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Efficacy of ultraviolet decontamination of *Clostridioides difficile* (*C. difficile*) spores on hospital materials, Personal Protective Equipment and clinical surfaces.

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Scope & Objectives

Clostridioides difficile is a spore-forming nosocomial pathogen that is the causative agent of antibiotic associated diarrhoea in susceptible patients. Patients usually acquire infection from ingestion of *C. difficile* spores from the faecal to oral route. The spores of the organism are highly resistant to desiccation, chemical disinfection and drying; thus they are able to persist in the environment for months (Joshi et al., 2012). *C. difficile's* spores have also shown a propensity to adhere to clinical surfaces, such as stainless steel, particularly well and survive disinfection with chlorine releasing agents (Dyer et al., 2019). Research is currently ongoing to determine whether this observed survival is indicative of genetic chlorine resistance in *C. difficile*. Thus, as spores of *C. difficile* have proven difficult to eradicate, it is of significant interest to find an alternate effective means of surface disinfection for *C. difficile* spores within healthcare and community environments. The use of UV-C light represents an alternative solution to decontaminate surfaces effectively and eliminate the risks of transmission. The approach of this study was to investigate C. difficile spore survival on clinical "high touch" surfaces and PPE after exposure to the Zeus UV-C cabinet (253.7 nm germicidal wavelength) at a range of spore concentrations and UV-C time regimens. This study has sought to determine the UV-C exposure regimen required for effective disinfection of *C. difficile* from these surfaces.

Objectives:

- Determine the efficacy of UV-C light administered by the Zeus Smart UVC cabinet to decontaminate clinical surfaces soiled with clinical pathogens (*C. difficile spores*).
- Clinical surfaces tested include patient gowns, surgical scrubs, disposable privacy curtains, vinyl flooring, steel disks and surgical masks.
- View materials in scanning electron microscope (SEM) to investigate any morphological effects of UVC on *C. difficile* spores.

Outline of test method

A range of concentrations of *C. difficile* spores were inoculated on to each surface type at a volume of 100 µl suspensions. Surfaces included sterile patient gowns, surgical scrubs, disposable privacy curtains, vinyl flooring, steel disks and surgical masks which were inoculated in $2cm^2$ squares. Inoculated materials were left to air-dry in a lateral flow cabinet overnight to allow for deposition of the spores, and then surfaces were tested and exposed to either 60, 90, 120, or 240 seconds of UV-C in the Zeus cabinet. Treated samples were then pressed onto agar for 15 seconds, 15 times (following Dyer *et al.,* 2019) using a 100g weight, to replicate 'hand touch pressure'. This was followed by 48h incubation in anaerobic conditions and colony counting.

Acceptance Criteria

To determine the efficacy of sporicidal activity with UVC, an ideal >log6 reduction of spores (spore forming units- sfu) will be recovered after a short exposure period, ideally after 60 seconds for concentrations above the infectious dose (1×10^6 sfu/ml).

Test Information		Deviation
Name of UV system	Zeus Smart UVC Cabinet	
Project start	19/10/20	
Project End	08/01/21	26/02/21
Manufacturer/Supplier	Finsen Tech	
Storage conditions	Ambient	
Experimental conditions	Aseptic	
Experimental surfaces	• NHS standard patient gown • Surgical scrubs • Disposable hospital privacy curtain • PolyVinylChloride (PVC) hospital flooring • Hospital Grade 2b Stainless Steel discs • Disposable surgical face mask type IIR	
Pathogen strains	*Clostridioides difficile* • R20291 (PCR Ribotype 027- hypervirulent) • CD630 (PCR Ribotype 012- Type strain)	
Incubation conditions	• Anaerobic growth chamber, 37 ^{o}C • 48 hours • Brain heart infusion (BHI) agar supplemented with 0.1% sodium taurocholate (ST), 1% L-Cysteine and 5% yeast extract (BHI + ST Agar)	
Treatment/exposure times	0, 60, 90, 120 & 240 second cycle	

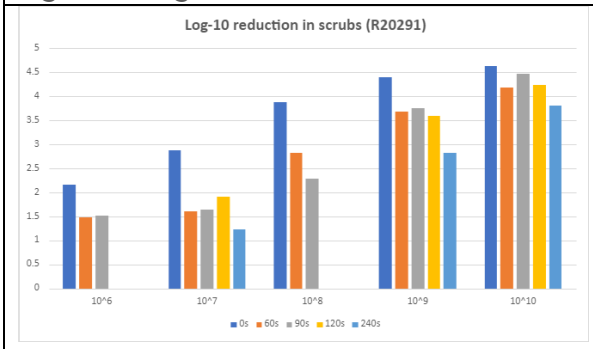
Deviations from standard method

Period of research extended by 8 weeks due to UK Government COVID19 lockdown restrictions which were unforeseeable.

