



Toulouse, June 9th 2022

ASSAY REPORT N° 22-1917

STUDY 20-2795

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
Efficacy and distribution tests

Client

Company registration SIREN 448974253
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Assay laboratory

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1. Test Laboratory

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2. Identification of the aerial disinfection system

Device : **Diffuser PX-00**
Serial number :172X731

Disinfectant : **Formula N-3**
Batch : A2910200S/1
Exp.: Oct/2022
Receipt : Nov/03/2020

Disinfectant : **Formula N-3**
Batch : A071220N+/1
Exp.: Oct/2022
Receipt : Jan/04/2021

Disinfectant : **Formula N-3**
Batch : A230222N+/1
Exp.: Feb/2024
Receipt : Feb/25/2022

Concentration of product: 3 mL/m³ or 5 mL/m³
One treatment - Waiting time 60 or 120 minutes after the end of diffusion
Amount of disinfectant diffusion ≈ 100 or 162,5 mL
Time of diffusion : 6 minutes or 9 minutes 45 seconds

Promotor : Company registration SIREN 448974253

Storage conditions: Ambient temperature
Period of testing: November 2020 - March 2022

Actives Substances: Hydrogen peroxide (12%)

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 :
 - o *Bacillus subtilis* CIP 52.62
 - o *Clostridium difficile* NCTCC 13366 (additional microorganism)
- Mycobactericidal activity :
 - o *Mycobacterium terrae* ATCC 15755
 - o *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-1-040221 (passage N°1) and SS-6-260421 (passage N°6)

Receiving cells

Origin: ATCC
ATCC reference: CCL-2
Batch number ATCC: 4440136
Internal number Batch: WCB-140613 (passage N°42)

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) and SS-4-271118 (passage 4)

Receiving cells

Origin : ATCC
ATCC reference: TIB-71
Batch number ATCC: 5822175
Internal number Batch: WCB-210912 (passage N°29)

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. 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 1600trs/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

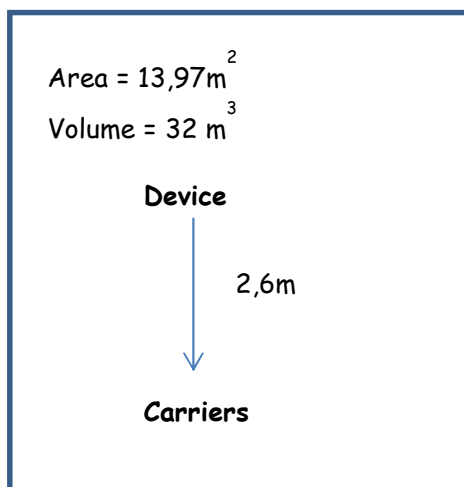
The estimated of infectious unite is determined by method KARBBER-SPAERMAN calculating the negative logarithm of 50% endpoint ($\lg\text{DICT}_{50}$) by the following formula:

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

4. Efficacy tests

a. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 50% to 67% (see results).

Initial temperatures ranging from 18,5°C to 20,4°C (see results).

Test room volume : 32m³.

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

b. Diluants, culture media and membranes

Interfering substances

1/20 reconstituted milk (Internal preparation - Batches 10219 Exp. Apr/09/2021 and 10280 Exp. May/13/2021)

BSA fraction V 0,3g/l (Internal preparation - Batches 351, 374, 379, 382, 384, 392 and 401)

Diluants

Suspension preparation : Water for Injectable Preparations (WIP)* (interference of product with Tryptone-salt) (Cooper - Batch 19MKA300 Exp. Sept/2021)

Diluant for *A. brasiliensis* (Internal preparation - Batch 53 Exp. May/26/21)

Recovery solution + 0,5% Tween80 (Internal preparation - Batches 9931, 10201, 10234, 10267 and 10284)

Recovery solution (viruses) EMEM (Internal preparation - batches N°2870 and N°2876)

Filtration membranes

Nitrocellulose membranes 0,45 µm (Millipore - white / Batches FOMB14755C and F05B62670C - black / Batches F9HA42174, FOMB71383C and F0KB98880C)

Culture media

Malt Extract agar (Internal preparation - Batch 10275 Exp. May/12/21)

Trypcase soy agar (Biomérieux - Batches 1008444040 Exp. June/09/2022 and 1008551380 Exp. Aug/10/2022)

Middlebrook agar + OADC (Internal preparation - Batches 9900 Exp. Dec/19/2020 and 10179 Exp. Mar/23/2021)

