

## IN VITRO ANTIMICROBIAL ACTIVITY OF UV LED 405 NM AS PATHOGEN INACTIVATION ON HUMAN PLASMA CONTAMINATED BY PSEUDOMONAS AERUGINOSA

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### Abstrak

Kontaminasi bakteri dapat berasal dari berbagai sumber, termasuk kulit donor yang tidak aseptik, bakteremia donor, dan proses pengolahan produk darah. Dibandingkan dengan infeksi virus, kasus kontaminasi bakteri memiliki risiko lebih tinggi untuk penularan infeksi melalui transfusi darah. Infeksi *Pseudomonas aeruginosa* telah didokumentasikan dalam kasus infeksi aliran darah, infeksi saluran kemih, dan infeksi tempat operasi. Beberapa penelitian telah mengkaji potensi penggunaan UV-LED 405 nm (Ultra Violet - Light Emitting Diodes) sebagai pilihan tanpa perlu photosensitizer, dan dapat diterapkan dengan aman pada manusia. Studi ini menggunakan desain eksperimen sejati dan dilakukan pada Agustus hingga September 2023 di Laboratorium Teknologi Bank Darah STIKES Rajekwesi Bojonegoro, Indonesia. Sampel yang digunakan adalah bakteri *Pseudomonas aeruginosa* ATCC 27853 dan plasma dari donor darah dengan desain kelompok kontrol hanya pada uji pasca. Ketika paparan UV LED diterapkan pada plasma yang terkontaminasi dengan *P. aeruginosa*, terdapat perbedaan yang signifikan dalam pertumbuhan koloni pada kultur NA. Pertumbuhan bakteri berkurang seiring dengan peningkatan waktu paparan cahaya UV LED, dan ada perbedaan yang signifikan antara kelompok yang terpapar UV LED dan kelompok kontrol (nilai  $p < 0,05$ ). Setelah 60 menit paparan, terjadi penurunan sebesar 90% dalam pertumbuhan bakteri dibandingkan dengan kelompok kontrol, yang tidak menerima paparan UV LED 405 nm.

**Keywords:** *Pseudomonas aeruginosa*, plasma, pathogen inactivation, UV LED 405nm

### Abstract

Origins of bacterial contamination may arise from non-aseptic donor skin, donor bacteremia, and the processing of blood products. Blood transfusions spread infections more often from cases of bacterial contamination than from viral infections. *Pseudomonas aeruginosa* infections have previously been connected to surgical site, urinary tract, and bloodstream infections. Without the need for additional photosensitizers, several studies have been able to advance the use of 405 nm UV-LEDs (Ultra Violet Light Emitting Diodes) as a viable option that can be applied to people in a safe manner. The Blood Bank Laboratory of STIKES Rajekwesi Bojonegoro, Indonesia, hosted this study from August to September 2023, employing a true experimental design. The sample was the bacterium *Pseudomonas aeruginosa* ATCC 27853 and Liquid Plasma from Blood Donor using Posttest-Only Control. The exposure of UV LED light on Contaminated Plasma with *P. aeruginosa* showed different count of colony growth on NA culture. The bacterial growth decreased following the time exposure to UV LED light and showed significant difference between the treated groups and control ( $p$  value  $< 0.05$ ). The reduction in 60 minutes exposure reached 10% when compared to the control group, where the control group was not exposed to 405 nm UV LED.

**Keywords:** *Pseudomonas aeruginosa*, plasma, pathogen inactivation, UV LED 405nm

## 1. INTRODUCTION

Ultraviolet light-emitting diodes (UV-LEDs) are small, mercury-free devices with a flexible and adjustable design. UV-LEDs can be used without a warming up period,

enabling diverse application of this device such as on-demand operation. The effectiveness of UV-LEDs at various wavelengths for water disinfection has been demonstrated in many studies, with most studies investigating surrogate micro-

organisms such as the indicator bacterium *Escherichia coli*; indicator viruses such as bacteriophage MS2, Qb, and T7; and aerobic spore-forming bacteria (Ragupathy et al., 2022; Rattanakul & Oguma, 2018; Stewart, Ralston, et al., 2022).

