

Report on Sporicidal Efficacy of TECcare® T.U.F. System on *C. difficile* Spores

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Introduction

Clostridium difficile (*C. difficile*) is a naturally occurring anaerobic, gram negative bacterium which can be found in the gastrointestinal tract (GI tract) of mammals and the general environment. It is estimated that *C. difficile* can be found in 2 -3 % of the human population¹. Most individuals colonised by *C. difficile* will be unaware of its presence, however when the normal competing bacteria found in the GI tract (normal gut flora) are depleted, i.e. through use of antibiotics, the organism can cause severe diarrhoea and other potentially life threatening intestinal diseases.

When removed from the optimum growing conditions (anaerobic, moist, warm) found in the GI tract and placed under stress, *C. difficile* forms a spore which can tolerate extreme conditions that the active bacteria (flora) cannot, allowing it to survive dormant in the open environment for extended periods of time.

Once an area has become contaminated, usually after the stay of an infected person, a deep clean process must be undertaken to remove and or destroy any spores that may be present. However many traditional cleaning and disinfection products are ineffective at killing the pathogenic spores and may actually proliferate the spread.

TECcare® ULTRA is a safe², new ultra high level disinfectant / cold sterilant³ that in laboratory conditions has proved to be a highly effective sporicidal agent, consistently producing >8 log reductions in less than 10 minutes contact time⁴.

Traditional EN13704 test methods use a dilution process to test the sporicidal efficacy of many disinfectants, this procedure may distort the test results in favour of the product being tested, as spores in dilution naturally transform from their 'spore' state to flora, which is relatively easy to kill.

A new test method has been developed to measure sporicidal efficacy in the environment, using dried *C. difficile* spores retained on Petri dishes which are placed strategically within a test area to mimic those conditions where spores are naturally found. This testing method was used to examine the sporicidal

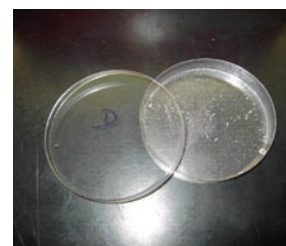
TECcare®
ULTRA

efficacy of vapourised TECcare® ULTRA using the T.U.F. System.

Preparation of the *C. difficile* Spores

A BHI broth was inoculated with a *Clostridium difficile* colony from a Columbia Agar plate and incubated for 10 days. The broth was reduced to a 10th of the volume by centrifuge and diluted with saline solution.

1ml of the test mixture was mixed with 9ml ethanol and allowed to evaporate in a 9cm diameter Petri dish. The spores were dried on the test Petri dish.



Method

The testing method was designed to mimic 'normal' conditions found within a healthcare facility but with the controls in place to ensure an accurate representative sample could be taken and the efficacy of TECcare® ULTRA mist, deployed via the T.U.F. System could be measured.

The test was conducted in a two bedded room with toilet facilities within the hospital. The test sample Petri dishes containing a defined quantity of *C. difficile* spores were placed in specified points within the room (see *Diagram 1*). A control sample was held in similar conditions to the test samples, but without exposure to the TECcare® ULTRA mist, to measure the volume of *C. difficile* that could be found in either flora or spore state.

The room was a total of 54m² and consisted of 3 areas;

- Bedroom with 2 beds - 36m²
- Bathroom - 6m²
- Entrance - 12m²

The TECcare® T.U.F. System was placed in the bedroom. Prior to treatment the air conditioning vent and entrance door were sealed up with tape to prevent the mist escaping.



TUF
SYSTEM

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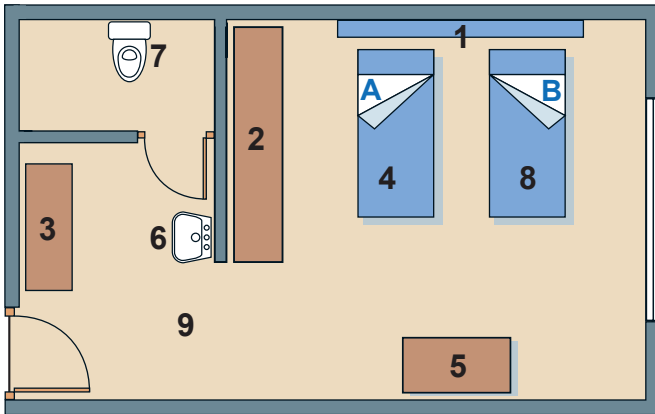


Diagram 1

The details of application were as follows:

- 27 minutes treatment time + 1 hour of mist settlement.
- 972ml dispensed at 1/15 concentration.

After treating the room, 10ml of Létheen broth was added to the Petri dishes and left to mix for 10 minutes. 200 µl of various dilutions were added to CCFA+T, medium containing bile salts in order to encourage de-sporification. After 48 hours in an anaerobic state at 37°C the number of colonies per Petri dish was counted.

Results

PETRI DISH LOCATION	NO. OF COLONIES PER ML		
	C. DIFF STRAIN NAP1A / 027 (1067)	C. DIFF STRAIN VPI 10463	C. DIFF STRAIN ATCC 43598
Control	14,500,000	16,300,000	17,600,000
Headboard (1)	0*	0*	0*
Wardrobe Bedroom (2)	0*	0*	0*
Wardrobe Entrance (3)	90*	110*	120
On top of Bed A (4)	0*	0*	0*
Table (5)	0*	0*	0*
Wash Basin Entrance (6)	60*	70*	75*
Toilet (7)	80*	85*	90*
Underneath Bed B (8)	0*	0*	0*
Floor Entrance (9)	20*	15*	30*

* >6 Log Reduction - this was achieved in all locations including the more isolated test points within the room.

Conclusion

The T.U.F. System utilising TECcare® ULTRA reduced the sporicidal activity within the test area by a 6 log at a dilution of 1:15.

It is concluded from these results that the T.U.F. System using TECcare® ULTRA at a 1:15 dilution is highly effective at decontaminating environmental areas after contamination with *C. difficile* spores.

The Controls used within this test procedure verify the accuracy of these results.

Recommendations

The T.U.F. System at 1:15 dilution is highly effective but a greater mist settlement time was required compared with that originally suggested by the manufacturer.

The manufacturer has, subsequent to this study amended their recommended treatment and mist settlement times. In addition, an angle bracket is now provided with the T.U.F. System to elevate the dispensing nozzle into the centre of the room for a more effective spread of the TECcare® ULTRA mist.

A decontamination kit is now also provided by the manufacturer comprising a T.U.F. bottle pre-dosed with TECcare® ULTRA, 10 validation test strips and a roll of room sealant tape.

References

1. Ryan K.J., Ray C.G. (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp.322-4
2. Material Safety Data Sheet (available from Talley Environmental Care Ltd)
3. Independent Microbial Testing (available from Talley Environmental Care Ltd)
4. Rapport de l'action sporicide du produit TECcare, Cliniques universitaires St-Luc, Faculté de médecine, Unité de Microbiologie, Centre de référence *C. difficile*, Brussels, Belgium

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