



Toulouse, June 10th 2015

STUDY 15-1823

Determination of sporicidal activity for aerial surface disinfection processes
According to the method described
in the standard NF T 72-281 (November 2014)

Medical area

Additional Conditions: *Clostridium difficile* (spore) - 6 days of treatment

Promotor

OXY'PHARM
917 rue Marcel Paul
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Test laboratory

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1. Test Laboratory

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2. Identification of the aerial disinfection system

Apparatus: NOCOSPRAY

Serial number: 37S347

Disinfectant: NOCOLYSE®

Batch: 070415N (Expiry date: 04/2017)

Concentration of product in the room: 1 mL/m³

Each day, one treatment with 30 minutes of wait (5 carriers recovery after waiting)

Amount of disinfectant diffusion \approx 52 mL/treatment of 1 mL/m³.

Promotor : OXY'PHARM

Storage conditions: Ambient temperature

Period of testing: May - June 2015

Actives Substances: Hydrogen peroxide

3. Experimental Conditions

a. Tests micro-organisms

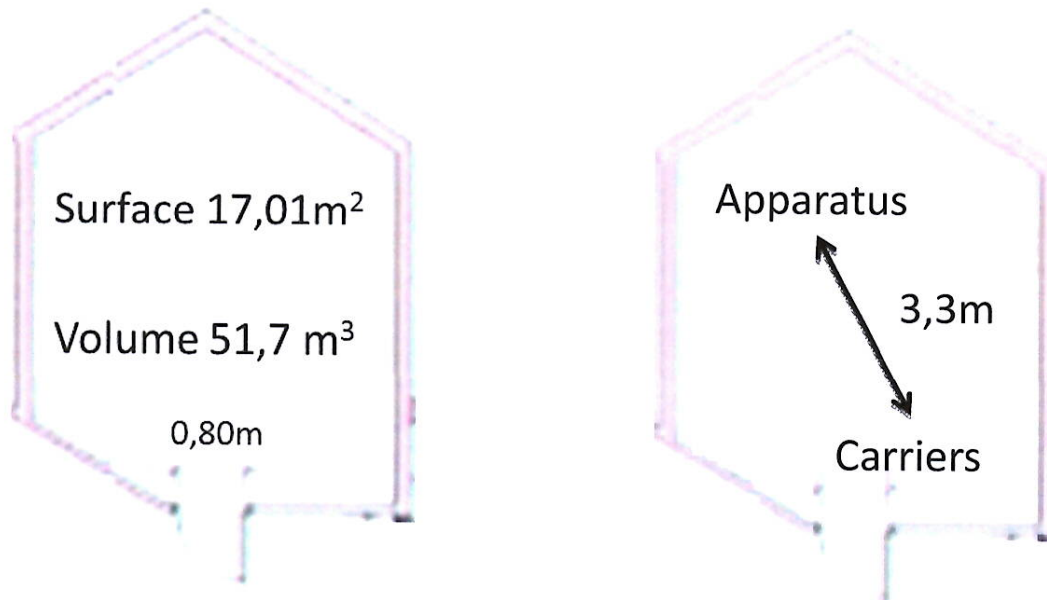
- Sporicidal activity :
 - o *Clostridium difficile* (spore) NCTC 13366

b. Carriers

The selected tests surfaces are stainless steel discs, flats, corresponding to the requirements of paragraph 5.2.3.1 of the standard. The supplier is CONTIGIANI (Toulouse).

c. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 44% to 62%.

Initial temperatures ranging from 19,4°C to 20,5°C.

Test room volume : 51,7m³.

