (Cohen et al., 2015). Antibiotic therapy is currently the key to reducing morbidity and mortality of sepsis (Thompson, Venkatesh, & Finfer, 2019). However, there is concern that the rate of antibiotic development may not be adequate to address the increasing levels of multidrug-resistant bacteria found in sepsis cases (Goldstein et al., 2019).

Antibiotic resistance has been associated with negative outcomes for patients with sepsis. Sepsis caused by multidrug-resistant bacteria is associated with higher mortality in patients (Capsoni et al., 2019). Additionally, patients with infections that are complicated by multidrug-resistance are more likely to have longer and more costly hospitalisations (Adams, Susi, & Nylund, 2019), highlighting a need for a novel treatment modality for multidrug-resistant sepsis.

Ultraviolet blood irradiation, where blood is exposed to ultraviolet C (UVC) light outside the body, was a therapy used to treat infections over 80 years ago (Hamblin, 2017). During treatment, blood was withdrawn from the body, exposed extracorporeally to ultraviolet light, and then reinfused back into the patient. Multiple studies in this era reported the resolution or marked improvement of patients with sepsis under ultraviolet blood irradiation treatment (Miley & Christensen, 1947; Rebbeck, 1942, 1951).

The treatment of patients with multidrug-resistant sepsis is a complex problem. However, the effective treatment of spiked blood products could potentially indicate the suitability of a method as a treatment for multidrug-resistant sepsis. Thus, a device for the treatment of infected blood products is under development in this project to evaluate the suitability of UVC irradiation as a therapeutic modality for multidrug-resistant sepsis.

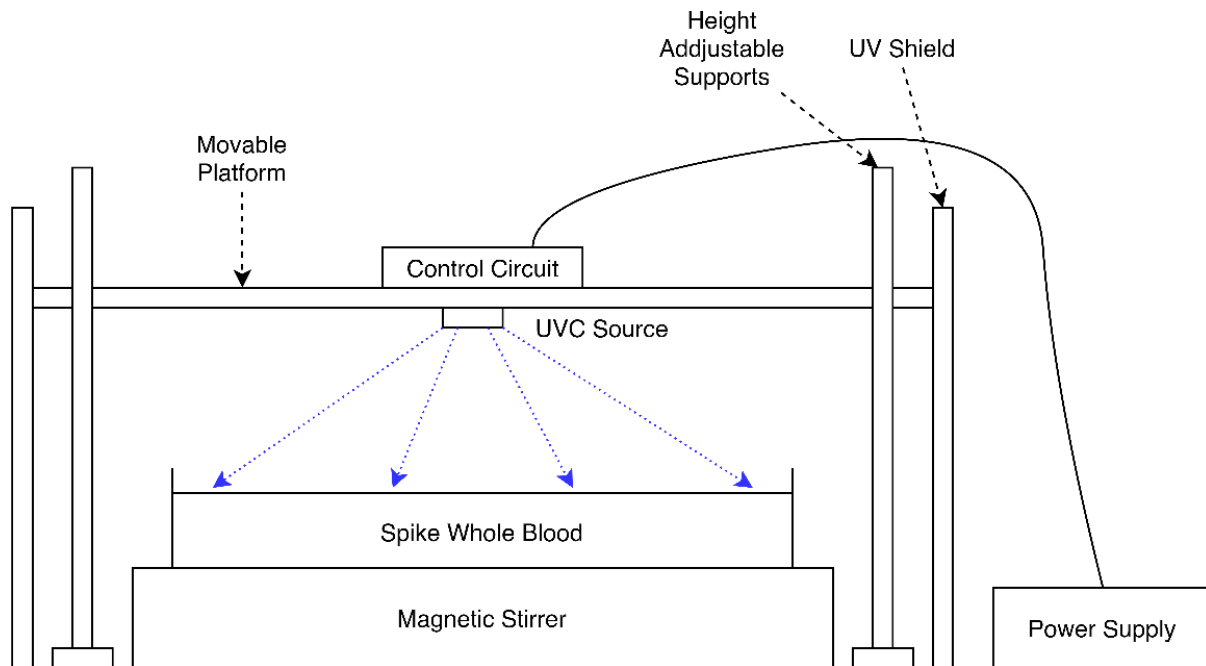


Figure 1: Experimental setup used during the irradiation experiments.

## MATERIALS AND METHODS

Approvals from both the Human Research Ethics Committee (411/2021) and the Health Sciences Faculty Biosafety Committee (SS/001a/2021-2024) of the University of Cape Town were obtained before initiating the testing in this project.

### i. Material Sourcing and Preparation

Expired whole blood was obtained from the Western Cape Blood Services and stored between 2°C and 6°C at the Division of Medical Microbiology Laboratory, at the University of Cape Town. Trypticase soy agar (TSA, Biolab) was prepared according to the manufacturer's instructions. *Staphylococcus aureus* ATCC25923 was used to spike the whole blood. The *S. aureus* isolate was maintained on Brucella agar containing horse blood. For the experiments, the isolate was streaked on blood agar plates and incubated overnight (for 18 to 20 hours). Bacterial cells from the overnight colonies were resuspended in phosphate-buffered saline (PBS) to obtain a concentration of approximately  $10^6$  colony forming units per ml (CFU/ml).