Test result summary

The tested product achieved sporicidal activity in spore concentrations of $1x10^6$ – $1x10^8$ sfu/ml after 60 seconds of treatment on curtains, vinyl and masks. For higher concentrations of $1x10^9$ and $1x10^{10}$ sfu/ml sporicidal activity was not seen above a 1-log reduction in any tested surfaces. This is, however, a higher load which is unlikely to be encountered in clinical environments. Scrubs and gown exhibited the least reduction in spores. To summarise; higher concentrations showed the least efficacy in all materials/surfaces, and scrubs showed the highest spore recovery across all exposures in both strains. **Raw data is shown in figures 1 – 5**. SEM of the curtain at a concentration of $1x10^9$ exposed for 90 seconds shows a spore which appears to be destroyed physically by the ultraviolet light. This is a novel finding and may require further exploration **(figure 6)**.

Figure 1: Log-reduction in scrubs



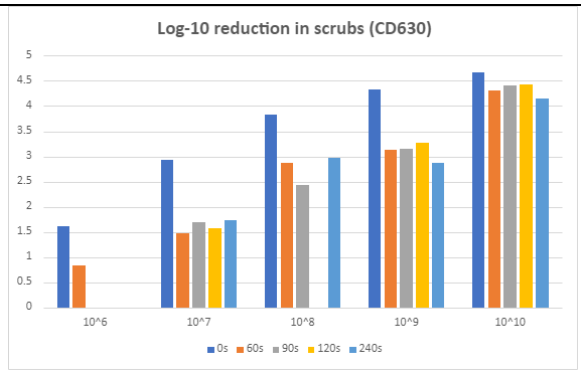
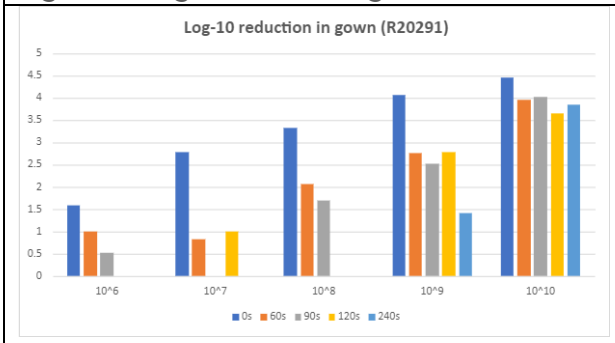


Figure 2: Log-reduction in gown



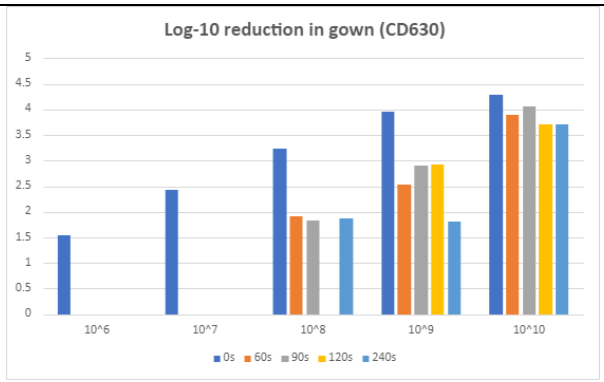
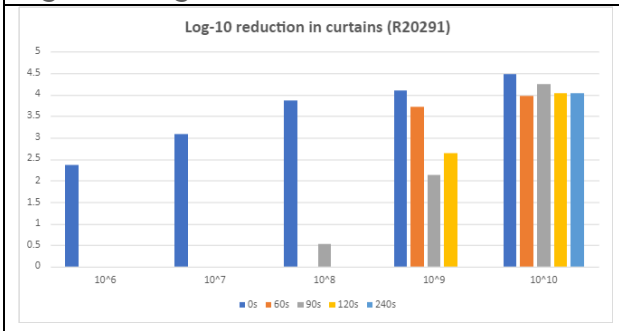