Distance between the apparatus and the carriers : 3,3m (tableau B.1).

d. Diluants and culture media

Interfering substances

1/20 reconstituted milk (Internal preparation - Batch 5972 Exp. June/27/2015)

Diluants

Suspension preparation: EPPI (Cooper - Batch 19HD03GA Exp. March 2017)

Recovery solution (Internal preparation - Batches 5978 and 5990)

Filtration membranes

Nitrocellulos membranes 0,45µm (Millipore, batch F4SA32924 Exp. December 2016)

Culture media

Medium for *Clostridium difficile* (Internal preparation - Batches 5973 Exp. June/27/2015 and 5982 Exp. July/01/2015)

4. Assays

- Treatment 1 mL / m³ – waiting 30 minutes. One treatment per day and for 6 days

<i>Clostridium difficile</i>	N Test suspension (CFU/mL)	Preliminary assay			T Control (CFU/spot – 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction – Mean
		n1/N1	n2/N2	n3/N1			
	2.10 ⁵ – 5.10 ⁵	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁴		
DAY 1 Date May/28/15 B: 19,4°C/44% RH E: 19,5°C/47% RH	2,79.10 ⁵	d1 : 54/45 d2 : 64/45	d1 : 50/46 d2 : 51/46	d1 : 66/45 d2 : 59/45	d1 : 2,63.10 ⁴ d2 : 3,00.10 ⁴ T = 2,82.10 ⁴	d1 : 110 + 1 d2 : 220 + 1 d3 : 195 + 0	R1 : 2,40 R2 : 2,11 R3 : 2,16 R = 2,22
DAY 2 Date May/29/15 B: 19,6°C/46% RH E: 19,6°C/49% RH	2,79.10 ⁵	d1 : 35/53 d2 : 36/53	d1 : 40/39 d2 : 40/39	d1 : 50/53 d2 : 28/53	d1 : 2,11.10 ⁴ d2 : 1,95.10 ⁴ T = 2,03.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 4,31 R2 : 4,31 R3 : 4,31 R > 4,31
DAY 3 Date June/01/15 B: 19,9°C/49% RH E: 20°C/53% RH	2,79.10 ⁵	d1 : 21/34 d2 : 22/34	d1 : 31/28 d2 : 30/28	d1 : 27/34 d2 : 23/34	d1 : 0,44.10 ⁴ d2 : 0,38.10 ⁴ T = 0,41.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 3,61 R2 : 3,61 R3 : 3,61 R > 3,61

<i>Clostridium difficile</i>	N Test suspension (CFU/mL)	Preliminary assay			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration - disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	2.10 ⁵ - 5.10 ⁵	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁴		
DAY 4 Date June/02/15 B: 20°C/52% RH E: 20,1°C/55% RH	2,79.10 ⁵	d1 : 44/20 d2 : 45/20	d1 : 32/32 d2 : 20/32	d1 : 48/20 d2 : 38/20	d1 : 0,88.10 ⁴ d2 : 0,91.10 ⁴ T = 0,90.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 3,95 R2 : 3,95 R3 : 3,95 R > 3,95
DAY 5 Date June/03/15 B: 20,2°C/54% RH E: 20,2°C/58% RH	2,79.10 ⁵	d1 : 28/24 d2 : 28/24	d1 : 25/30 d2 : 30/30	d1 : 26/24 d2 : 27/24	d1 : 0,7.10 ³ d2 : 0,6.10 ³ T = 0,65.10 ³	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 2,81 R2 : 2,81 R3 : 2,81 R > 2,81*
DAY 6 Date June/04/15 B: 20,4°C/59% RH E: 20,5°C/62% RH	2,79.10 ⁵	d1 : 21/28 d2 : 30/28	d1 : 27/25 d2 : 24/25	d1 : 30/28 d2 : 30/28	d1 : 0,50.10 ³ d2 : 0,38.10 ³ T = 0,44.10 ³	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 2,64 R2 : 2,64 R3 : 2,64 R > 2,64*

* With control < 10³ UFC/spot, the maximum log reduction is less than 3 log.

T: counting of micro-organisms on the discs.

