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Enhanced decontamination of *C. difficile* spores on surfaces via the synergistic action of 405nm light and disinfectants

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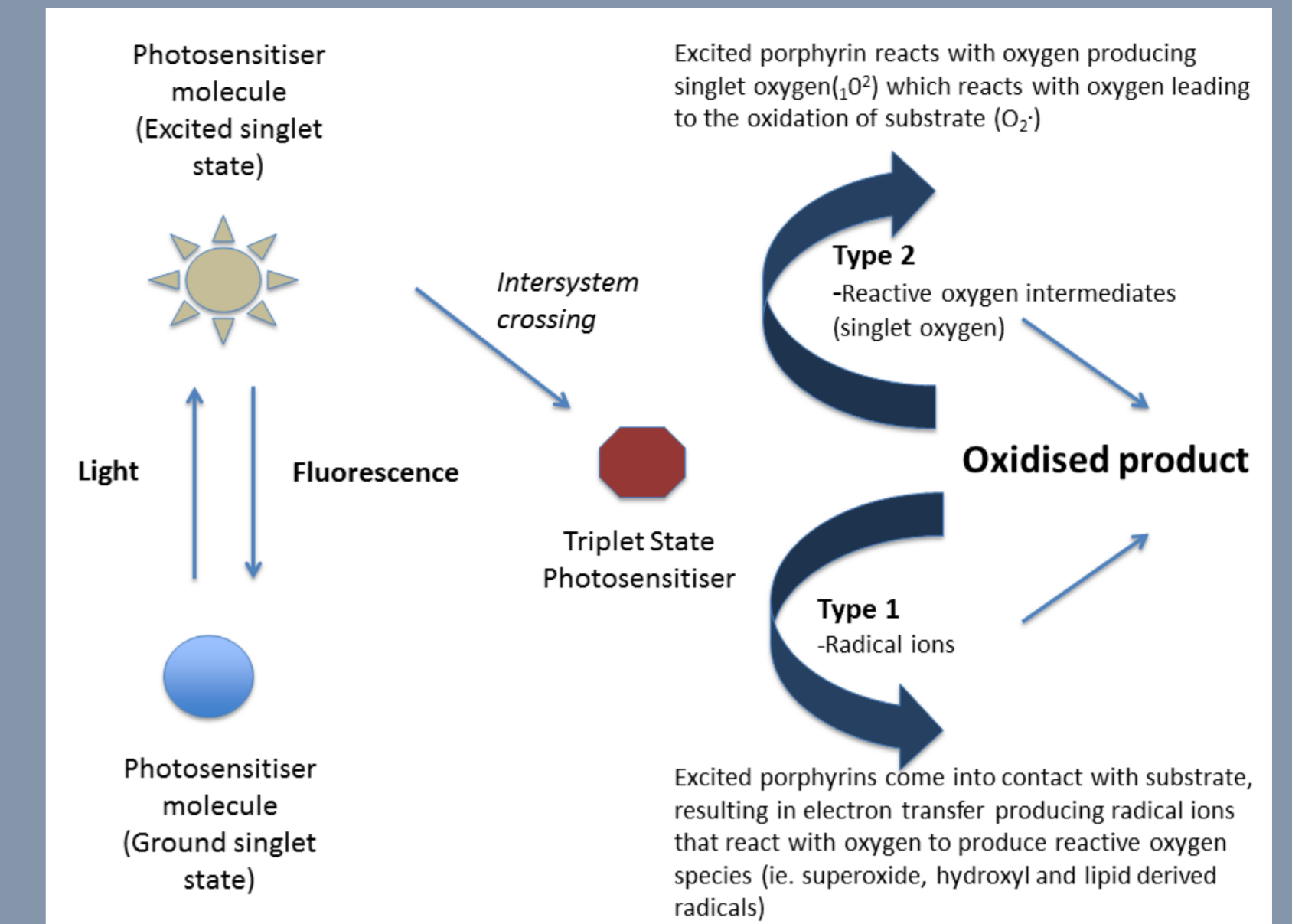
Clostridium difficile

- C. difficile* is a spore forming bacteria that is one of the major causes of antibiotic-associated diarrhoea in the western world.
- C. difficile* spores can survive in the environment for up to 5 months, with the rooms of patients with CDI reaching contamination rates of up to 50%.
- Chlorinated disinfectants are recommended for environmental cleaning in the presence of CDI. However, these have several drawbacks including their corrosive nature and the release of irritating vapours affecting healthcare workers.

