



Toulouse, June 4th 2021

ASSAY REPORT N° 21-1716

STUDY 20-2794

STANDARD NF EN 17272 (Avril 2020)

Chemical disinfectants and antiseptics -

Methods of airborne room disinfection by automated process - Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities

Medical area

Clean conditions

Client

OXY'PHARM

829 rue Marcel Paul

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FRANCE

Assay laboratory

FONDEREPHAR

Faculté des Sciences Pharmaceutiques

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1. Test Laboratory

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2. Identification of the aerial disinfection system

Device : **NOCOSPRAY 2**
Serial number :172X731

Disinfectant : **NOCOLYSE NEUTRAL 6%®**
Batch : A281020N/1
Exp.: 10/2022
Receipt : Nov/03/2020

Disinfectant : **NOCOLYSE NEUTRAL 6%®**
Batch : A050121N/2
Exp. : 01/2023
Receipt: Jan/08/2021

Concentration of product: 5mL/m³
One treatment - Waiting time 120 minutes after the end of diffusion
Amount of disinfectant diffusion \approx 162,5 mL
Time of diffusion : 9 minutes 45 secondes

Promotor : OXY'PHARM

Storage conditions: Ambient temperature
Period of testing: November 2020 - May 2021

Actives Substances: Hydrogen peroxide (6%)

3. Experimental Conditions

a. Tests micro-organisms

- Bactericidal activity :
 - *Acinetobacter baumannii* CIP 7034
 - *Staphylococcus aureus* CIP 4.83
 - *Enterococcus hirae* CIP 58.55
 - *Escherichia coli* CIP 54.127
- Fungicidal activity :
 - *Candida albicans* DSM 1386
 - *Aspergillus brasiliensis* CBS 733.88

- Sporicidal activity :
 - *Bacillus subtilis* CIP 52.62
 - *Clostridium difficile* NCTCC 13366
- Mycobactericidal activity :
 - *Mycobacterium terrae* ATCC 15755
 - *Mycobacterium avium* ATCC 15769
- Virucidal activity (virus/receiving cells):

Adenovirus/HELA Cells

Virus

Origin: ATCC
ATCC reference: VR-5
Batch number supplier: 58486654
Internal number Batch: SS-7-180611 (passage N°7)

Receiving cells

Origin: ATCC
ATCC reference: CCL-2
Batch number ATCC: 4440136
Internal number Batch: WCB-140613 (passage N°30)

Murine Norovirus souche S99/RAW264.7 cells:

Virus

Origin : Friedrich Loeffler Institut Berlin
Supplier reference: RVB-651
Batch number supplier: 4/200409/220409
Internal number Batch: SS-5-110419 (passage N°5)

Receiving cells

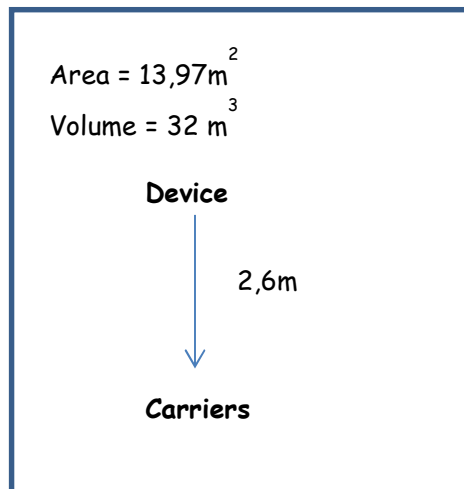
Origin : ATCC
Designation : RAW264.7
ATCC reference: TIB-71
Batch number ATCC: 5822175
Internal number Batch: WCB-210912 (passage N°35)

b. Carriers

The selected tests surfaces are stainless steel discs, flats, corresponding to the requirements of paragraph 5.2.3.2 of the standard. The supplier is MERCIER CLAUSSE (France).

c. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 50% to 64% (see results).

Initial temperatures ranging from 19,3°C to 20,5°C (see results).

Test room volume : 32m³.