Figure 3: Log-reduction in curtain



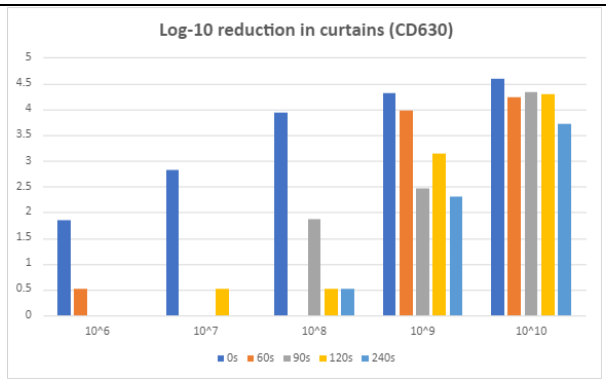
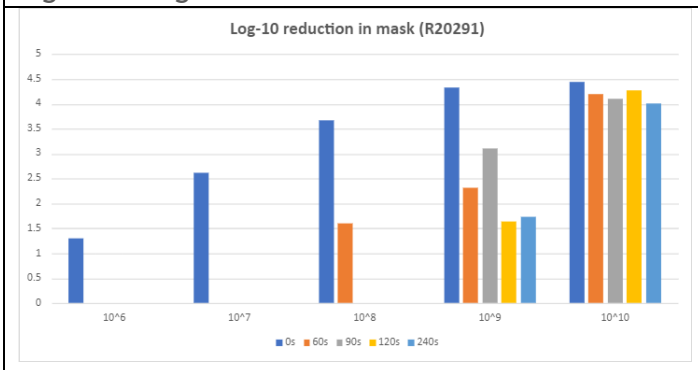


Figure 4: Log-reduction in masks



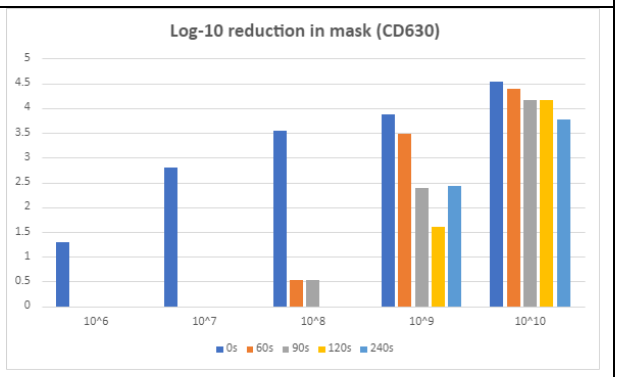
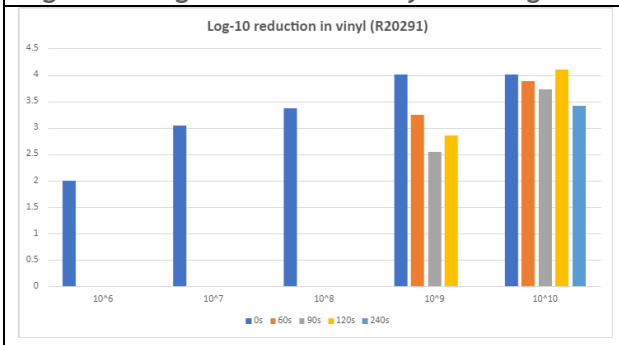