BHIYT-L Agar (Counting of *Clostridium difficile*) (Internal preparation - Batch 10276 Exp. May/12/2021)

EMEM (Internal preparation - batches N°2870 and N°2876)

c. Results

c1. Bactericidal activity

- 3 mL / m³ - waiting 60 minutes - Batch A071220N+/1

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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. hirae</i> Assay Apr/14/2021 20,1°C / RH 54%	2,87.10 ⁸	d1 : 33/31 d2 : 36/31	d1 : 38/31 d2 : 33/31	d1 : 41/31 d2 : 34/31	d1 : 8,50.10 ⁶ d2 : 1,10.10 ⁷ T = 9,75.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,99 R2 : 6,99 R3 : 6,99 R = 6,99
<i>S. aureus</i> Assay Mar/30/2021 19,7°C / RH 50%	5,55.10 ⁸	d1 : 58/56 d2 : 60/56	d1 : 59/54 d2 : 60/54	d1 : 52/56 d2 : 59/56	d1 : 1,25.10 ⁷ d2 : 1,30.10 ⁷ T = 1,28.10 ⁷	d1 : 48 + 0 d2 : 0 + 0 d3 : 16 + 0	R1 : 5,43 R2 : 7,11 R3 : 5,90 R = 6,15
<i>A. baumannii</i> Assay Mar/30/2021 19,7°C / RH 50%	6,60.10 ⁸	d1 : 60/66 d2 : 65/66	d1 : 57/70 d2 : 64/70	d1 : 69/66 d2 : 74/66	d1 : 4,00.10 ⁶ d2 : 3,35.10 ⁶ T = 3,68.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,57 R2 : 6,57 R3 : 6,57 R = 6,57

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

FONDEREPHAR

6/16

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Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Apr/15/2021 19,2°C / RH 62%	4,00.10 ⁹	d1 : 37/40 d2 : 40/40	d1 : 35/41 d2 : 36/41	d1 : 33/40 d2 : 37/40	d1 : 1,09.10 ⁶ d2 : 1,73.10 ⁶ T = 1,41.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,15 R2 : 6,15 R3 : 6,15 R = 6,15

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

c2. Fungicidal activity

- **Treatment 3 mL / m³ - waiting 60 minutes - Batch A071220N+/1**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Apr/14/2021 20,1°C / RH 54%	5,70.10 ⁷	d1 : 50/57 d2 : 47/57	d1 : 51/60 d2 : 46/60	d1 : 43/57 d2 : 48/57	d1 : 5,95.10 ⁵ d2 : 6,80.10 ⁵ T = 6,38.10 ⁵	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,80 R2 : 5,80 R3 : 5,80 R = 5,80

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

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Apr/14/2021 20,1°C / RH 54%	7,60.10 ⁶	d1 : 59/76 d2 : 63/76	d1 : 30/49 d2 : 47/49	d1 : 61/76 d2 : 59/76	d1 : 6,95.10 ⁵ d2 : 6,70.10 ⁵ T = 6,83.10 ⁵	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,83 R2 : 5,83 R3 : 5,83 R = 5,83

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

c3. Sporicidal activity

- Treatment 3 mL / m³ - waiting 60 minutes - Batch A071220N+/1

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Apr/22/21 20,4°C/RH 52%	2,65.10 ⁶	d1 : 33/28 d2 : 34/28	d1 : 18/14 d2 : 26/14	d1 : 23/28 d2 : 24/28	d1 : 8,85.10 ⁴ d2 : 9,30.10 ⁴ T = 9,08.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 4,96 R2 : 4,96 R3 : 4,96 R = 4,96
<i>C. difficile</i> Assay Apr/22/21 20,4°C/RH 52%	4,00.10 ⁶	d1 : 33/40 d2 : 48/40	d1 : 30/37 d2 : 34/37	d1 : 44/40 d2 : 39/40	d1 : 1,12.10 ⁵ d2 : 1,22.10 ⁵ T = 1,17.10 ⁵	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,07 R2 : 5,07 R3 : 5,07 R = 5,07

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

c4. Mycobactericidal activity

- Treatment 3 mL / m³ - waiting 120 minutes - Batches A071220N+/1 (*M. terrae*) and A2910200S/1 (*M. avium*)

