TEST REPORT

**TEST REPORT**: Determination of the sporidical activity of Clinell Sporicidal against *Clostridium difficile* rybotype 027.

**Test laboratory**: Welsh School of Pharmacy, Cardiff University, King Edward VII avenue, Cardiff CF10 3NB, UK

**CLIENT**

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**Disclaimer**: this work was conducted as part of a wider investigation about the efficacy of sporidical wipe commonly used in the UK. This report is an extract of this work. This work resulted in a peer-reviewed publication: Siani H, Cooper CJ and Maillard J-Y. (2011) Efficacy of 'sporicidal' wipes against Clostridium difficile. American Journal of Infection Control, 39(3), 212-218.

**Identification of sample**

- Name of product: Clinell sporidical wipe
- Manufacturer: GAMA Healthcare Ltd

**Test method**

Preparation of spores: A single colony was inoculated to 50 mL of reduced Brain Heart Infusion (BHI) broth (Oxoid, UK) and cultured for 10 days at 37°C under anaerobic conditions (5% H2:10% CO2:85% N2) in a Electrotek GW200 workstation (Electrotek, Shipley, UK). The broth culture was then centrifuged at 5000 g for 15 min at 4°C (Heraeus Primo R, Thermo, UK). The supernatant was discarded and the pellet re-suspended in 2.5 mL sterile ice cold water and 10 mL absolute ethanol before incubation at room temperature for 1 h. The suspension was centrifuged twice as above and washed with chilled (4°C) sterile de-ionized water between centrifugations. Four spore preparations were then pooled, heated to 80°C for 10 min prior to centrifugation at 5000 g for 10 min at 4°C and re-suspended in 1 mL sterile de-ionized water.
Enumeration of spores: the spore suspension was diluted in tryptone sodium chloride (TSC; 1 g/L tryptone (Oxoid) and 8.5 g/L sodium chloride (Fisher Scientific)) and dilutions plated onto BHI agar supplemented with 0.1% (w/v) sodium taurocholate (Fisher Scientific). All media used for the enumeration of *C. difficile* were reduced for 24 h prior to use.

Test protocol: The Clinell sporicidal wipe sample was directly inoculated with 20 µL of the test spore suspension (~7 log_{10} CFU). Following 5 min exposure, wipe sample was transferred into glass bottles containing neutralizer (10 mL) and glass beads (5 g; 3 mm diameter). After 1 min vortex mixing and neutralization for 5 min, the suspension was serially diluted in TSC and used to inoculate BHI agar plates containing 0.1% (w/v) of sodium taurocholate. Bacterial colonies were counted after 48 h anaerobic incubation at 37°C.

**Experimental conditions**

- **Product diluent used:** none
- **Product test sample:** 2 x 2 cm² aseptically cut
- **Contact time:** 5 min
- **Test temperature:** 22 ± 1°C
- **Interfering substance:** none
- **Neutralising solution:** Saponin (Sigma) 30 g/L, L-histidine (Sigma) 1 g/L, polysorbate 80 (Sigma) 30 g/L, azolectin from soybean (Sigma) 3 g/L and sodium thiosulphate (Fisher) 5 g/L prepared in TSC.

**Identification of bacteria:** *Clostridium difficile* 20291, ribotype 027

**Reproducibility:** three replica were performed

**Test Results**

The Clinell sporicidal wipe reduced the number of spores of *C. difficile* 20291 (ribotype 027) by 3.74 Log_{10} within 5 min contact time (Table 1).

**Table 1 Sporicidal activity against *C. difficile* 20291 (ribotype 027) (n=3)**
Sporicidal effect
(log_{10} reduction ±SD)

| Clinell sporicidal wipe | 3.74 (± 2.26) |

Conclusions

The Clinell sporicidal wipes showed a sporicidal activity against *C. difficile* 20291 (ribotype 027) within 5 min contact time at 20°C. The large standard deviation observed is caused by 1 value out of the 3 repeats; that log_{10} reduction value is lower than the other 2 values obtained.

Signed

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