Microbiological efficacy of disinfecting the outer surface of packaged medical devices with UV-C using the UVSmart D25

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BACKGROUND

In many hospitals, packaged disposable medical devices that have not been opened, but have been stored in a patients room, are discarded once the patient is discharged. This is due to a lack of appropriate disinfection methods for these packaging. Potentially, a loss 10 thousands of euros per year and a lot of plastic waste is involved. Studies at the UMCG have already shown that paper & plastics packages can be effectively disinfected using the UVSmart D25 (see appendix-1). UVSmart has received approval from the supplier of these disposables to disinfect the packaging with UVC.

This laboratory study focuses on demonstrating the microbiological effectiveness of UVC disinfection of packaging for medical disposables; it concerns the outside of the packaging of disposable medical devices.
The current experiment was designed to study the microbiological efficacy of disinfecting the outer surface of packaged medical devices with UV-C using the D25.

MATERIALS

- D25, based on ECD (ECDV100001)
- Various packaged small medical devices (manufacturer: Fygon)
- Clinical isolates of the following micro-organisms[1]:

Claim	Micro-organism to be tested	ATCC reference strain	Required log reduction
Bactericidal	1. Staphylococcus aureus	25923	>Log 6
	2. Pseudomonas aeruginosa	9027	>Log 6
	3. Escherichia coli	25922	>Log 6
	4. Streptococcus pneumoniae	49619	>Log 6
Fungicidal	5. Candida albicans	10231	>Log 4
Sporicidal	6. Bacillus subtilis	11778	>Log 4

[1] *All micro-organisms were isolated from clinical samples submitted to a NEN-EN-ISO 15189:2012 certified clinical microbiology laboratory. Identification was performed using standard Maldi-tof determination testing with ATCC strains as reference micro-organisms.*

- Tryptone Soy Agar + 5% Sheep blood (TSA-SB) agar (Thermo Fisher Scientific)
- Sabouraud Dextrose (Sab) Agar (Thermo Fisher Scientific)
- E-swabs with amies medium (Copan)
- 35°C incubator
- Sterile NaCL suspension

METHODS

General

This study was performed using techniques in accordance with the ASTM E2111-12(2018) standard (Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemicals).
All experiments, including examination and interpretation of agar plates and colony counts, were performed by trained technicians with over 10 years of experience in clinical bacteriology. Tests were performed in a clinical microbiology laboratory certified according to the NEN-EN-ISO 15189:2012.

Preparing inocula of micro-organisms and Simulating package surface contamination

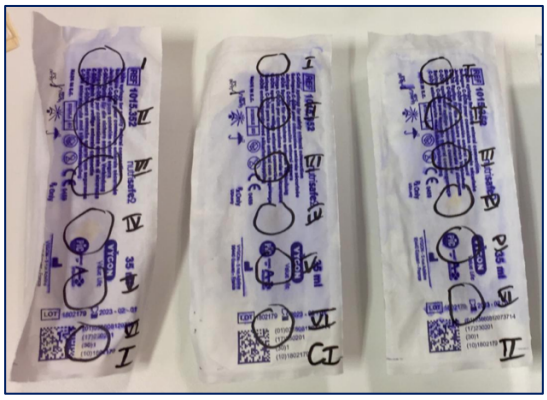
Micro-organisms were grown overnight on TSA-SB agar (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Streptococcus pneumoniae*) or Sabouraud agar (*Candida albicans*) to receive fresh cultures. These fresh cultures were used to make 0.5 McFarland suspensions (corresponding to 1-2 x 10^8 CFU/mL) for the quantitation standard curves.