N₁: counting of test suspension by pour plate technique - N₂: counting of test suspension by filtration method

n₁: counting to search inhibitor effect in agar medium - n₂: counting to search inhibitor effect on membrane filtration - n₃: counting to search inhibitor effect after inclusion of disc in agar medium

n'₁: number of survival micro-organisms in 100mL of tryptone-salt - n'₂: number of micro-organisms after inclusion of the disc in agar medium.

n'₁ + n'₂: total number of survival micro-organisms on the carrier surface.

d1: disc N°1 / d2: disc N°2 / d3: disc N°3

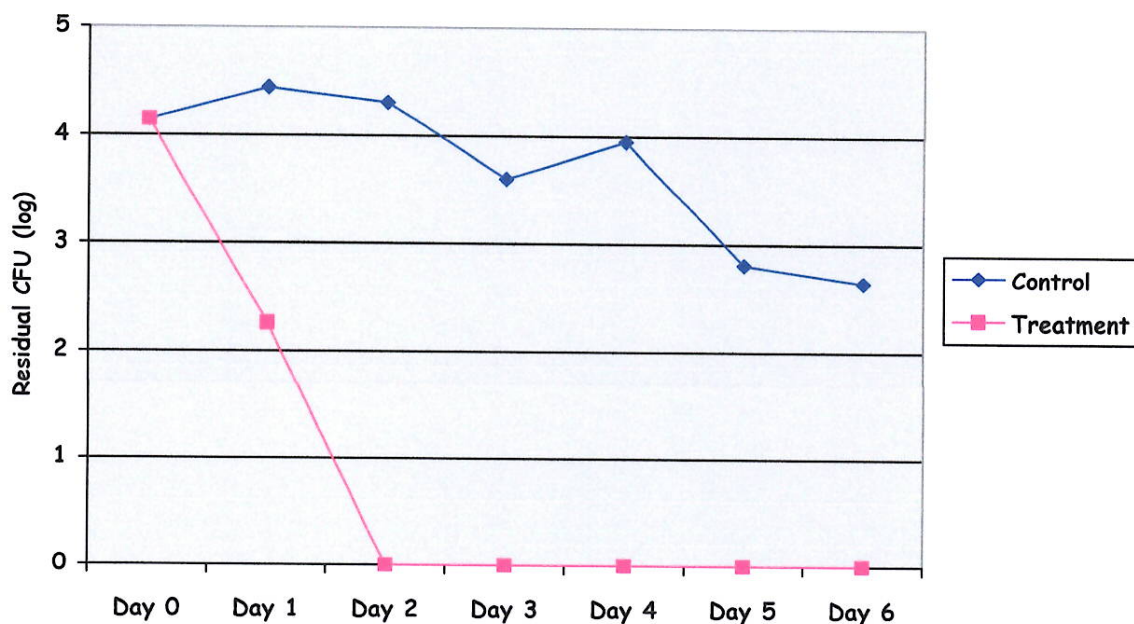
5. Conclusion

According to the conditions of test, the couple apparatus/product led to:

- A sporicidal activity (log reduction ≥ 3)
 - o After only 2 days with 1 treatment/ day with $1\text{mL}/\text{m}^3$ treatment - 30 minutes of wait on the following strain :
 - *Clostridium difficile* (spore) NCTC 13366

The log reductions were determined according to the standard (i.e. versus T: counting of microorganisms on the discs after drying and waiting time).

To better interpret the effect of cumulative treatments, the evolution of residual viable counts was presented for T and (n'1 + n'2) on the following graph:



Evolution of residual viable counts according to the duration of experiment

This graph underlines:

- a progressive decrease of CFU for the T discs even for *C. difficile* under spores. Controls performed on the initial suspension indicate that spores represent more than 99% of the cells. This observation may suggest that in normal conditions (temperature, RH), one part of the spores progressively return to a vegetative form not able to resist on the discs.
- a rapid and dramatic activity of cumulative treatments even at very low concentration with no detection of viable residual cells only after the second treatment at $1\text{L}/\text{m}^3/\text{day}$.