### ii. Experimental Setup and Method

A testing rig (Figure 1) was developed to provide a UVC source for the irradiation experiments. The testing rig's platform was adjusted so that the UVC source was 20mm above the surface of the blood. During the experiments, the spiked blood was placed in a Petri dish and stirred on a magnetic stirrer. The experimental conditions for each of the conducted experiments are summarised in Table 1. For each test, samples were taken from the blood before irradiation, after irradiation (plated immediately) and at an additional sampling time after irradiation (Figure 2).

Table 1: Summary of the experimental conditions used for each successive experiment.

Experimental Condition	Experiment 1	Experiment 2	Experiment 3
Quantity of Blood Used	25ml	25ml	5ml
Magnetic Stirring	200rpm	200rpm	Off
Blood Age	55 days	61 days	67 days
Irradiation Duration	100 s	300 s	300 s
Approximate UVC Dose	20 mJ/cm <sup>2</sup>	60 mJ/cm <sup>2</sup>	60 mJ/cm <sup>2</sup>
Additional Sampling Times	90, 180 seconds	30 mins	30 mins
Control Medium	-	PBS	Trypticase Soy Broth

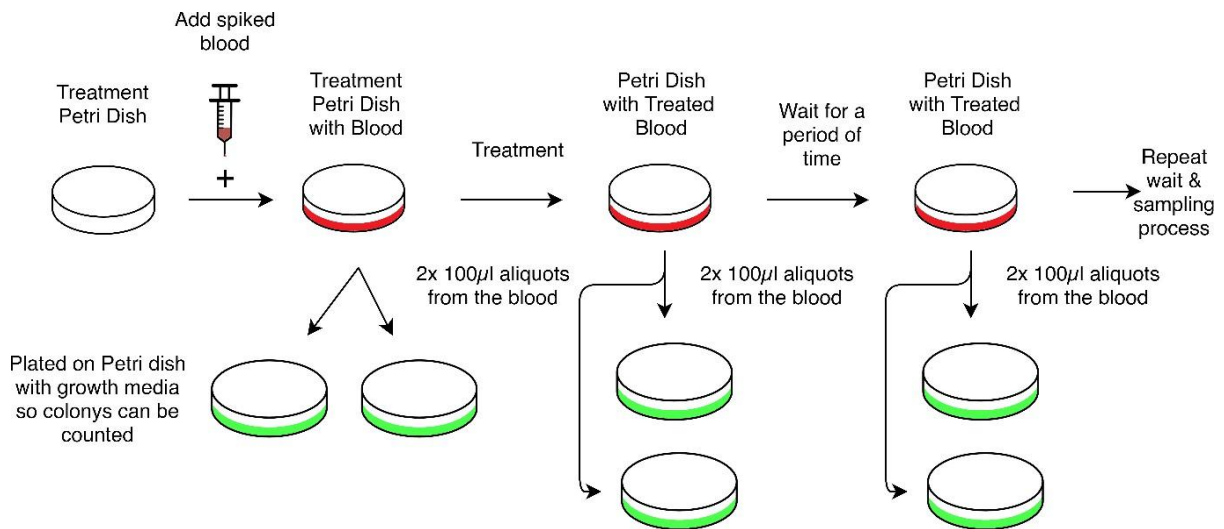


Figure 2: Flowchart of the experimental procedure.

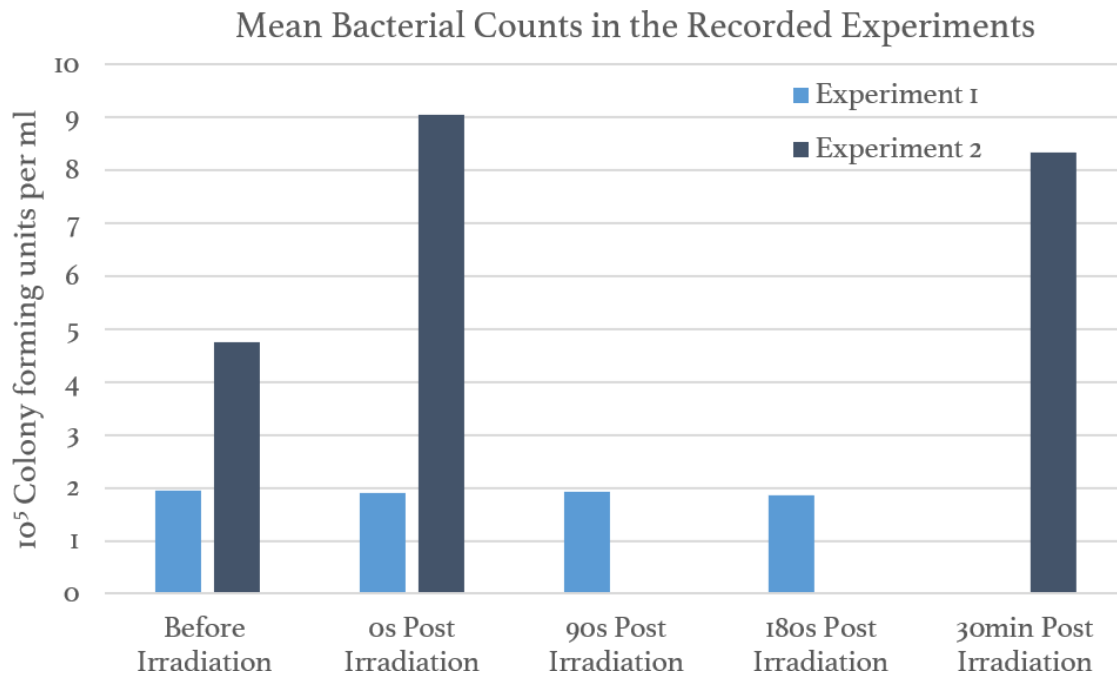
When sampling the blood for measurement, aliquots were serially diluted in PBS and plated in duplicate technical repeats on TSA plates. The TSA plates were incubated at 37°C under aerobic conditions for 24 hours. Colony forming units were then manually counted and recorded. The final colony counts were determined by the average of plates with between 30 and 300 colonies for each sample.