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Mar/16/21 19,4°C/RH 55%	1,73.10 ⁷	d1 : 50/65 d2 : 53/65	d1 : 36/39 d2 : 42/39	d1 : 26/65 d2 : 40/65	d1 : 4,01.10 ⁶ d2 : 3,86.10 ⁶ T = 3,94.10 ⁶	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,59 R2 : 6,59 R3 : 6,59 R = 6,59
<i>M. avium</i> Assay Nov/30/20 19,1°C/RH 50%	7,25.10 ⁷	d1 : 193/233 d2 : 175/233	d1 : 97/129 d2 : 97/129	d1 : 103/233 d2 : 132/233	d1 : 3,90.10 ⁶ d2 : 3,83.10 ⁶ T = 3,86.10 ⁶	d1 : 14 + 0 d2 : 16 + 0 d3 : 6 + 0	R1 : 5,44 R2 : 5,38 R3 : 5,81 R = 5,54

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

c5. Virucidal activity

Treatment 3 mL / m³ - waiting 120 minutes - Batch A071220N+/1

- Adenovirus type 5

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

Assay June/14/2021 18,5°C/RH 61%	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1		
Carrier 2	7.13	
Average	7.38	
- Without traitement (S2)	7.23	
Carrier 1		
	7.00	Difference <1 lg.
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	6.88	
	7.13	
Carrier 2	7.00	
Average		
- Without traitement (D2)	7.00	
Carrier 1		Difference <0,5 lg.
Test control		
Carrier1	6.63	
Carrier 2	6.88	
Average	6.76	
Assay		
Support 1	≤0.5	
Support 2	≤0.5	
Support 3	≤0.5	
Average	≤0.5	≥ 6.26

- **Murine Norovirus**

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

Assay June/21/2021 19.9°C/RH 67%	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus - With treatment (S1) Carrier 1 Carrier 2 Average - Without traitement (S2) Carrier 1	 6.25 6.63 6.44 6.88	 Difference <1 lg.
Efficiency for suppression of disinfectant activity - With treatment (D1) Carrier1 Carrier 2 Average - Without traitement (D2) Carrier 1	 6.88 6.75 6.82 6.63	 Difference <0,5 lg.
Test control Carrier1 Carrier 2 Average	 7.00 6.75 6.88	
Assay Support 1 Support 2 Support 3 Average	 ≤0.5 ≤0.5 ≤0.5 ≤0.5	 ≥ 6.38

5. Distribution test

a. Conditions of aerial disinfection system use

- Room : same room as for the efficacy tests (area = 13,97m² ; volume = 32m³)

Relative humidity: 38% (see results).

Initial temperature: 19,4°C (see results).

The positioning of the carriers in relation to the apparatus shall be as indicated in Table A.2 of the standard.

b. Diluants, culture media and membranes

Interfering substances

BSA fraction V 0,3g/l (Internal preparation - Batch 461)

Diluants

Suspension preparation : Water for Injectable Preparations (WIP)* (interference of product with Tryptone-salt) (Cooper - Batch 19QEAGF0 Exp. Apr/2024)

Recovery solution + 0,5% Tween80 (Internal preparation - Batch 10964)

Filtration membranes

Nitrocellulose membranes 0,45 µm (Millipore - white / Batch F1JB715006)

Culture media

Trypcase soy agar (Biomérieux - Batch 1009034820) Exp. Apr/26/2023)

c. Results

- 5 mL / m³ - waiting 120 minutes - Batch A230222N+/1

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 CFU/ 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 Mar/29/22 19,4°C / RH 38%	5,60.10 ⁸	d1 : 55/56 d2 : 52/56	d1 : 47/51 d2 : 49/51	d1 : 54/56 d2 : 47/56	d1 : 1,29.10 ⁷ d2 : 1,40.10 ⁷ T = 1,35.10 ⁷	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0 d4 : 0 + 0 d5 : 0 + 0 d6 : 0 + 0 d7 : 1 + 0 d8 : 0 + 0	R1 : 7,13 R2 : 7,13 R3 : 7,13 R4 : 7,13 R5 : 7,13 R6 : 7,13 R7 : 7,13 R8 : 7,13

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...

6. Conclusion

The device/product combination: diffuser PX-00 serial number 172X731 / Formula N-3 (batches A2910200S/1 Exp. Oct/2022, A071220N+/1 Exp. Oct/2022 et A230222N+/1 Exp. Feb/2024), for use in clean conditions, in the medical area, meets the criteria of standard NF EN 17272 (April 2020) for bactericidal, fungicidal, sporicidal, mycobactericidal and virucidal efficacy tests and for distribution test (*S. aureus* CIP 4.83) after treatment at 5 mL/m³ - waiting time 120 minutes.

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