Origins of bacterial contamination may arise from non-aseptic donor skin, donor bacteremia, and the processing of blood products (Btari et al., 2020). In addition, environmental conditions during storage, procedural methodologies involving porous bag processing, and the incorporation of preservatives into storage receptacles can serve as potential energy sources for bacteria, thereby promoting enhanced growth of contaminating bacterial strains (Benjamin et al., 2017; Jones et al., 2018.).

Cases of bacterial contamination have a higher risk of infection transmitted through blood transfusions than viral infections (Li et al., 2021). Moreover, bacterial contamination stands as the second most prevalent cause of mortality, primarily attributed to the heightened risk of bacterial sepsis associated with transfusions (Lanteri et al., 2020). This correlation extends to recipients of transfusions who exhibit immunosuppressive conditions, further amplifying susceptibility to bacterial contamination and subsequent risks of bacterial sepsis. Previous studies revealed that gram-positive and gram-negative bacteria were known to be present in 9.2% of 196 blood products. (Stewart, Ralston, et al., 2022).

The identification results showed that there were *Staphylococcus*, *Bacillus sp.*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* bacteria in the stored blood products. More than 50% of the bacteria detected in plasma blood products are gram-positive bacteria which can cause transfusion reactions, while gram-negative bacterial contamination is typically less prevalent; however, when it does occur, there is an elevated risk of transfusion-related complications and potential fatality (Maclean et al., 2016).

Several bacterial species that have been isolated in liquid plasma products include *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus caprae*, *Micrococcus luteus*, and *Acinetobacter*

*lwoffii* (Da Silva et al., 2023). *Pseudomonas aeruginosa* is a Gram-negative bacteria classified as an opportunistic pathogen because it cause a variety of infections in both immunocompetent and immunocompromised people, and can transmitted during transfusion (Li et al., 2021).

*P. aeruginosa* is also a microbe that exhibits high resistance to a great number of conventional antibiotics and disinfectants. during the procedur in Blood Donor Unit(Rattanakul & Oguma, 2018). Disinfectant is a must instrument to apply before blood donor started. Biofilm formation makes it difficult for residual chlorine to diffuse and inactivate microorganisms in inner layers, and can result in recontamination of drinking water (Petrini et al., 2017).

Infections caused by *Pseudomonas aeruginosa* have been documented in cases of contaminated drinking water ingestion, with more severe infection incidents primarily occurring within hospital settings (Bahar-e-Mustafa et al., 2021). These infections manifest as pneumonia, bloodstream infections, urinary tract infections, and surgical site infections. The Centers for Disease Control and Prevention (CDC) in the United States reported 51,000 infections of *P. aeruginosa* per year with approximately 6700 cases of multi-drug resistance resulting in 400 deaths per year (CDC, 2013). As a result, the World Health Organization (WHO) has listed *P. aeruginosa* as a critical priority (WHO, 2017).

Numerous strategies have been suggested to address *Pseudomonas aeruginosa*, targeting molecular or cellular structures (Mullaiselvan et al., 2020). One such approach is Photodynamic Antimicrobial Chemotherapy (PACT), a technique involving the generation of Reactive Oxygen Species (ROS) through the interaction of light, a photosensitizing molecule, and oxygen (Ragupathy et al., 2022). The photosensitized molecule undergoes an excited state transition at a particular light wavelength, resulting in the production of singlet oxygen. This singlet oxygen is highly reactive with cellular components, ultimately causing cellular

demise through oxidative damage (Stewart, Ralston, et al., 2022).

Several studies have been able to develop the use of 405 nm UV-LED (Ultra Violet – Light Emitting Diodes) as a potential candidate without relying on the addition of photosensitizers and can be applied safely to humans (Mohr et al., 2009; Stewart, Ralston, et al., 2022; Stewart, Tomb, et al., 2022). UV LED 405 nm has the ability to inactivate bacteria in biological fluids, rabbit plasma and human plasma with 99.9% of samples showing a decrease in the number of bacteria after being exposed for a certain time (Barneck et al., 2016a).

Direct exposure to 405 nm UV LED is capable of decontaminating plasma in storage bags and does not require a photo sensitizer, indicating that 405 nm UV LED is able to reduce the number of bacteria in body fluids contaminated with bacteria which is closely related to transfusion medicine (Barneck et al., 2016b). Stewart et al (2022) showed that exposure to 405 nm UV LED was able to reduce the level of bacterial contaminants in plasma products after exposure for 1 hour with an efficiency of 99%-100% (Stewart, Ralston, et al., 2022). This shows that one hour is sufficient time to eliminate bacteria, so several modifications to the exposure time are needed to determine the optimal time needed to reduce bacterial contamination.