Distance between the appartus and the carriers : 2,6m (tableau B.1), 1,15m from floor.

d. Diluants, culture media and membranes

Interfering substances

1/20 reconstituted milk (Internal preparation - Batches 9780 Exp. Nov/14/2020, 9883 Exp. Dec/16/2020, 10001 Exp. Jan/15/21 and 10155 Exp. Mar/15/21)

BSA fraction V 0,3g/l (Internal preparation - Batches 351, 361, 362, 367, 368, 383, 390)

Diluants

Suspension preparation: Tryptone salt (Biomérieux - Batches 1843680 Exp. Jun/22/2021 and 1856370, Exp. Jul/16/2021) or Water for Injectable Preparations (WIP)* (interference of product with Tryptone-salt) (Cooper - Batch 19MKA300 Exp. Sept/2021)

Diluant for *A. brasiliensis* (Internal preparation - Batch 52 Exp. Mar/07/21)

Recovery solution + 0,5% Tween80 (Internal preparation - Batches 9851, 9869, 9880, 10008, 10029, 10075, 10096, 10097)

Recovery solution (viruses): EMEM (Batches N°2848, N°2849, N°2856 and 2857)

Filtration membranes

Nitrocellulose membranes 0,45 µm (Millipore - white / Batches F0JB71371C and FOMB14755C - black / Batch F9HA42174)

Culture media

Malt Extract agar (Internal preparation - Batches 10015 Exp. Jan/29/21, 10030 Exp. Feb/04/21 and 10156 Exp. Mar/15/21)

Trypcase soy agar (Biomérieux - Lot 1008219370 Exp. Jan/30/2022 and 1008383190 Exp.

May/05/2022)

Middlebrook agar + OADC (Internal preparation - Batches 9775 Exp. Nov/14/2020, 9850 Exp. Dec/04/2020 and 9875 Exp. Dec/12/2020)

BHIYT-L Agar (Counting of *Clostridium difficile*) (Internal preparation - Batch 10080 Exp. Feb/18/2021)

EMEM (Batches N°2848, N°2849, N°2856 and 2857)

e. Virucidal activity: validation and titration

Control of sensitivity of cells to virus

- Add one volume of solution S or PBS + one volume of cellular suspension at 2.10^5 cells/ml for one hour in water bath at $36^\circ\text{C} \pm 1^\circ\text{C}$
- The cells are centrifuged at 1600tr/min for 10 min and resuspended in culture media
- The virus is diluted from 1/10 to 1/10 on a 96-well microplate (10 dilutions)
- Add 100 μl of cell suspension treated (Solution S) or not treated (PBS control) to each well of the microplate
- Incubate for 72 hours

The difference of title reduction between cells treated by the solution S and cells treated by PBS shall be $< 1 \lg$.

Control of efficiency for suppression of disinfectant activity

- Add 1 volume of BSA + 1 volume of virus suspension + 1 volume of solution S or distilled water
- Leave the mixture in the ice bath for 60 min at room temperature

Titration method

- Titrate the virus (method titration on cell in suspension) by following steps:
- Serial dilutions (1/10) are realized with culture medium in the glass tube
- Transfer 0,1 ml of each dilution into eight wells of a microplate plaque
- The last row of eight wells will receive 0,1 ml of culture medium (control untreated cells)
- Add 0,1 ml of cell suspension at 2.10^5 cell/ml.
- Incubate for 72 hours at $36^\circ\text{C} \pm 1^\circ\text{C}$ under $5\% \text{CO}_2 \pm 2\%$.
- The viral cytopathic effect is read by using an inverted microscope

The estimated of infectious unite is determined by method KARBES-SPAERMAN calculating the negative logarithm of 50% endpoint ($\lg\text{DICT}_{50}$) by the following formula:

$\lg\text{DICT}_{50} = \text{negative logarithm of the highest concentration of virus} - [(\text{Sum of \% affected to each dilution}/100 - 0.5) \times (\lg \text{dilution})]$

