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***In Vitro* Microbiological Evaluation of UV-HEROES**

Introduction

Microbiological tests were carried out in specialized laboratories to evaluate the effectiveness of UV-HEROES in reducing microbial contamination on the surface of the stethoscope diaphragm. The tests were performed using a broad spectrum of microorganisms, including *Escherichia coli* (ATCC 8739), methicillin-resistant *Staphylococcus aureus* (ATCC 43300 - MRSA), and *Pseudomonas aeruginosa* (ATCC 27853), which are representative of the most common pathogens found in healthcare environments. The microorganisms were inoculated onto the simulated diaphragm surface using the drop method.

Justification for microorganism selection

Escherichia coli and *Pseudomonas aeruginosa* are Gram-negative bacteria commonly found in hospital environments and associated with healthcare-associated infections. *Staphylococcus aureus* (including the MRSA variant) is a Gram-positive bacterium responsible for numerous skin and systemic infections. The inclusion of MRSA, an antibiotic-resistant strain, allows for assessing UV-HEROES' effectiveness against particularly challenging microorganisms in clinical settings.

Methodology

Microbiological tests were conducted using the following materials and protocol.

Microorganisms used

S. aureus ATCC 43300 (MRSA)

E. coli ATCC 8739

P. aeruginosa ATCC 27853

Media and solutions used

Phosphate-buffered saline (PBS), pH 7.4: suspension medium

Plate Count Agar (PCA): for all bacteria

Preparation of microorganisms

For each microorganism tested, a starting inoculum was prepared in a 10 ml PBS suspension at a concentration of 0.5 McFarland, corresponding to approximately $\pm 1.5 \times 10^8$ CFU/ml. Serial dilutions were then performed until a final concentration of approximately $\pm 1.5 \times 10^4$ CFU/ml was achieved.

Inoculum application

To simulate real-world conditions, different UV-HEROES configurations were tested, including variants with different numbers of LEDs and different sample application positions, as schematically shown in Figure 1, illustrating the various possible placements. For each configuration, 50 μ l aliquots (drops) of each microorganism were applied and exposed to UV light for a defined period (Fig. 1)

Irradiation

The quartz disc with droplets is placed at the same distance from the UV-C source as the membrane of the stethoscope during normal use of the device and is irradiated with the three different UV-HEROES models.



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The irradiation times correspond to those of a sanitization cycle, namely 3 minutes 30 seconds and 2 minutes 30 seconds for the UV-HEROES lite and UV-HEROES models, respectively.

Microorganism Recovery, Incubation, and Counting

Each droplet was subsequently recovered using a precision pipette (by aspiration) and plated on 90 mm Petri dishes containing the appropriate culture medium. For all bacteria, the plates were incubated at 37°C for 48 hours and counted using a manual colony counter.

Controls

Negative controls were included (quartz discs inoculated with microorganisms but not irradiated) to assess the viability of the microorganisms, calculate the percentage reduction by comparing treated and untreated samples, and detect any environmental contamination.

Acceptance criteria

Test results were considered valid if the negative controls showed growth consistent with the inoculum concentration.

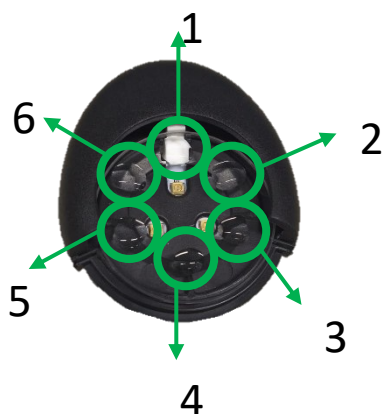


Figure 1 – Arrangement of the inoculum droplets on the quartz disc

Table 1 – Microbiological Reductions: decrease in CFU compared to positive controls

Micro-organisms	Model	Action time	Initial concentration (UFC/mL)	Log ₁₀ reduction	Inactivation %
<i>Escherichia coli</i>	UV-HEROES lite	3' 30"	10 ⁴	>2	>99%
	UV-HEROES	2' 30"	10 ⁴ e 10 ⁵	>3	>99,9%
<i>Staphylococcus aureus - MRSA</i>	UV-HEROES lite	3' 30"	10 ⁴	>2	>99%
	UV-HEROES	2' 30"	10 ⁴ e 10 ⁵	>3	>99,9%
<i>Pseudomonas aeruginosa</i>	UV-HEROES lite	3' 30"	10 ⁴	>2	>99%
	UV-HEROES	2' 30"	10 ⁴ e 10 ⁵	>3	>99,9%



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Results

The results obtained with this protocol indicate a microbial inactivation greater than 99% and 99.9% for the UV-HEROES lite (1 LED) and UV-HEROES (3 LED) models, respectively.

Conclusions

Although this in-vitro test provides an indication of the device's microbial reduction capabilities, it is essential to understand that there is no internationally recognized regulatory standard that defines a universally accepted testing protocol for evaluating the effectiveness of UV-C devices like UV-HEROES. Consequently, the results obtained with this specific protocol do not constitute regulatory evidence of these levels of inactivation and may not be directly comparable to those obtained using other verification methods.

Moreover, it is important to consider that real-world environmental conditions—such as the presence of organic matter, biofilm formation, and variability in microbial susceptibility—can significantly influence the effectiveness of microbial reduction in clinical settings.

Therefore, under Regulation (EU) 2017/745 (MDR), the classification standard for medical devices intended for the disinfection or sterilization of other medical devices is not applicable. As such, UV-HEROES is classified as a Class I medical device, designed to support hygiene practices and significantly contribute to the reduction of microbial contamination risk.