## RESULTS AND DISCUSSION

Firstly, these results are from a series of preliminary tests and are intended for internal testing of the methodology and equipment. Thus, any insights and conclusions should be considered preliminary pending further investigation. The blood samples in Experiment 3 were contaminated with other microbes and, thus, there were no quantitative results from Experiment 3. The results from the other two recorded experiments are presented in Figure 3.

Overall, the results in Figure 3 that there was no substantial reduction in the quantity of viable bacteria in the sample either after irradiation or after waiting for an additional delay post- irradiation as specified in Table 1.

Additionally, while the samples in Experiment 3 were contaminated and not counted, it was noted that there was not any discernible qualitative reduction in the quantity of colony growths on the plates. The trypticase soy broth control in Experiment 3 was not contaminated, but also showed no inactivation of bacteria. However, there was complete inactivation of bacteria in phosphate-buffered saline (PBS) control in Experiment 2.



*Figure 3: Mean bacterial counts in the whole blood samples at different time points during the experiments conducted in this study. Experiments 1 and 2 had different post-irradiation sampling times. The mean CFU/ml was determined by averaging the plates with between 30 and 300 colonies for each blood sample.*

The inactivation of *S. aureus* has been demonstrated by other studies in various mediums under different conditions (Kim & Kang, 2018; Terpstra et al., 2008). The PBS control in Experiment 2 demonstrated the capability of the experimental setup to inactivate *S. aureus* under the conditions described in Table 1. Despite this, the bacteria were not inactivated in the whole blood samples or the trypticase soy broth control. This suggests that compounds in these mediums could be absorbing the UVC light and impacting the bactericidal effect of the UVC light.

One of the developers of the historic ultraviolet blood irradiation therapy reported the ability of UVC light to inactivate *S. aureus* in whole blood (Knott, 1948), and the therapy developed from his work was subsequently used to treat *S. aureus* infections (Rebeck, 1942).

Therefore, the lack of inactivation seen so far in this project could be due to the combination of conditions used in these experiments. The experimental conditions which could be affecting the inactivation results are listed in Table 2.

*Table 2: List of experimental conditions which could affect the inactivation of bacteria in whole blood*

<b>Key Conditions of Interest</b>		<b>Additional Conditions</b>
UVC dose	UVC wavelength	Quantity of blood used
Age of blood	Quantity of bacteria	Duration for additional sampling
Container and blood flow characteristics during irradiation		Temperature of blood

The key conditions of interest all have a large potential to affect the experimental outcome. As it is hypothesised that ultraviolet blood irradiation produces some form of an immune-modulating effect (Boretti, Banik, & Castelletto, 2020), the age of the blood could make a significant difference to the results. This is because various cells of the blood break down over time which could change the immune response of the blood to the UVC radiation. While spiking the blood with high quantities of bacteria ( $10^6$  CFU/ml) allows for a measurement of the log inactivation achieved by the device, clinically relevant levels of bacteria are much lower at 1-100 CFU/ml (Lamy, Dargère, Arendrup, Parienti, & Tattevin, 2016; Reimer, Wilson, & Weinstein, 1997). Thus, changing the quantity of bacteria used to spike the blood could also affect the results. Finally, the wavelength of UVC light, dose of UVC light, the container holding the blood, and the way the blood flows in the container could all affect how the light is absorbed by the blood and, thereby, the inactivation results. The ongoing work in this project will vary these conditions of interest to attempt to determine the conditions required to inactivate bacteria in whole blood.

## **CONCLUSION**

The initial findings of this project have shown the ability of the experimental setup to inactivate *S. aureus* in phosphate-buffered saline in an initial test. However, the lack of bacterial inactivation in the blood samples suggests that the inactivation of bacteria in whole blood may be dependent on a variety of conditions. Further work is being done to identify the relevant conditions and then quantify the effect of these conditions. These findings will then be utilised to guide the further testing and development of the device design proposed in this project.

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