4. Assays

a. Bactericidal activity

- 5 mL / m³ - waiting 120 minutes - Batch A050121N/2

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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			
	5.10 ⁷ - 2.10 ⁹	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁶		
<i>S. aureus</i> * Assay Feb/17/2021 20,2°C / HR 53%	4,90.10 ⁸	d1 : 56/49 d2 : 53/49	d1 : 61/54 d2 : 54/54	d1 : 58/49 d2 : 46/49	d1 : 7,85.10 ⁶ d2 : 9,25.10 ⁶ T = 8,55.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,93 R2 : 6,93 R3 : 6,93 R = 6,93
<i>A. baumannii</i> * Assay Feb/17/2021 20,2°C / HR 53%	5,80.10 ⁸	d1 : 54/58 d2 : 52/58	d1 : 51/61 d2 : 55/61	d1 : 70/58 d2 : 51/58	d1 : 6,90.10 ⁶ d2 : 5,70.10 ⁶ T = 6,30.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,80 R2 : 6,80 R3 : 6,80 R = 6,80

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 mL / m³ - waiting 120 minutes - Batch A281020N/1

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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			
	5.10 ⁷ - 2.10 ⁹	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁶		
<i>E. coli</i> Assay Nov/17/2020 19,5°C / RH 58%	2,63.10 ⁹	d1 : 29/32 d2 : 28/32	d1 : 31/45 d2 : 34/45	d1 : 33/32 d2 : 30/32	d1 : 8,35.10 ⁶ d2 : 9,60.10 ⁶ T = 8,98.10 ⁶	d1 : 0 + 0 d2 : 1 + 0 d3 : 0 + 0	R1 : 6,95 R2 : 6,95 R3 : 6,95 R = 6,95
<i>E. hirae</i> Assay Nov/17/2020 19,5°C / RH 58%	3,17.10 ⁸	d1 : 75/34 d2 : 70/34	d1 : 79/39 d2 : 108/39	d1 : 95/34 d2 : 98/34	d1 : 1,19.10 ⁷ d2 : 0,93.10 ⁷ T = 1,06.10 ⁷	d1 : 0 + 0 d2 : 810 + 0 d3 : 470 + 0	R1 : 7,03 R2 : 4,12 R3 : 4,35 R = 5,17

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

b. Fungicidal activity

- **Treatment 5 mL / m³ - waiting 120 minutes - Batch A281020N/1**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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 ⁷ - 1.10 ⁸	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁵		
<i>C. albicans</i> Assay Jan/06/21 19,5°C / RH 50%	6,40.10 ⁷	d1 : 57/64 d2 : 62/64	d1 : 62/66 d2 : 53/66	d1 : 61/64 d2 : 52/64	d1 : 8,65.10 ⁵ d2 : 8,60.10 ⁵ T = 8,63.10 ⁵	d1 : 30 + 0 d2 : 33 + 0 d3 : 7 + 0	R1 : 4,46 R2 : 4,42 R3 : 5,09 R = 4,66

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

- Treatment 5 mL / m³ - waiting 120 minutes - Batch A050121N/2

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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			
	5.10 ⁶ - 1.10 ⁷	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁵		
A. brasiliensis Assay Feb/17/2021 20,2°C / RH 53%	1,12.10 ⁷	d1 : 50/45 d2 : 51/45	d1 : 29/30 d2 : 23/30	d1 : 29/45 d2 : 38/45	d1 : 1,04.10 ⁶ d2 : 1,03.10 ⁶ T = 1,04.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,02 R2 : 6,02 R3 : 6,02 R = 6,02

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

c. Sporocidal activity

- Treatment 5 mL / m³ - waiting 120 minutes - Batch A050121N/2

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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 ⁶ - 5.10 ⁶	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 ⁵		
<i>B. subtilis</i> * Assay Jan/26/21 19,9°C/RH 57%	4,30.10 ⁶	d1 : 40/43 d2 : 38/43	d1 : 37/28 d2 : 35/28	d1 : 50/43 d2 : 37/43	d1 : 1,23.10 ⁵ d2 : 1,37.10 ⁵ T = 1,30.10 ⁵	d1 : 11 + 0 d2 : 8 + 0 d3 : 7 + 0	R1 : 4,07 R2 : 4,21 R3 : 4,27 R = 4,18
<i>C. difficile</i> * Assay Jan/21/21 19,3°C/RH 60%	2,05.10 ⁶	d1 : 15/23 d2 : 19/23	d1 : 18/18 d2 : 17/18	d1 : 13/23 d2 : 13/23	d1 : 0,61.10 ⁵ d2 : 0,58.10 ⁵ T = 0,60.10 ⁵	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 1	R1 : 4,78 R2 : 4,78 R3 : 4,78 R = 4,78