405 nm light

- 405nm light is a novel decontamination method which inactivates bacteria by oxidative damage, caused by photoexcitation of porphyrin molecules within microbial cells.
- Low irradiance 405 nm light has proven effective for continuous environmental decontamination whilst being safe for human exposure.
- Efficacy of 405nm light against *C. difficile* spores has not yet been fully established.

Mechanism of inactivation



Aims

- To establish whether spore susceptibility can be enhanced by combining 405nm light with low concentration chlorinated disinfectants: 0.1% sodium hypochlorite, 0.001% Actichlor and 0.0001% Tristel.
- Both 405nm light and the selected disinfectants cause lethal oxidative damage to microorganisms, therefore their combined use has the potential to deliver enhanced sporicidal effects.

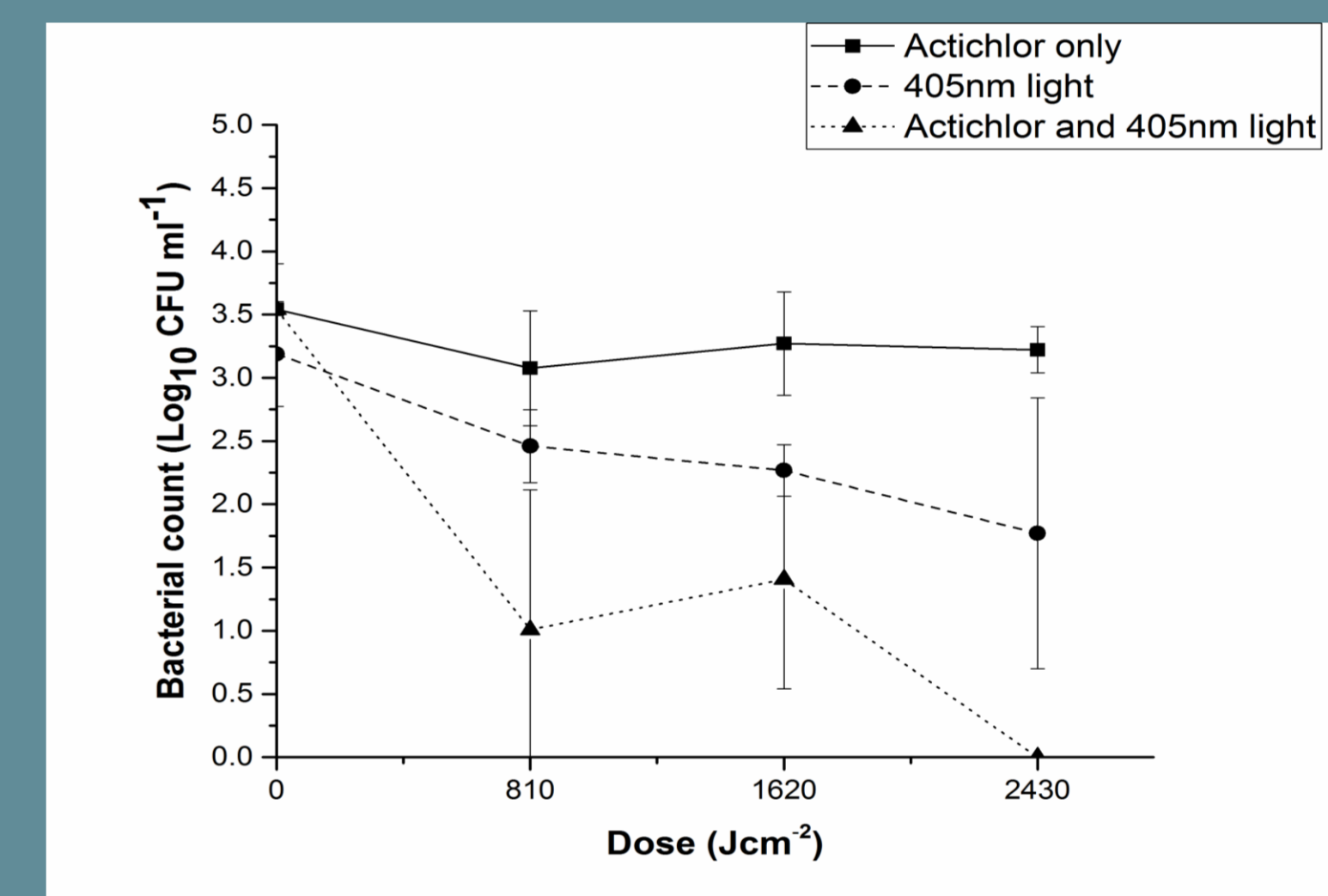
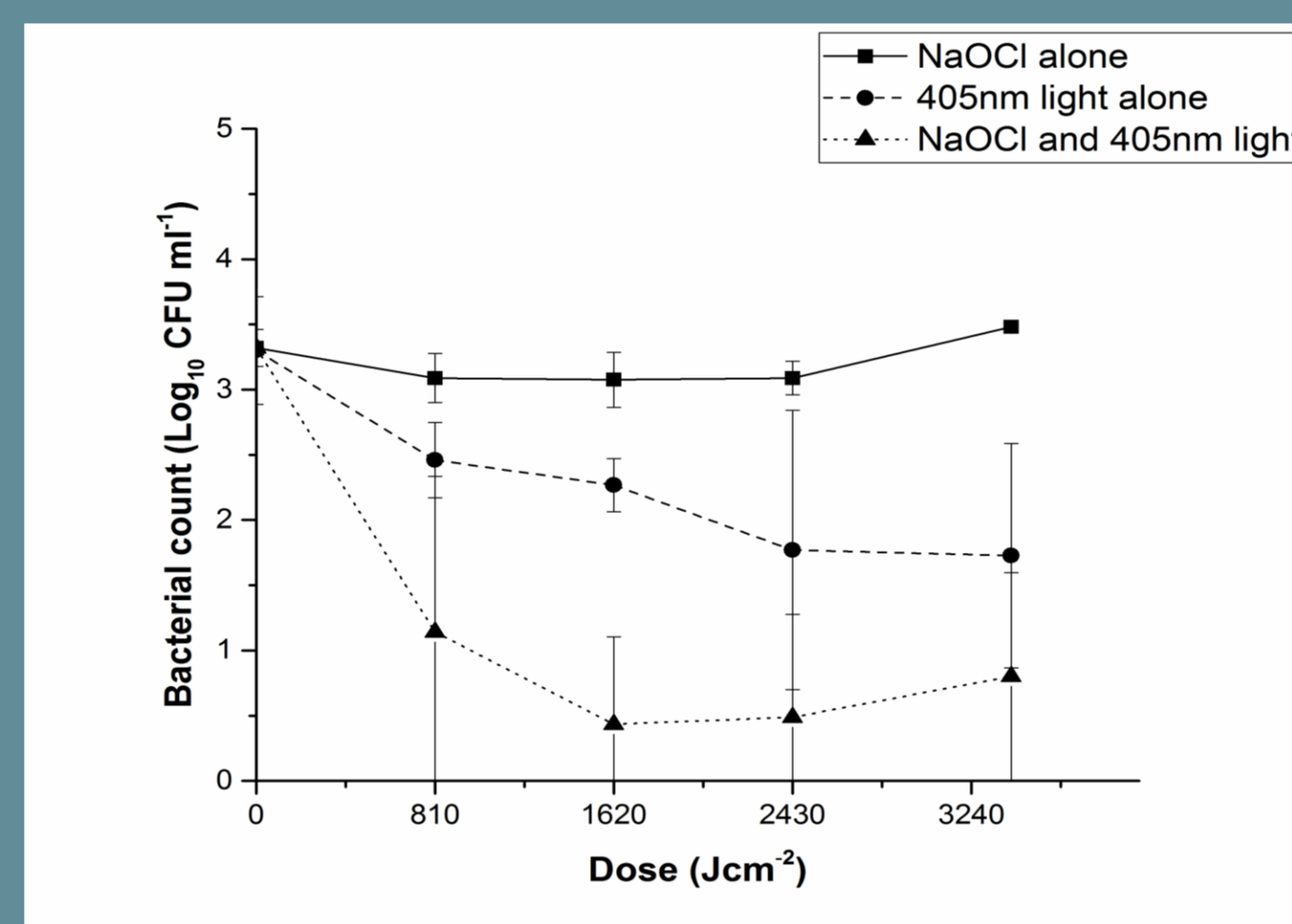
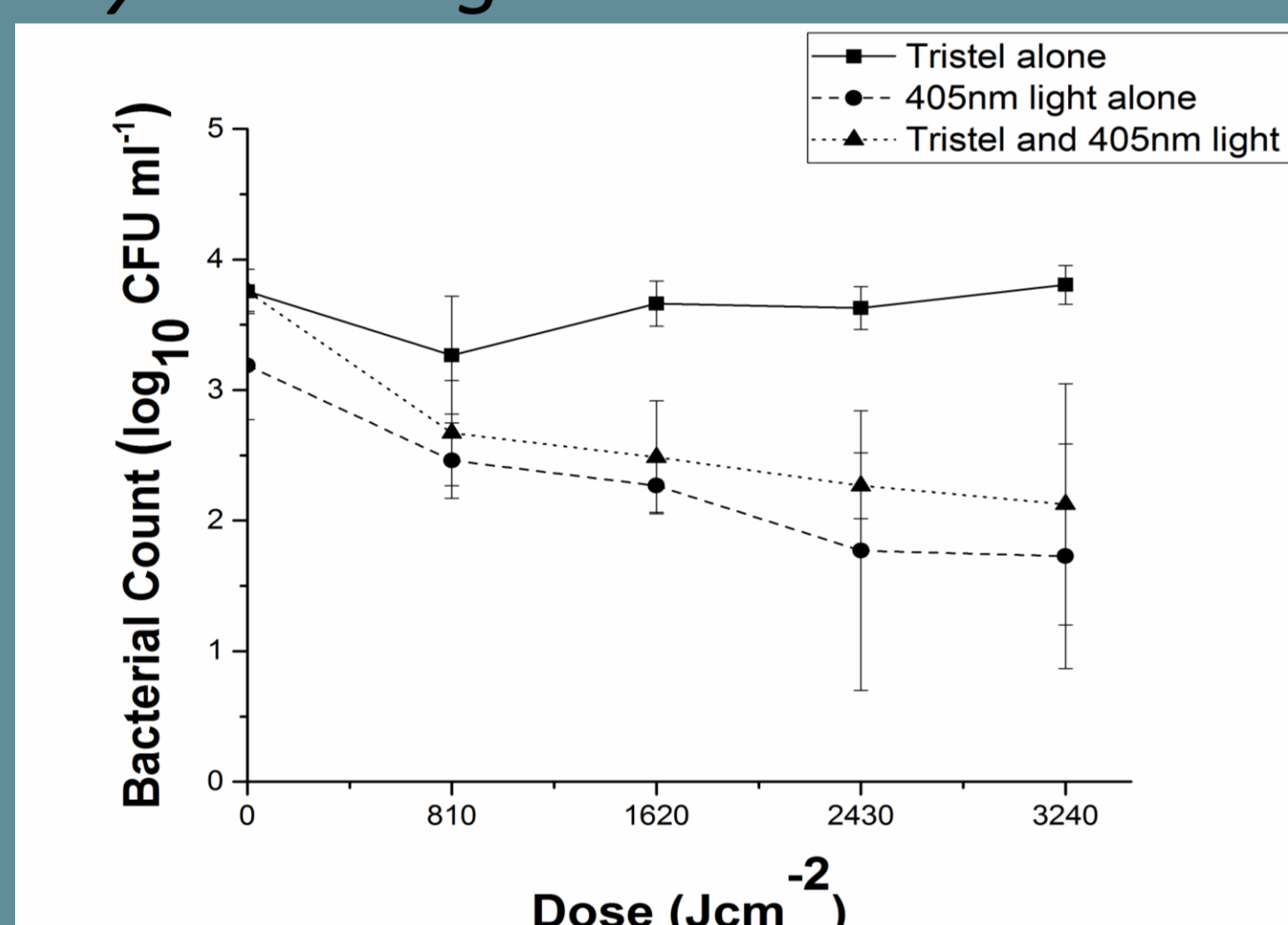
Materials and methods