## 2. RESEARCH METHOD

This study was true experimental design and conducted on August to September 2023 at the Blood Bank Laboratory of STIKES Rajekwesi Bojonegoro, Indonesia. The sample used was the bacterium *Pseudomonas aeruginosa* ATCC 27853 and Liquid Plasma from Blood Donor using Post test-Only Control. The Ethics Committee decision is required for the study used human materials (Ethical Clearance Number 031/PRO/KEPK/VIII/2023). All procedure to human plasma collection were conducted at Blood Donor Unit Bojonegoro and directly used after the collection.

### Sample Preparation

*P. aeruginosa* samples were prepared by incubating an aliquot of stock solution in nutrient broth at 37°C for 24 h. Nutrient broth media was used to measure the number of *P. aeruginosa* in a CFU assay at 37°C and 24 h incubation following the method provided by ATCC. Liquid plasma collected from conventional blood donor which processed through Standard Operational Procedure for Blood Separation using double blood bag. The collection procedure handled by professional blood technician in Blood Donor Unit of Bojonegoro.

### The Inoculation of *Pseudomonas aeruginosa* on Human Plasma

*P. aeruginosa* which has been prepared one day before on Nutrient Broth media then diluted using saline to reach the exact concentration about 10<sup>8</sup> CFU/ml. Inoculate the bacterial isolates onto sterile tubes by adding 1 ml of bacterial isolate and 9 ml of liquid plasma and homogenize the inoculants. All procedures were done under sterile condition.

### Pathogen Inactivation Procedure

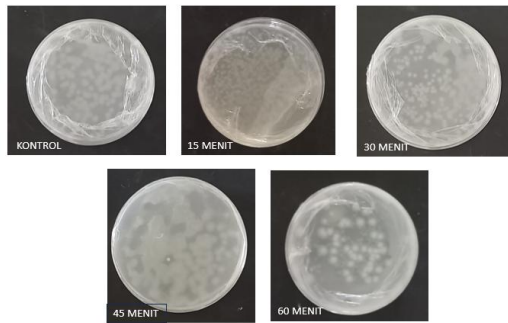
The following procedure were moved the 200 µl contaminated-liquid plasma into 96-well and applied Pathogen Inactivation protocol using UV LED instrument for 15, 30, 45, and 60 minute. Each treated groups and control were replicated about 6 well each. A mixture of liquid plasma and bacterial suspension that had been exposed by UV LED light then inoculated into Nutrient Agar Media and incubated at 37°C and 24 h. The growth of *P. aeruginosa* been identified vary from all treatment groups and control.

### Statistical Analysis

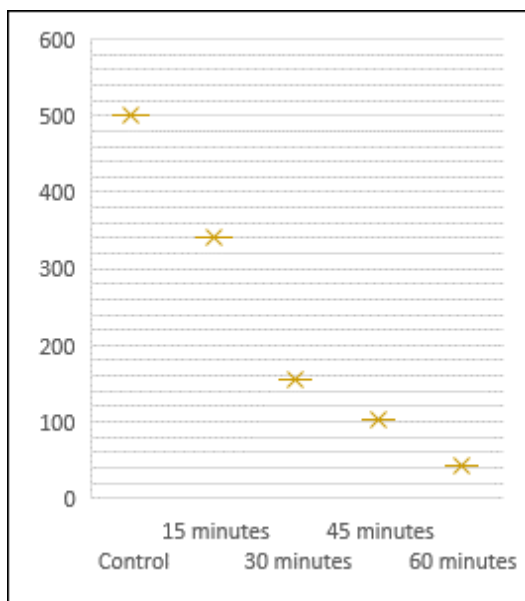
Data were analyzed using SPSS 25 software (IBM, USA) and analyzed by one way ANOVA after been known data were normal distribution by Kolmogorov-Smirnov Test. A pos hoc LSD test was performed to determine the differences between treatment groups and the significant value decided less than 0.05.