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

d. Mycobactericidal activity

- Treatment 5 mL / m³ - waiting 120 minutes - Batch A281020N/1

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			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		
<i>M. terrae</i> Assay Nov/09/20 18,8°C/RH 51%	3,10.10 ⁷	d1 : 73/76 d2 : 76/76	d1 : 57/67 d2 : 58/67	d1 : 54/76 d2 : 54/76	d1 : 3,23.10 ⁶ d2 : 3,33.10 ⁶ T = 3,28.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 1 + 0	R1 : 6,52 R2 : 6,52 R3 : 6,52 R = 6,52
<i>M. avium</i> Assay Nov/16/20 18,8°C/RH 63%	3,92.10 ⁷	d1 : 85/88 d2 : 57/88	d1 : 65/61 d2 : 63/61	d1 : 77/88 d2 : 69/88	d1 : 2,66.10 ⁶ d2 : 2,35.10 ⁶ T = 2,51.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,40 R2 : 6,40 R3 : 6,40 R = 6,40

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

e. Virucidal activity

- **Treatment 5 mL / m³ - waiting 120 minutes - Batch A050121N/2**
- **Adenovirus type 5**

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection.

Assay May/11/2021 20,5°C/RH 64%	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	7.88	
Carrier 2	7.63	
Average	7.76	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	7.75	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	7.63	
Carrier 2	7.75	
Average	7.69	Difference <0,5 lg.
- Without traitement (D2)		
Carrier 1	7.50	
Test control		
Carrier1	6.25	
Carrier 2	6.88	
Average	6.57	
Assay		
Support 1	1.50	
Support 2	1.50	
Support 3	1.50	
Average	1.50	5.07

- Murine Norovirus

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection.

Assay April/29/2021 19,4°C/RH 64%	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	6.88	
Carrier 2	6.88	
Average	6.88	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	7.00	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	6.50	
Carrier 2	6.63	
Average	6.57	Difference <0,5 lg.
- Without traitement (D2)		
Carrier 1	6.38	
Test control		
Carrier1	6.13	
Carrier 2	6.38	
Average	6.26	
Assay		
Support 1	1.50	
Support 2	2.00	4.55
Support 3	1.63	
Average	1.71	

5. Conclusion

According to the conditions of standard NF EN 17272 (April 2020), the couple device/product: NOCOSPRAY 2 serial number 172X731 / NOCOLYSE NEUTRALL 6%® (Batches A281020N/1 Exp. Oct/2022 and A050121N/2 Exp. Jan/2023), for a use in clean conditions, in medical area, led to:

- A **bactericidal** activity (log reduction ≥ 5) after a 5 mL/m³ treatment and 120 minutes of wait on the following strain:
 - *A. baumannii* CIP 7034
 - *E. coli* CIP 54.127
 - *E. hirae* CIP 58.55
 - *S. aureus* CIP 4.83
- A **fungicidal** activity (log reduction ≥ 4) after a 5 mL/m³ treatment and 120 minutes of wait on the following strain:
 - *C. albicans* DSM 1386
 - *A. brasiliensis* CBS 733.88
- A **sporocidal** activity (log reduction ≥ 4) after a 5 mL/m³ treatment and 120 minutes of wait on the following strain:
 - *Bacillus subtilis* CIP 52.62
 - *C. difficile* NCTCC 13366
- A **mycobactericidal** activity (log reduction ≥ 4) after a 5 mL/m³ treatment and 120 minutes of wait on the following strain:
 - *M. terrae* ATCC 15755
 - *M. avium* ATCC 15769
- A **virucidal** activity (log reduction ≥ 4) after a 5 mL/m³ treatment and 120 minutes of wait on the following strain:
 - *Adenovirus* type 5 ATCC VR-5
 - *Norovirus* Murin souche S99

The results hold only for the device/product under assay and apply to the sample as received.