

Toulouse, February 20th 2020

STUDY 19 - 2571

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

Human Healf

***Mycobacterium terrae* ATCC 15755 - 6 days of treatment**

Client

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1. Test Laboratory

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2. Identification of the aerial disinfection system

Device: NOCOSPRAY
Serial number: 172X731

Disinfectant: NOCOLYSE NEUTRAL 6%
Batch: 141119N/2 (Expiry date: November 2021)
Packaging : 1L

Concentration of product in the room: 1 mL/m³
Each day, one treatment with 1H of wait (5 carriers recovery after waiting)
Amount of disinfectant diffusion \approx 32 mL/treatment of 1 mL/m³.

Promotor : OXYPHARM

Storage conditions: Ambient temperature
Period of testing: January - February 2020
Actives Substances: Hydrogen peroxide (6%)

3. Experimental Conditions

a. Tests micro-organisms

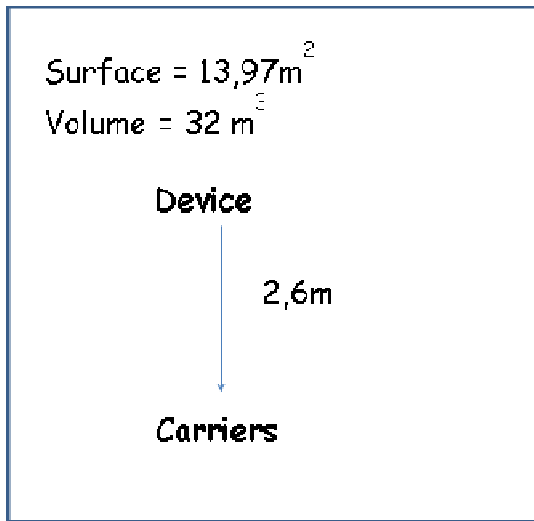
- Mycobactericidal activity :
 - o *Mycobacterium terrae* ATCC 15755

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 MERCIER CLAUSESE.

c. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 35% to 46%.

Initial temperatures ranging from 18,2°C to 19,6°C.

Test room volume : 32m³.

- Carriers

Distance between the device and the carriers: 2,6m (Annexe B - Table B.1).

The carriers were placed at a high of 1,15m, in vertical position, inoculum opposite to the device.

d. Diluants and culture media

Interfering substances

1/20 reconstituted milk (Internal preparation - Batch 9115 Exp. Feb/03/2020)

Diluants

Suspension preparation: EPPI (Cooper - Batch Lot 19MKA300 Exp. September 2021)

Recovery solution (Internal preparation - Batches 9114 Exp. Feb/03/2020 and 9123 Exp. Feb/07/2020)

Filtration membranes

Nitrocellulose membranes 0,45µm (Millipore - Batch F8NA26143E Exp. October 2020)

Culture media

Middlebrook Agar medium + OADC (Internal preparation - Batches 9113 Exp. Feb/03/2020, 9122 Exp. Feb/07/2020 and 9128 Exp. Feb/09/2020)

4. Assays

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

<i>Mycobacterium terrae</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			
	1.10 ⁷ - 1.10 ⁸	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≥ 10 ⁵		
DAY 1 Date Jan/06/2020 19,5°C/46% RH	4,09.10 ⁷	d1 : 139/187 d2 : 129/187	d1 : 106/100 d2 : 83/100	d1 : 99/187 d2 : 103/187	d1 : 3,05.10 ⁶ d2 : 2,51.10 ⁶ T = 2,78.10 ⁶	d1 : 2,39.10 ⁴ + 49 d2 : 5.10 ³ + 4 d3 : 4,00.10 ⁴ + 15	R1 : 2,06 R2 : 2,74 R3 : 1,84 R = 2,21
DAY 2 Date Jan/07/2020 19,6°C/45% RH	-	d1 : 97/118 d2 : 95/118	d1 : 102/93 d2 : 91/93	d1 : 64/118 d2 : 80/118	d1 : 1,48.10 ⁶ d2 : 2,58.10 ⁶ T = 2,03.10 ⁶	d1 : 1,38.10 ³ + 2 d2 : 1,13.10 ³ + 0 d3 : 8,70.10 ² + 1	R1 : 3,17 R2 : 3,25 R3 : 3,37 R = 3,26
DAY 3 Date Jan/08/2020 19,6°C/45% RH	-	d1 : 59/105 d2 : 59/105	d1 : 47/79 d2 : 98/79	d1 : 131/105 d2 : 76/105	d1 : 5,23.10 ⁵ d2 : 6,82.10 ⁵ T = 6,03.10 ⁵	d1 : 89 + 0 d2 : 1,43.10 ² + 0 d3 : 3,0.10 ² + 0	R1 : 3,83 R2 : 3,62 R3 : 3,30 R = 3,58

<i>Mycobacterium terrae</i>	N Test suspension (CFU/mL)	Preliminary assay			T Control (CFU/spot - 50 μ L)	n'1 + n'2 UFC/ spot 50 μ L (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	$\approx 10^4$		
DAY 4 Date Jan/09/2020 19,2°C/44% RH	-	d1 : 109/124 d2 : 99/124	d1 : 85/94 d2 : 87/94	d1 : 64/124 d2 : 68/124	d1 : 1,17.10 ⁶ d2 : 1,49.10 ⁶ T = 1,33.10 ⁶	d1 : 82 + 0 d2 : 46 + 0 d3 : 63 + 0	R1 : 4,21 R2 : 4,46 R3 : 4,32 R = 4,33
DAY 5 Date Jan/10/2020 18,2°C/45% RH	-	d1 : 59/107 d2 : 69/107	d1 : 82/89 d2 : 80/89	d1 : 84/107 d2 : 87/107	d1 : 8,50.10 ⁵ d2 : 1,57.10 ⁶ T = 1,21.10 ⁶	d1 : 46 + 0 d2 : 16 + 0 d3 : 3 + 0	R1 : 4,42 R2 : 4,88 R3 : 5,61 R = 4,97
DAY 6 Date Jan/13/2020 19,2°C/35% RH	-	d1 : 81/156 d2 : 89/156	d1 : 103/102 d2 : 86/102	d1 : 78/156 d2 : 113/156	d1 : 4,20.10 ⁵ d2 : 1,43.10 ⁶ T = 9,25.10 ⁵	d1 : 22 + 0 d2 : 1 + 0 d3 : 85 + 1	R1 : 4,62 R2 : 5,97 R3 : 4,03 R = 4,87

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 device (NOCOSPRAY)/product (NOCOLYSE NEUTRAL 6%) led to:

- A mycobactericidal activity (log reduction ≥ 4)
 - o After 4 days with 1 treatment/ day with 1mL/m³ treatment - 1H of wait on the strain :
 - *Mycobacterium terrae* ATCC 15755

According to the conditions of test, control carriers do not indicate significant loss of viability (< 1 log).