Same fresh cultures were used to prepare 2.0 McFarland suspensions to inoculate on the outer surface of the packages. Three similar packages were used to be contaminated with each micro-organism: two to be used for separate disinfection with D25 and the other one as a non-disinfected control. The outer surfaces were inoculated on two different sites:

1. At the plastic side of the package

***Inoculation of the plastic side of the packages**. I and II: packages used for 2 separate UVC disinfection experiments. CI: package not treated with UVC (controls). Circles depicted with numbers 1 – 6 indicate the sites where the six micro-organism strains were inoculated.*

2. At the paper side of the package

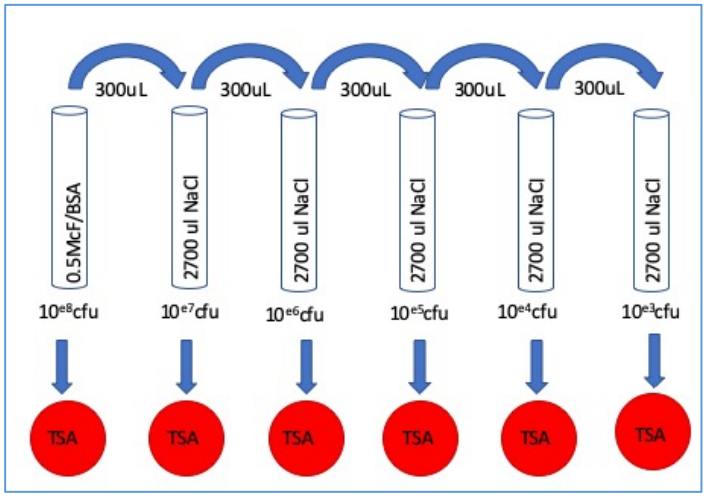


***Inoculation of the paper side of the packages**. I and II: packages used for 2 separate UVC disinfection experiments. CI: packages not treated with UVC (controls). Circles depicted with numbers 1 – 6 indicate the sites where the six micro-organism strains were inoculated.*

Quantitation standard curve

For quantitation purposes, McFarland standard curves were created for all tested micro-organisms. This was done by making 10-fold dilution series of the 0.5 McFarland/BSA suspensions (see figure 1). The McFarland dilutions were inoculated on TSA-SB agar (*S. aureus, E. coli*, *P. aeruginosa* and *B. cereus*), Chocolate agar (*S. pneumoniae*) or Sabouraud dextrose agar (*C. albicans*).

Figure 1: McFarland standard curves from 1-2 x 10^8 CFU/mL to 1-2 x 10^3 CFU/mL were created using 10-fold dilutions series from a 0.5 McFarland suspension.



Exposure of the contaminated package surface to UVC radiation with D25

Following inoculation of the package surfaces, one of the two similar disposables was placed into the D25 and disinfected for 25 seconds, according to the manufacturers' instructions (figure 2). The other contaminated disposable was left outside the D25 to function as a control. After disinfection, the contaminated sites of both, the disinfected and non-disinfected packages were thoroughly swabbed and cultured on TSA-SB agar (*S. aureus, E. coli* and *B. cereus*), Chocolate agar (*Streptococcus pneumoniae*) or Sabouraud dextrose agar (*C. albicans*) in order to recover the inoculated micro-organisms.
All agar plates were examined after 24 and 48 hours. Colony counts were be compared between the disinfected and non-disinfected packages and quantitation was determined using the McFarland standard curves.

Disinfection with D25 was repeated 2 times twith two separate packaged to examine reproducibility.

Figure 2: The contaminated packages were be placed in the D25 to receive optimal UVC exposure at either the plastic sides, the paper side or the at the opening tab.

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Validation study: **Microbiological efficacy of disinfecting the outer surface of packaged medical devices with UV-C using the UVSmart D25**

RESULTS

McFarland standard curves

McFarland standard curves ranging from 1-2 x 10^8 CFU/mL to 1-2 x 10^2 CFU/mL were created for all micro-organisms mentioned in table-1. The standard curves demonstrated a gradually declining colony count from ~10^8 CFU/mL to ~10^3 CFU/mL (Figure 4.)

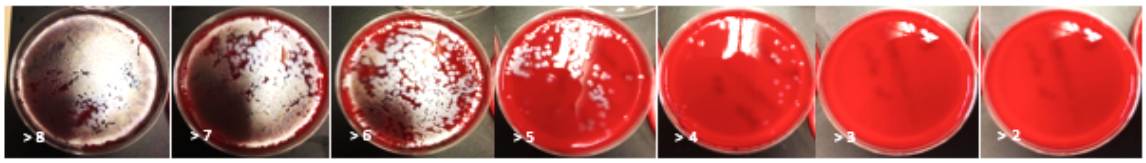
Figure 3. McFarland standard curves.

