

Toulouse, May 6th 2015

STUDY 15-1780

**Determination of bactericidal, fungicidal, sporicidal, mycobactericidal and virucidal 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)

Promotor

OXY'PHARM
917 rue Marcel Paul
94500 CHAMPIGNY SUR MARNE

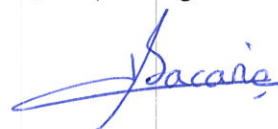
Test laboratory

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1. Test Laboratory

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2. Identification of the aerial disinfection system

Appartus : NOCOSPRAY
Serial number : 37S347

Disinfectant : NOCOLYSE ONE SHOT
Batches : 160315N+F (Expiry date: 03/2017)

Concentration of product in the room : 3 ml/m³
One treatment with one hour of wait (carriers recovery after waiting)
Amount of disinfectant diffusion \approx 155 mL/treatment of 3mL/m³.

Promotor : OXY'PHARM

Storage conditions: Ambient temperature
Period of testing: April 2015
Actives Substances: Hydrogen peroxide

3. Experimental Conditions

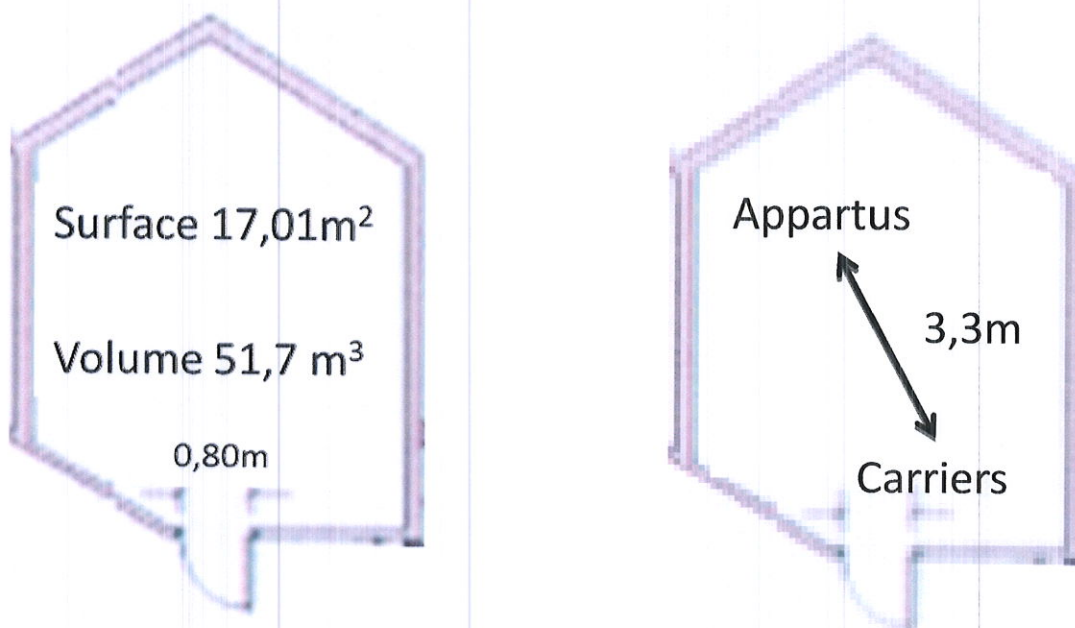
a. Tests micro-organisms

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

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).

b. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 50% to 65%.

Initial temperatures ranging from 19°C to 20°C.

Test room volume : 51,7m³.

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

Diluants and culture media

Interfering substances

1/20 reconstituted milk (Batch 5866 Exp. May/13/2015)

Diluants

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

Recovery solution (Batch 5881)

Filtration membranes

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

Culture media

Medium for Clostridium difficile (Batch 5892 Exp. May/20/2015)

4. Assays

a. Sporocidal activity

- Treatment 3 mL / m³ - waiting 1H

Tests	N	Preliminary assay			T	n'1 + n'2 UFC/ spot 50µL (dilution/filtration - disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
microorganisms	Test suspension (CFU/mL)				Control (CFU/spot - 50µL)		
	2.10 ⁵ - 5.10 ⁵	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁴		
<i>C. difficile</i>							
Date Apr/21/2015					d1 : 2,8.10 ⁴		R1 : 4,58
B: 19,4°C/RH 54%	4,6.10 ⁵	d1 : 24/46	d1 : 29/42	d1 : 27/46	d2 : 4,75.10 ⁴	d1 : 0 + 0	R2 : 4,58
E: 19,4°C/RH 63%		d2 : 28/46	d2 : 34/42	d2 : 35/46		d2 : 0 + 0	R3 : 4,58
					T = 3,78.10 ⁴	d3 : 0 + 0	R = 4,58

T: counting of micro-organisms on the discs.

N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method

n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : 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 3 mL/m³ treatment - 1 hour of wait on the following strain :
 - *Clostridium difficile* (spore) NCTC 13366