### 3. RESULT AND DISCUSSION

The exposure of UV LED light on Contaminated Plasma with *P. aeruginosa* showed different count of colony growth on NA culture. The bacterial growth decreased following the time exposure to UV LED light and showed no significant difference between the treated groups and control ( $p$  value  $> 0.05$ ).



Picture 1. *P. aeruginosa* cultures



Picture 2. Colony count of *P. aeruginosa* exposure by UV LED light

Transfusion-Transmitted Infections (TTIs). Application on human plasma that had been inoculated with *P. aeruginosa* showed a decrease in contamination at exposure times of 15, 30, 45, and 60 minutes. The results of inoculant cultures that had been exposed to UV LED 405 nm for 60 minutes showed a significant reduction compared to the 15, 30, and 45

minutes exposure groups. The reduction in 60 minutes exposure reached 90% when compared to the control group, where the control group was not exposed to UV LED 405 nm. This data showed UV LED 405 nm has a role in reducing the amount of *P. aeruginosa* contamination in donor plasma, and further testing is needed at a longer exposure time to determine the exposure time that can reduce and inhibit the growth of *P. aeruginosa* in blood plasma. UV LED is an instrument composed of several mercury lamps which are flexible, adjustable and can be applied directly without the heating process of the instrument.

The effectiveness of UV LED exposure at several wavelengths has been proven in several studies, using samples such as food, water and biological fluids (Rattanakul & Oguma, 2018) *P. aeruginosa* is an opportunistic gram-negative bacterium that can thrive in various types of natural environments and human biological fluids, including blood. Previous research stated that UV LED was effective in reducing *P. aeruginosa* contamination and had antibacterial capabilities by killing 95.1% at a wavelength of 405 nm and 100% at a wavelength of 470 nm. Several other studies regarding the inactivation of bacterial contaminants in blood products have shown a reduction in various contaminants (Barneck et al., 2016a; Guffey & Wilborn, 2006; Li et al., 2021; Petrini et al., 2017; Rattanakul & Oguma, 2018). Research by Lu et al showed the antimicrobial effectiveness of 405 nm UV LED on gram-positive bacteria which tend to be more susceptible, but this research uses a simpler buffer to determine the direct interaction between light photons and bacterial cells. (Lu et al., 2020) In this study, *P. aeruginosa* was suspended in human plasma and this had an effect on the susceptibility of the bacteria. The application of UV light exposure has been shown to be associated with several possible therapies. The application of UV has a bactericidal effect by using exposure to blue light which produces a bactericidal effect on aerobic organisms (Barneck et al., 2016a; Li et al., 2021; Petrini et al., 2017). The bactericidal mechanism of UV LED is by means of light that is absorbed by porphyrin produced by bacteria or produces free radicals, affecting

cytoplasmic membrane proteins and DNA, and direct effects on bacterial photolabile pigments (Chen et al., 2023; Huang et al., 2020; Li et al., 2021; Petrini et al., 2017)

Previous research showed that UV LED was more effective at killing *P. aeruginosa* than *S aureus* (Guffey & Wilborn, 2006). *S aureus* is more resistant if eliminated using light energy at this wavelength, and several other organisms do not show a response to light therapy at the wavelengths tested, but appear to be able to stimulate growth in *P. acne* cultures and are in line with Guffey's research comparing wavelengths 405 nm and 470 nm (Petrini et al., 2017). *Pseudomonas aeruginosa*, classified as a Gram-negative bacterium, is alleged an opportunistic pathogen due to its capacity to initiate various infections in Immunocompetent and immunocompromised individual. It can also be transmitted through blood transfusions. Particularly, *P. aeruginosa* demonstrates considerable resistance to a wide array of conventional antibiotics and disinfectants. Consequently, the use of disinfectants is imperative in the Blood Donor Unit as a mandatory measure prior to the commencement of blood donation procedures.

#### 4. CONCLUSION AND SUGGESTION

We can conclude that 405 nm UV-LED light exposure resulted in pathogen inactivation effect on *Pseudomonas aeruginosa* as a contaminant in blood plasma. These can be a breakthrough to improve blood service quality and decrease pathogen infection in blood donor. However, further research is necessary to conduct to obtain more holistic information about duration and intensity of UV light to reach optimum antibacterial effect without affecting blood quality.

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