- C. difficile* spores were seeded onto surfaces, and incubated at 50°C for 25 min to allow drying. 100µl disinfectant was added to the surface, and then light exposed to high irradiance (225 mW/cm²) or low irradiance (0.4 mW/cm²) 405 nm light.
- Surviving spores were recovered by resuspension in PBS, and enumerated after anaerobic incubation on blood agar (2 days at 37°C).

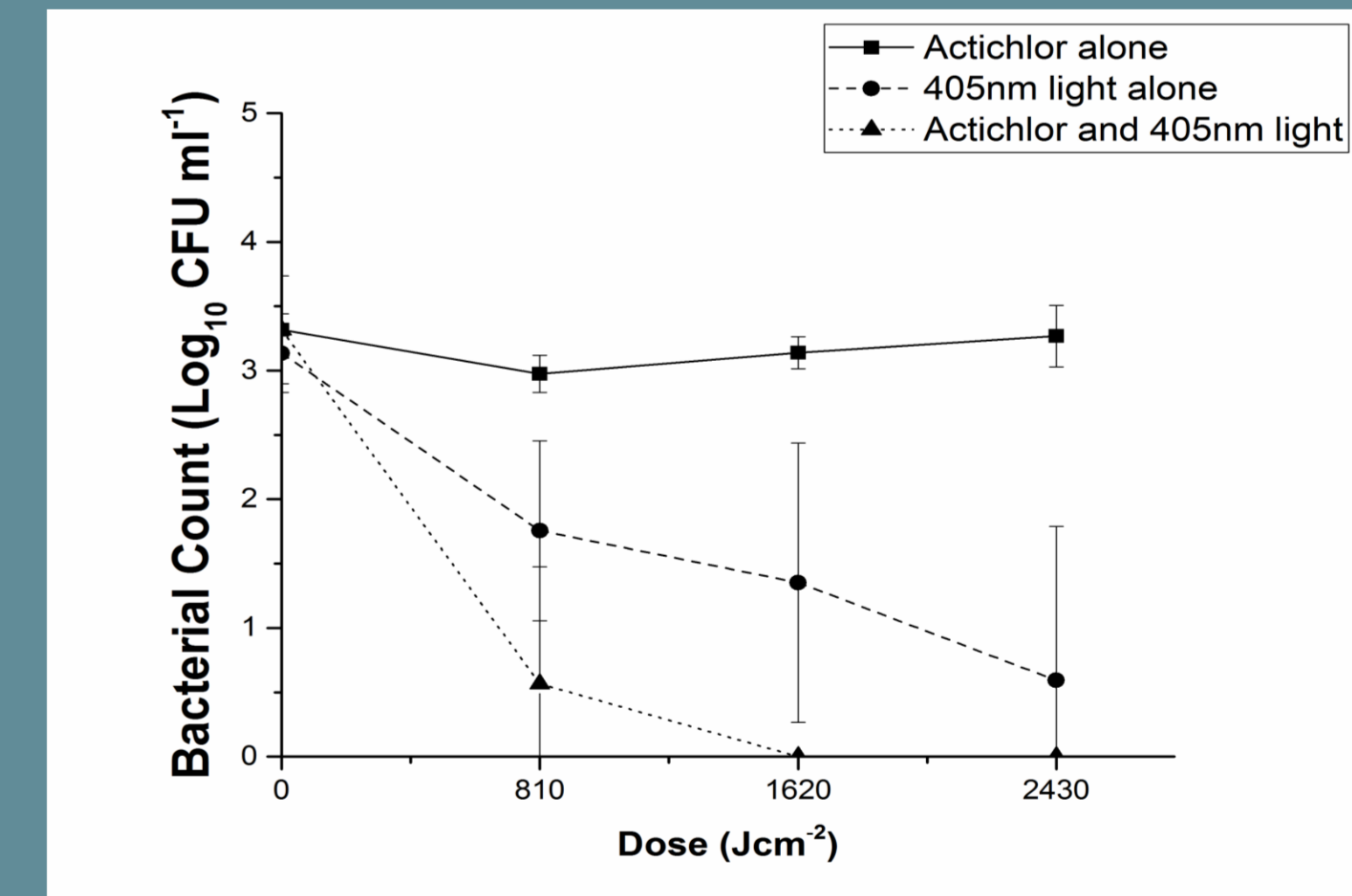
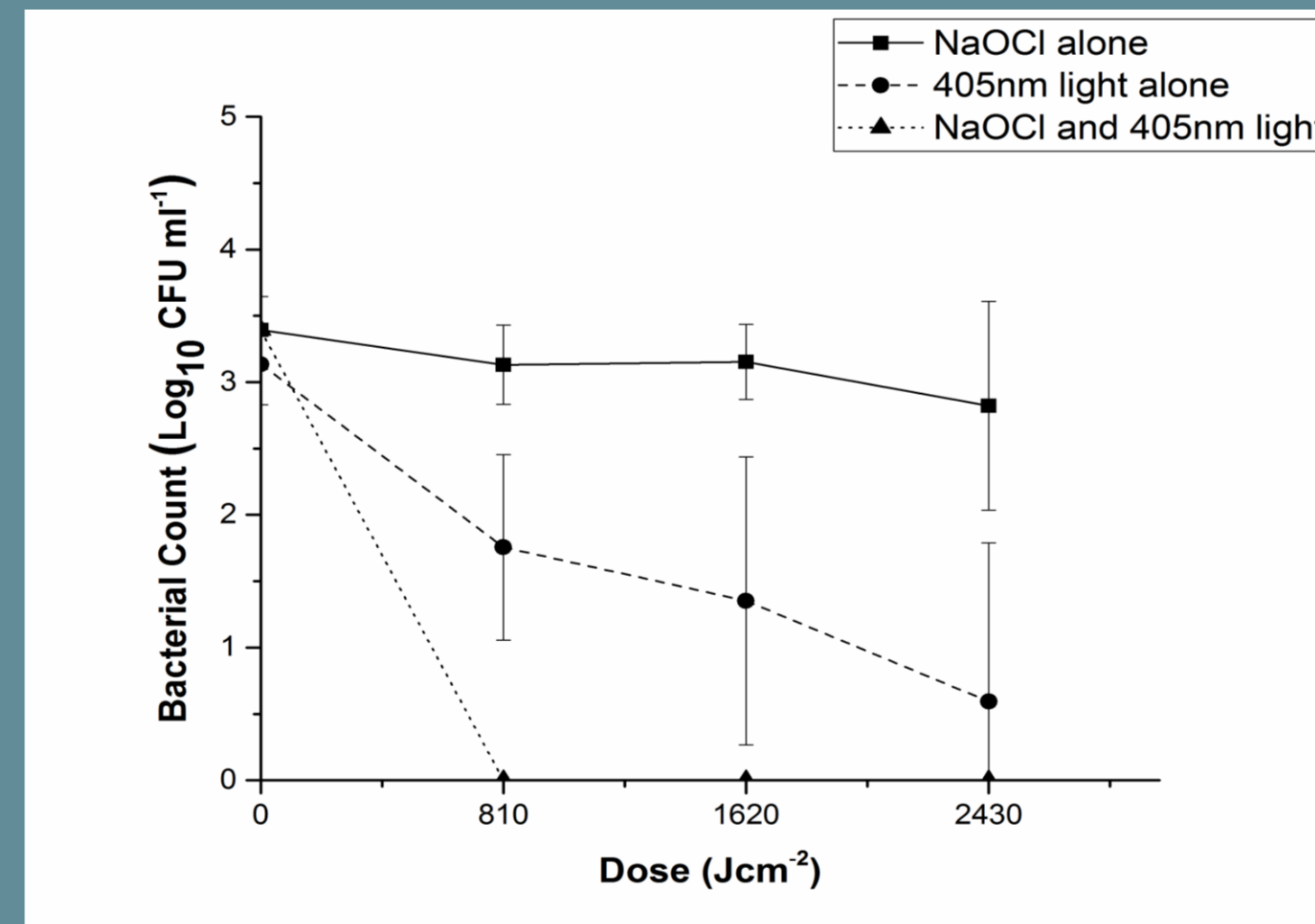
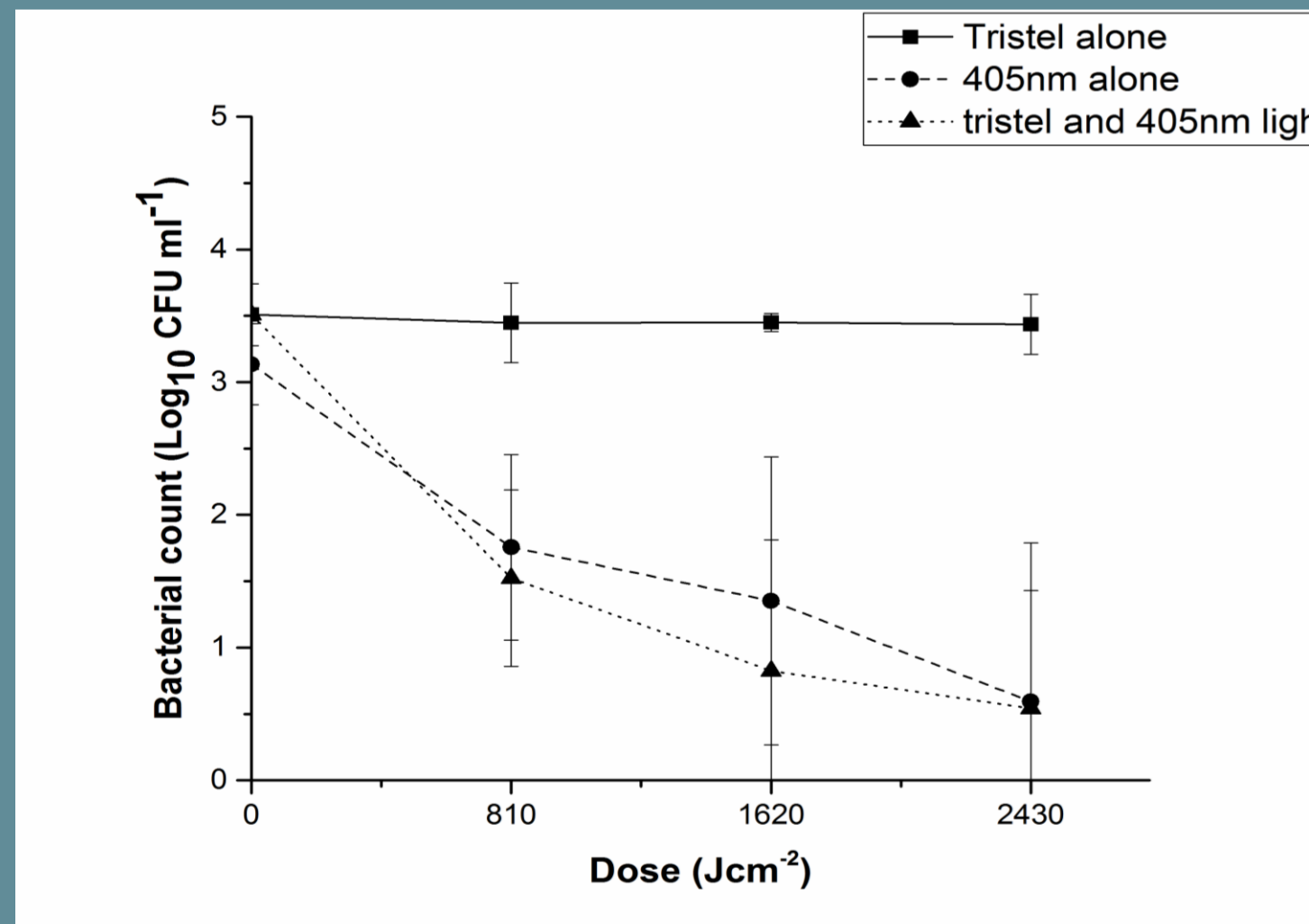
Results