S. aureus

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL

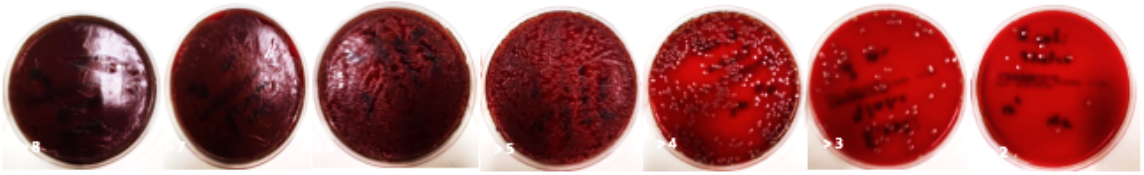
P. aeruginosa

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL



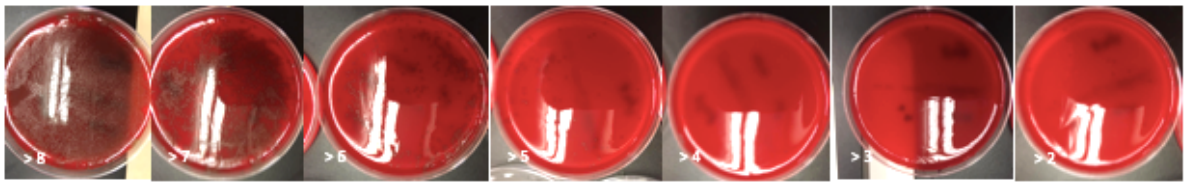
E. coli

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL



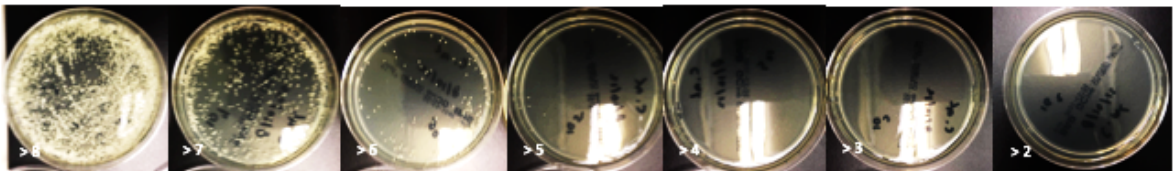
S. pneumoniae

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL



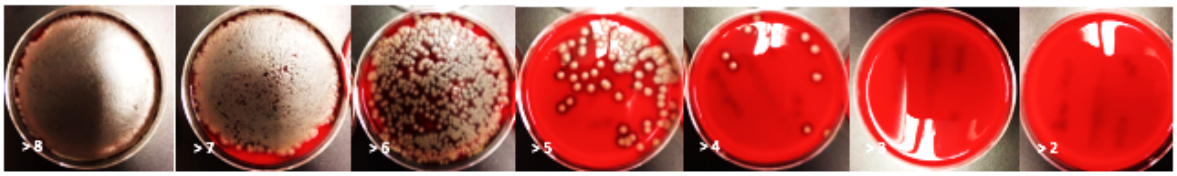
C. albicans

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL



B. subtilis

10^8CFU/mL 10^7 CFU/mL. 10^6 CFU/mL 10^5 CFU/mL 10^4 CFU/mL 10^3 CFU/mL 10^2 CFU/mL



