



Toulouse, June 24th 2022

STUDY 22 - 3162

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

Food, industry and collectivity areas
Clean conditions

Additional assays: *L. monocytogenes*, *B. cereus* - 3 days of treatment

Client

Company Registered SIREN 448974253
829 rue Marcel Paul
94500 CHAMPIGNY SUR MARNE
France

Test laboratory

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 TOULOUSE cedex 9
France

Dr Catherine FEUILLOLAY
Study Manager

Dr Jocelyne BACARIA
Quality Manager

1. Test Laboratory

Fondation pour le Développement de la recherche en Pharmacie (FONDEREPHAR)
Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers 31062 Toulouse cedex 9,
France

2. Identification of the aerial disinfection system

Device : Diffuser PX-00
Serial number: 172X731

Disinfectant: Formula N-5
Batch: A040522FD/1
Exp. : May/2024
Packaging : 1L
Receipt : May/10/2022

Concentration of product in the room: 1 mL/m³

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

Amount of disinfectant diffusion \approx 32 mL/treatment of 1 mL/m³.

Time of diffusion \approx 2 minutes

Promotor : Company Registered SIREN 448974253

Storage conditions: Ambient temperature
Period of testing: May 2022
Actives Substances: Hydrogen peroxide (7,9%)

3. Experimental Conditions

a. Tests micro-organisms

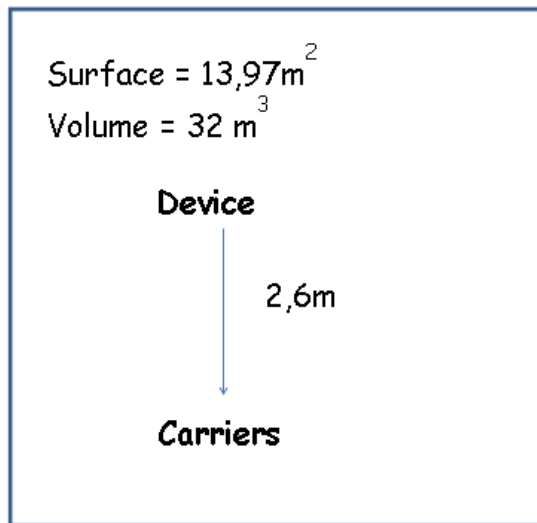
- Bactericidal activity :
 - o *Listeria monocytogenes* CIP 82.110T
- Sporicidal activity :
 - o *Bacillus cereus* CIP 105151 (spores)

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 40% to 51%.

Initial temperatures ranging from 19,3°C to 22°C.

Test room volume : 32m³.

- Carriers

Distance between the device and the carriers: 2,6m (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 11111 Exp. Jun/16/2022)

BSA fraction V 0,3g/l (Internal preparation - Batches 467 and 468)

Diluants

Suspension preparation: EPPI (Cooper - Batch Lot 19QEAGF0 Exp. April 2024)

Recovery solution (Internal preparation - Batch 11097 Exp. Jun/10/2022)

Filtration membranes

Nitrocellulose membranes 0,45µm (Millipore - Batch R1MB59701)

Culture media

Tryptase soy agar (Biomérieux - Batch 1009172670 Exp. Jul/19/2023)

4. Assays

a. Bactericidal activity

- Treatment 1 mL / m³ - waiting 30 minutes. One treatment per day and for 3 days

<i>Listeria monocytogenes</i> CIP 82.110T	N Test suspension (CFU/mL)	Preliminary assay			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 ⁵		
DAY 1 Date May/17/2022 22°C/40% RH	8,1.10 ⁸	d1 : 65/81 d2 : 72/81	d1 : 39/59 d2 : 49/59	d1 : 66/81 d2 : 57/81	d1 : 2,67.10 ⁵ d2 : 1,25.10 ⁵ T = 1,96.10 ⁵	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,29 R2 : 5,29 R3 : 5,29 R = 5,29
DAY 2 Date May/18/2022 21,1°C/44% RH	-	d1 : 68/58 d2 : 66/58	d1 : 47/56 d2 : 40/56	d1 : 62/58 d2 : 68/58	d1 : 4,95.10 ⁴ d2 : 3,25.10 ³ T = 2,64.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 4,42 R2 : 4,42 R3 : 4,42 R = 4,42
DAY 3 Date May/19/2022 19,3°C/51% RH	-	d1 : 54/44 d2 : 49/44	d1 : 54/45 d2 : 32/45	d1 : 61/44 d2 : 42/44	d1 : 9,60.10 ² d2 : 7,15.10 ² T = 8,38.10 ²	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 2,92 R2 : 2,92 R3 : 2,92 R = 2,92

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. Sporocidal activity

- Treatment 1 mL / m³ - waiting 30 minutes. One treatment per day and for 3 days

<i>Bacillus cereus</i> CIP 105151 (spores)	N Test suspension (CFU/mL)	Preliminary assay			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 ⁵ - 5.10 ⁵	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≥ 10 ⁵		
DAY 1 Date May/17/2022 22°C/40% RH	2,1.10 ⁵	d1 : 20/22 d2 : 19/22	d1 : 13/14 d2 : 12/14	d1 : 23/22 d2 : 24/22	d1 : 1,24.10 ⁴ d2 : 1,34.10 ⁴ T = 1,29.10 ⁴	d1 : 41 + 54 d2 : 49 + 74 d3 : 23 + 78	R1 : 2,13 R2 : 2,02 R3 : 2,11 R = 2,09
DAY 2 Date May/18/2022 21,1°C/44% RH	-	d1 : 31/29 d2 : 27/29	d1 : 27/20 d2 : 24/20	d1 : 29/29 d2 : 33/29	d1 : 1,04.10 ⁴ d2 : 1,11.10 ⁴ T = 1,08.10 ⁴	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 4,03 R2 : 4,03 R3 : 4,03 R = 4,03
DAY 3 Date May/19/2022 19,3°C/51% RH	-	d1 : 18/20 d2 : 21/20	d1 : 19/12 d2 : 11/12	d1 : 20/20 d2 : 22/20	d1 : 9,65.10 ³ d2 : 8,10.10 ³ T = 8,88.10 ³	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 3,95 R2 : 3,95 R3 : 3,95 R = 3,95

T: counting of micro-organisms on the discs.

N₁: counting of test suspension by pour plate technique - N₂: counting of test suspension by filtration methodn₁: 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 (EN 17272 - April 2020: additional assays), the couple device (Diffuser PX-00 Serial number 172X731) / product (Formula N-5 batch A040522FD/1) led to :

- A bactericidal activity (log reduction ≥ 5)
 - Since the first treatment with 1mL/m³ - 30 minutes of wait on the strain :
 - *Listeria monocytogenes* CIP 82.110T
- A sporicidal activity (log reduction ≥ 3)
 - After 2 treatments, one treatment / day, with 1mL/m³ - 30 minutes of wait, on the strain :
 - *Bacillus cereus* CIP 105151 (spores)

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