High intensity 405nm light – 225mW/cm²

A) Vinyl Flooring



B) PVC



Spores exposed to disinfectants (0.0001% Tristel, 0.1% sodium hypochlorite (NaOCl) and 0.001% Actichlor) in combination with 405nm light at an irradiance of 225mW/cm² on (A) vinyl flooring and (B) PVC. Control samples were exposed to 405nm light alone, and disinfectants alone, to establish the sporicidal activity of each agent, and to demonstrate the synergistic effect when combined (n>6).

Low irradiance 405 nm light– 0.4 mW/cm²

Surface	Dose (J/cm ²)	Tristel (CFU/ml)						Actichlor (CFU/ml)					
		405 nm light alone	SD	Tristel Alone	SD	405nm light and Tristel	SD	405 nm light alone	SD	Tristel Alone	SD	405nm light and Tristel	SD
Vinyl Flooring	0	3.71	0.06	3.71	0.06	3.71	0.06	3.54	0.15	3.54	0.15	3.54	0.15
	242	4.00	0.20	3.90	0.13	3.80	0.24	3.61	0.06	3.67	0.08	3.45	0.2
	484	3.50	1.2	4.00	0.18	3.03	1.28	3.76	0.08	3.29	0.17	2.63	0.2
PVC	0	3.67	0.17	3.66	0.17	3.66	0.17	3.59	0.12	3.59	0.12	3.59	0.12
	242	3.67	0.34	4.10	0.12	3.80	0.08	3.81	0.06	3.72	0.04	3.55	0.08
	484	3.21	0.17	3.92	0.12	3.21	0.65	3.77	0.12	3.00	0.14	2.64	0.14

Spores exposed to 0.0001% Tristel and 0.001% Actichlor and low irradiance 405nm light (~0.4mW/cm²) on Vinyl Flooring and PVC. Control samples were exposed to 405nm light alone, and disinfectants alone, to establish the sporicidal activity of each agent, and to demonstrate the synergistic effect when combined (n>6).

Discussion & Conclusions

- High irradiance 405nm light demonstrated increased sporicidal activity against *C. difficile* seeded on vinyl flooring and PVC surfaces when used in combination with low-concentration sodium hypochlorite and Actichlor. At the concentrations used in this study, Tristel demonstrated limited synergy on vinyl and PVC.
- Similarly, at the doses used in this study low irradiance 405nm light began to demonstrate slight synergy with Actichlor on both surfaces, whereas Tristel does not yet show enhanced inactivation. This work is ongoing and will continue to test increased doses similar to those found to be effective with the high irradiance light.
- The study has shown that the sporicidal efficacy of some chlorinated hospital disinfectants commonly used for surface cleaning can be enhanced when used alongside 405nm light. This has the potential to lead to a considerable reduction in exposure time and concentrations of disinfectant required to eliminate *C. difficile* spores.