Efficacy of disinfection outer surface of packaged medical devices with D25

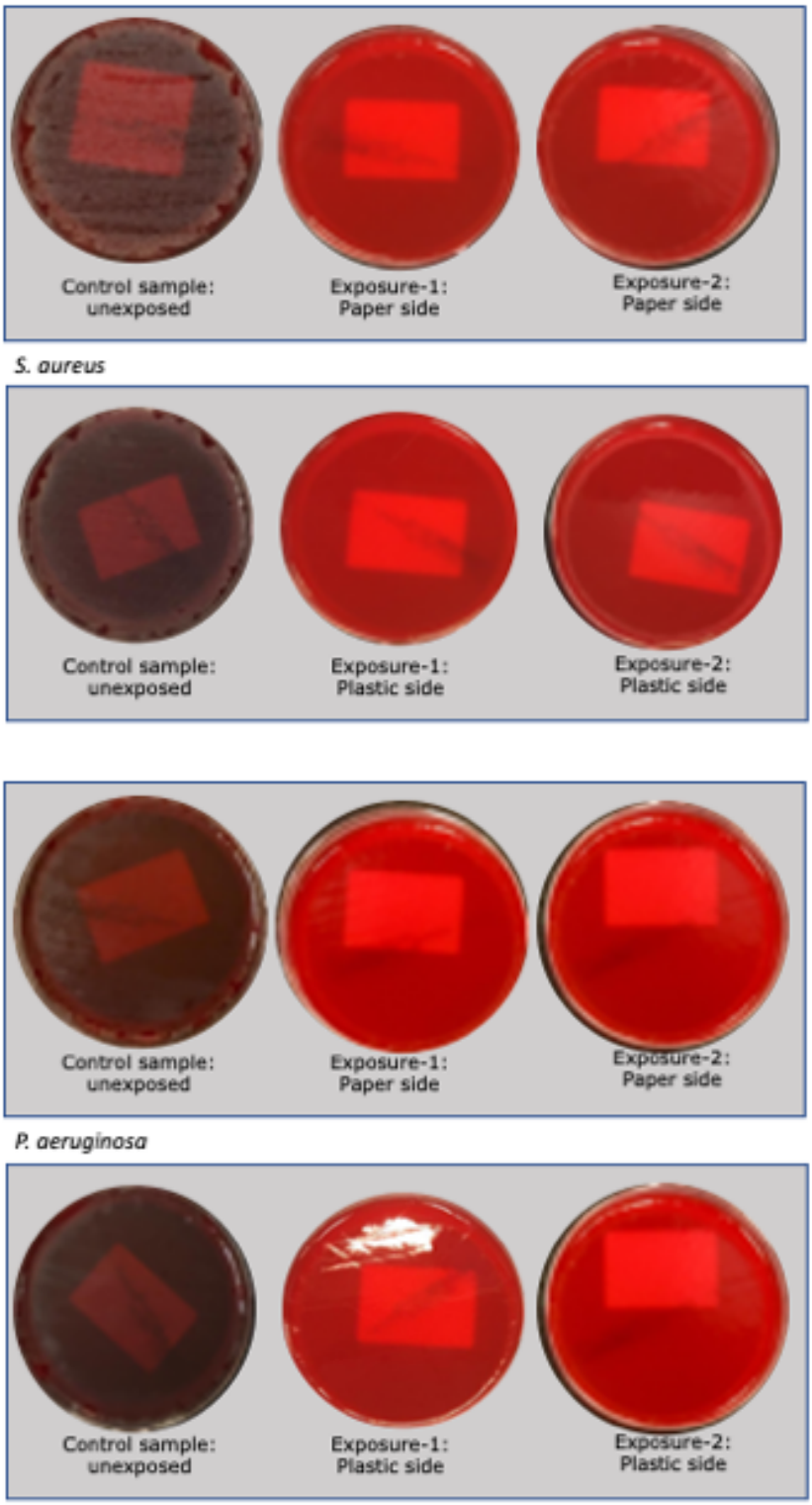
Inoculated micro-organisms were recovered from disinfected and non-disinfected surfaces of packages. The colony counts, estimated using the McFarland standard curves (Figure 3), were determined for the disinfected and non-disinfected packages. The results are summarized in table 1 and corresponding pictures of the agar plates are depicted in figure 4.