Figure 5: Log-reduction in vinyl flooring



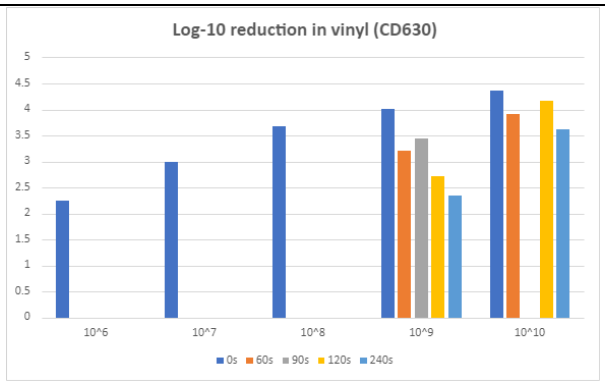
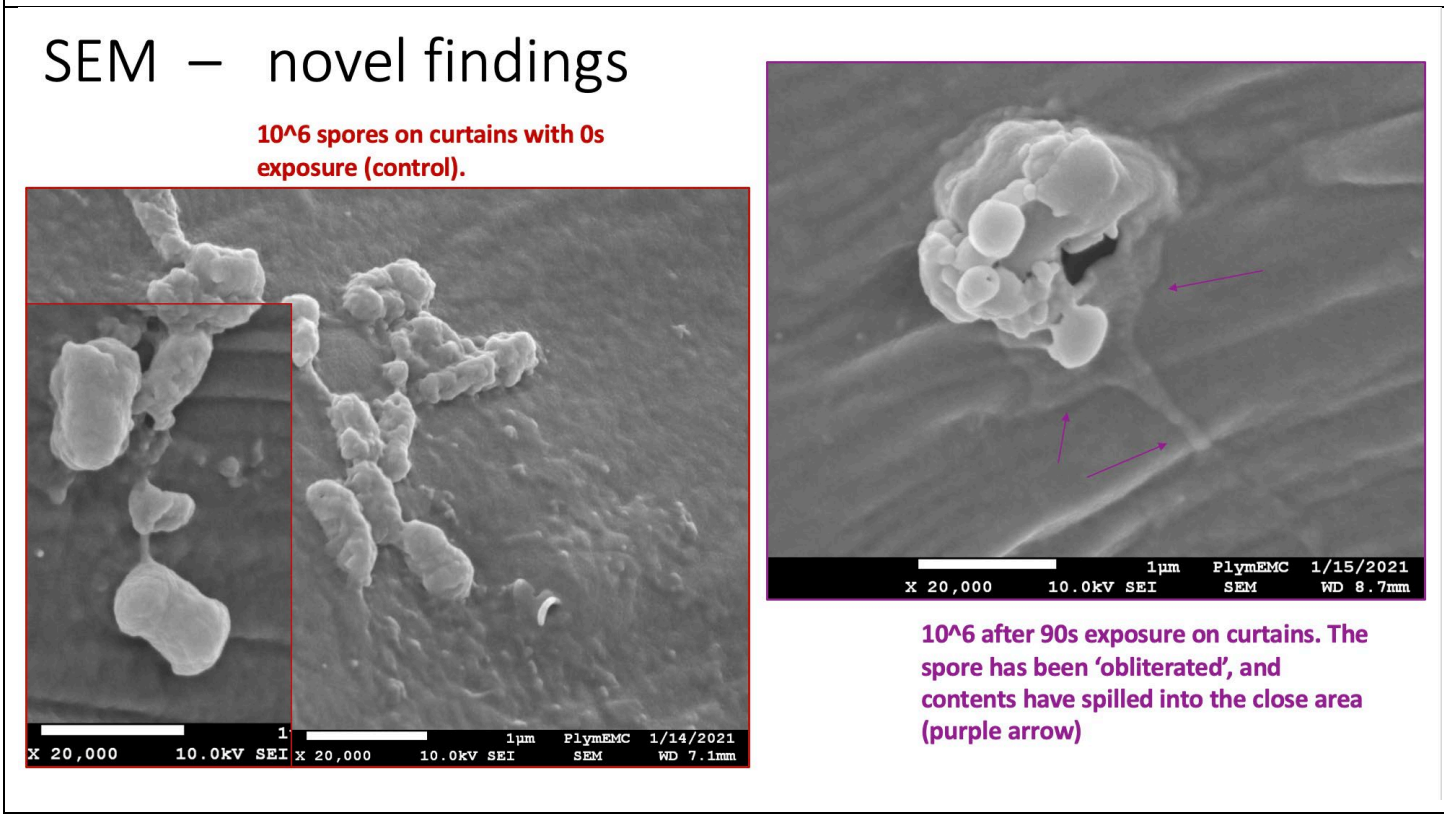


Figure 6: Scanning electron microscopy of an untreated and treated spore.



References

Dyer, C., Hutt, L.P., Burky, R. and Joshi, L.T., 2019. Biocide resistance and transmission of Clostridium difficile spores spiked onto clinical surfaces from an American health care facility. *Applied and environmental microbiology*, *85*(17), pp.e01090-19

Joshi LT, Phillips DS, Williams CF, Alyousef A & Baillie L 2012 'Contribution of Spores to the Ability of Clostridium difficile To Adhere to Surfaces' Applied and Environmental Microbiology 78, (21) 7671-7679