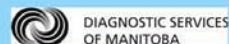


The Oxivir_{Tb} Formulation of Accelerated Hydrogen Peroxide (AHP) is Effective for Killing *Clostridium difficile* spores on Toilet Seat Surfaces

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Introduction:

Clostridium difficile associated diarrhea (CDAD) is a significant problem in healthcare facilities world-wide. The presence of *C. difficile* spores in toilets of patients with CDAD is thought to be a reservoir for the spread of the organism. Although bleach has been suggested as an efficient means of killing *C. difficile* spores it has a number of workplace safety concerns.

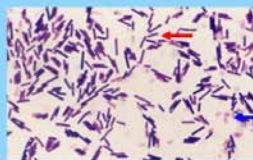
Aim of the study:

The aim of the study was to determine if any AHP formulations could provide an alternative to bleach for killing *C. difficile* spores on toilet surfaces in the presence of Artificial Test Soil (ATS) to provide an organic challenge.

Materials and Methods:

1. *Clostridium difficile* spore preparation:

Clostridium difficile clinical strain 765 was used for all studies. The vegetative form is indicated by red arrows and the spore form is indicated by blue arrows.



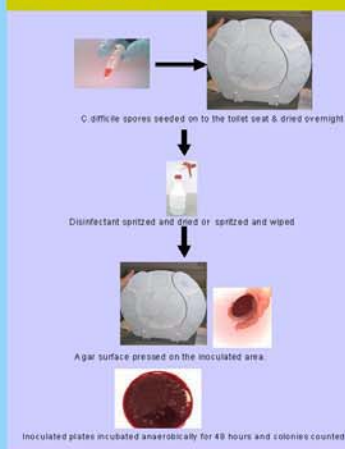
This organism was subcultured onto Anaerobic blood agar media (BAK) and allowed to incubate for 48 hours at 35°C, and then at room temperature for 2 weeks. The growth from 20 BAK plates was harvested using PBS and stored for 2 weeks in the refrigerator. The preparation was evaluated using Malachite green staining to ensure the predominance of the spore form.

The same spore preparation was used for all experiments.

2. Suspension testing:



3. Surface testing:



Results:

Table 1. Suspension testing: Effect of various disinfectants on spore kill

Time of exposure (minutes)	Average Log ₁₀ cfu/ml of <i>C. difficile</i> spores post-exposure to disinfectants:*				
	Oxivir _{Tb}	PerDiem	Bleach 500 ppm	Bleach 1000 ppm	Bleach 5000 ppm
1	3.84	6.04	5.22	3.40	0.33
5	3.54	5.92	0.19	0.36	0.17
10	3.49	4.42	0.00	0.30	0.17
15	2.45	4.47	0.00	0.38	0.12
20	2.43	4.59	0.22	0.40	0.12

*The suspension contained 5.9 Log₁₀ cfu/ml of *C. difficile* spores.

Table 2 Surface testing: Effect of various disinfectants on spore kill*

Disinfectant	Spritz and air dry	Spritz and wipe
	Number of tests showing ≥300 colonies of <i>C. difficile</i> (%)	Number of tests showing ≥300 colonies of <i>C. difficile</i> (%)
Oxivir _{Tb} (0.5% H ₂ O ₂)**	3/21 (14.36%)	3/18 (16.7%)
PerDiem*** (0.05% H ₂ O ₂)	21/21 (100%)	3/18 (16.7%)
Bleach 500 ppm	20/21 (95%)	2/18 (11.1%)
Bleach 1000 ppm	12/21 (57%)	1/18 (5.6%)
Bleach 5000 ppm	2/21 (9.5%)	0/18 (0%)

* Each site inoculated had 5 Log₁₀ cfu/ml of *C. difficile* spores

**Ready to use formulation

*** 0.05% achieved as per manufacturer's recommended use-dilution of 1:64

Discussion:

Current guidelines recommend that the physical action of cleaning is the most important parameter and that the microbial killing (i.e. surface disinfection) is important only in outbreak situations in healthcare environments.¹ Low level disinfectants are recommended for regular toilet cleaning. Recent reports regarding increased rates and severity of *C. difficile* associated disease have raised concern regarding the efficacy of using low level disinfectants for toilet cleaning.

Clostridium difficile spores in suspension were effectively killed by all concentrations of bleach evaluated. As the concentration increased, the time of exposure required to kill the spores decreased. Oxivir_{Tb} was more effective than PerDiem at the use-dilution tested (1:64). A major reduction in spore viability was achieved after a one minute exposure to Oxivir_{Tb}.

To reflect in-use conditions, disinfectants should be evaluated in the presence of organic material using surface testing methods. The killing action of surface disinfectants is hampered by the presence of residual organic material and may result in environmental *C. difficile* spore reservoirs that could contribute to nosocomial transmission.

Our study indicated in the presence of organic material (i.e. ATS), Oxivir_{Tb} and Bleach at 5000 ppm still killed spores efficiently when the agent was sprayed onto the toilet seat (i.e. no physical wiping). Our data support the importance of ensuring good physical action combined with a disinfectant agent with rapid sporicidal capacity for optimal removal of *C. difficile* spores from toilet seat surfaces.

Conclusions:

- Suspension testing indicated that bleach was the most effective at killing *C. difficile* spores.
- Bleach 5000 ppm is corrosive, and is an occupational hazard for health care workers. Oxivir_{Tb} is a possible alternative since it has a rapid sporicidal effect and is not harmful to personnel.
- Physical cleaning of the surface combined with an active disinfectant is the optimal approach for removing residual *C. difficile* spores from toilet seat surfaces.

References:

1. Infection Control Guidelines, Hand washing, cleaning, disinfection and sterilization in health care. CCDD 1997 vol 24S8