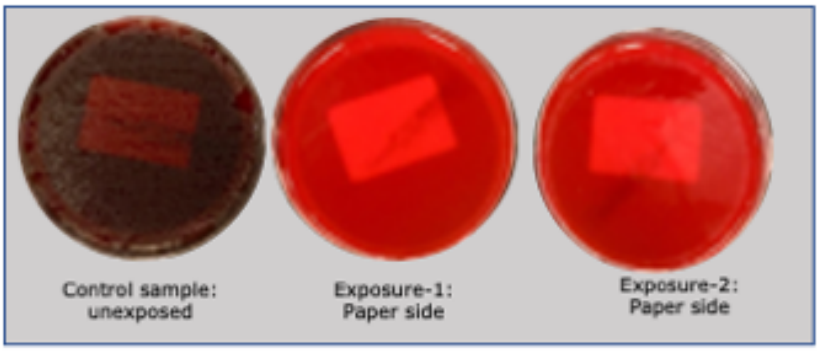
For all tested bacteria species an estimated 10^8 cfu/mL (at least) were recovered from the non-disinfected packages. After disinfection a log reduction of at least 10^6 cfu/mL was observed for all bacteria species. Spore forming *Bacillus* species are known to be more resilient to UVC than the other tested bacteria species. Nevertheless, elimination of *B. subtilis* was achieved at a level at least 10^6 cfu/mL. For the *C. albicans* species tested, a reduction of at least 10^4 cfu/mL was achieved.

Table 1: Overview of quantitative growth of micro-organisms recovered from disinfected (Disinfection-1 and Disinfection-2) and non-disinfected (control-1) outer surfaces of packages

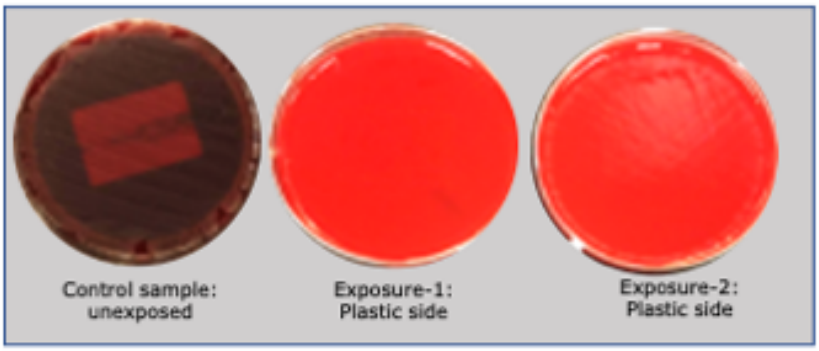
Micro-organism	Test specs	Semi-quantitative growth*1	Semi-quantitative growth*1	Log reduction (from paper and plastic side)
		From paper side	From plastic side	
S. aureus	Disinfection-1	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Control	10^8 cfu/mL (at least)	10^8 cfu/mL (at least)	
P. aeruginosa	Disinfection-1	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Control	10^8 cfu/mL (at least)	10^8 cfu/mL (at least)	
E. coli	Disinfection-1	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Control	10^8 cfu/mL (at least)	10^8 cfu/mL (at least)	
S. pneumoniae	Disinfection-1	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Control	10^6 cfu/mL (at least)	10^8 cfu/mL (at least)	
C. albicans	Disinfection-1	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^4 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^4 cfu / mL
	Control	10^5 cfu/mL (at least)	10^8 cfu/mL (at least)	
B. subtilis	Disinfection-1	0 colonies: < 10^1 cfu/mL	2 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Disinfection-2	0 colonies: < 10^1 cfu/mL	0 colonies: < 10^1 cfu/mL	>10^6 cfu / mL
	Control	10^8 cfu/mL (at least)	10^8 cfu/mL (at least)	

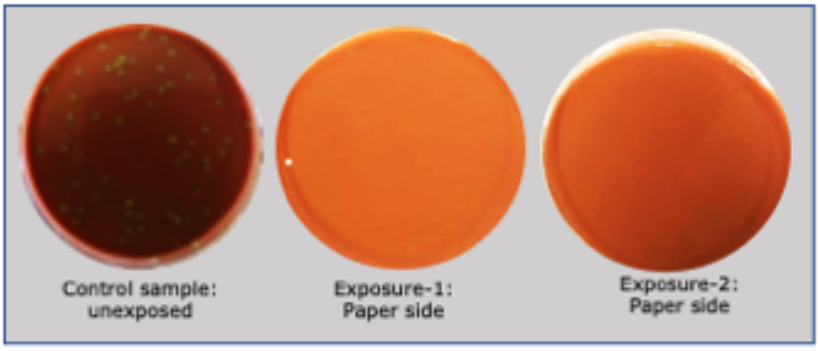
Figure 4. Recovery of exposed and unexposed (control) micro- organisms.



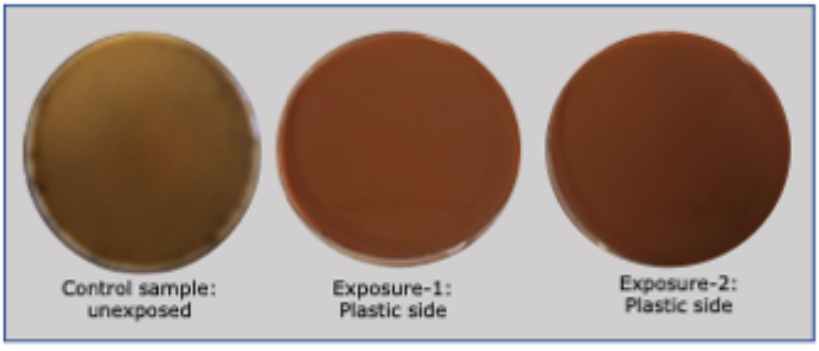


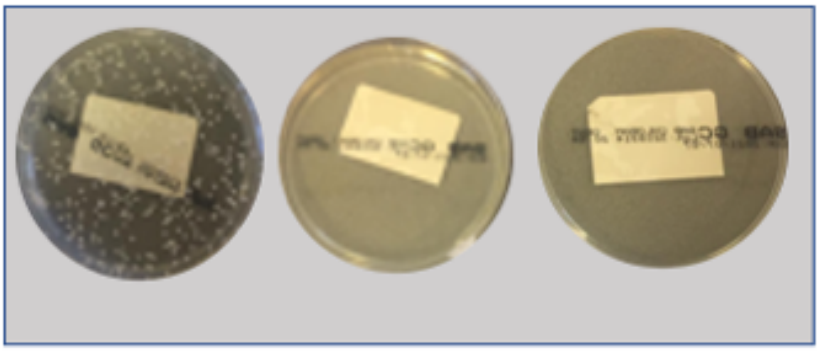
E. coli



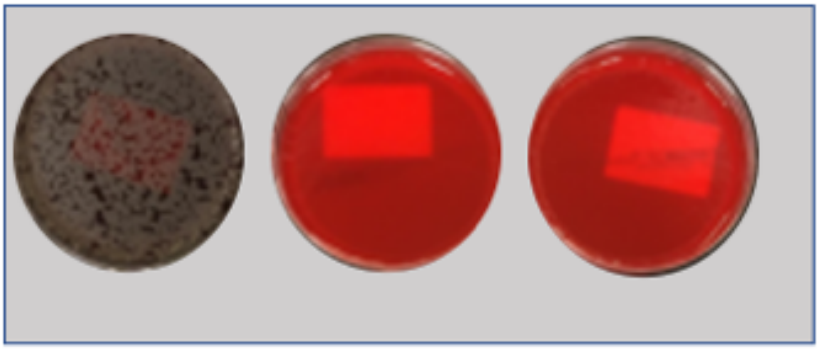


S. pneumoniae

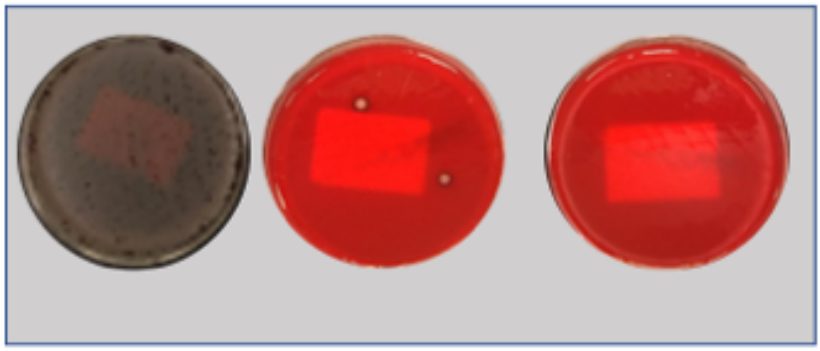




C. albicans



B. subtilis



Conclusion:

D25 from UV Smart is effective in reducing bacterial contamination on the outside of packagings for medical disposables medical devices with at least log-6 and log-4 for candida species.