



Toulouse, July 6<sup>th</sup> 2021

## ASSAY REPORT N° 21-1742

### 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**

**Client**  
**OXY'PHARM**  
**829 rue Marcel Paul**  
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**FRANCE**

**Assay laboratory**  
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## 1. Test Laboratory

Fondation pour le Développement de la recherche en Pharmacie (FONDEREPHAR)  
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## 2. Identification of the aerial disinfection system

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

Disinfectant : **NOCOLYSE PLUS/ONE SHOT®**  
Batch : A2910200S/1  
Exp.: Oct/2022  
Receipt : Nov/03/2020

Disinfectant : **NOCOLYSE PLUS/ONE SHOT®**  
Batch : A071220N+/1  
Exp.: Oct/2022  
Receipt : Jan/04/2021

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

Promotor : OXY'PHARM  
Storage conditions: Ambient temperature  
Period of testing: November 2020 - June 2021  
Actives Substances: Hydrogen peroxide

## 3. Experimental Conditions

### a. Tests micro-organisms

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

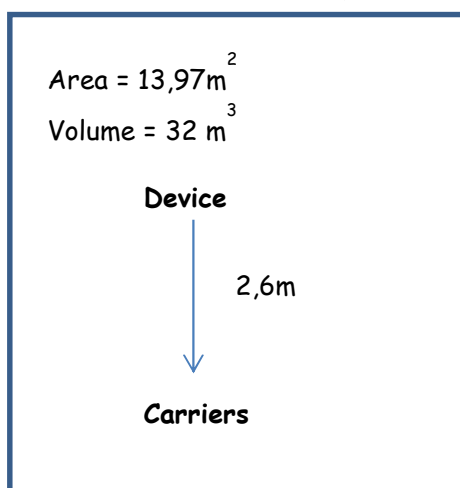
Origin : ATCC  
ATCC reference: TIB-71  
Batch number ATCC: 5822175  
Internal number Batch: WCB-210912 (passage N°29)

#### **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. 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 : 32m<sup>3</sup>.

Distance between the apparatus 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 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 FOKB98880C)

**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)

**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 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 (lgDICT50) by the following formula:

$\text{lgDICT50} = \text{negative logarithm of the highest concentration of virus} - [(\text{Sum of \% affected to each dilution}/100 - 0.5) \times (\text{lg dilution})]$

#### 4. Assays

##### a. Bactericidal activity

- 3 mL / m<sup>3</sup> - 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 <sup>7</sup> - 2.10 <sup>9</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>6</sup>		
<i>E. hirae</i> Assay Apr/14/2021 20,1°C / RH 54%	2,87.10 <sup>8</sup>	d1 : 33/31 d2 : 36/31	d1 : 38/31 d2 : 33/31	d1 : 41/31 d2 : 34/31	d1 : 8,50.10 <sup>6</sup> d2 : 1,10.10 <sup>7</sup>  T = 9,75.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,99 R2 : 6,99 R3 : 6,99 <b>R = 6,99</b>
<i>S. aureus</i> Assay Mar/30/2021 19,7°C / RH 50%	5,55.10 <sup>8</sup>	d1 : 58/56 d2 : 60/56	d1 : 59/54 d2 : 60/54	d1 : 52/56 d2 : 59/56	d1 : 1,25.10 <sup>7</sup> d2 : 1,30.10 <sup>7</sup>  T = 1,28.10 <sup>7</sup>	d1 : 48 + 0 d2 : 0 + 0 d3 : 16 + 0	R1 : 5,43 R2 : 7,11 R3 : 5,90 <b>R = 6,15</b>
<i>A. baumannii</i> Assay Mar/30/2021 19,7°C / RH 50%	6,60.10 <sup>8</sup>	d1 : 60/66 d2 : 65/66	d1 : 57/70 d2 : 64/70	d1 : 69/66 d2 : 74/66	d1 : 4,00.10 <sup>6</sup> d2 : 3,35.10 <sup>6</sup>  T = 3,68.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,57 R2 : 6,57 R3 : 6,57 <b>R = 6,57</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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

6/14

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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 <sup>7</sup> - 2.10 <sup>9</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>6</sup>		
<i>E. coli</i> Assay Apr/15/2021 19,2°C / RH 62%	4,00.10 <sup>9</sup>	d1 : 37/40 d2 : 40/40	d1 : 35/41 d2 : 36/41	d1 : 33/40 d2 : 37/40	d1 : 1,09.10 <sup>6</sup> d2 : 1,73.10 <sup>6</sup>  T = 1,41.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,15 R2 : 6,15 R3 : 6,15 <b>R = 6,15</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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 3 mL / m<sup>3</sup> - 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 <sup>7</sup> - 1.10 <sup>8</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>5</sup>		
<i>C. albicans</i> Assay Apr/14/2021 20,1°C / RH 54%	5,70.10 <sup>7</sup>	d1 : 50/57 d2 : 47/57	d1 : 51/60 d2 : 46/60	d1 : 43/57 d2 : 48/57	d1 : 5,95.10 <sup>5</sup> d2 : 6,80.10 <sup>5</sup>  T = 6,38.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,80 R2 : 5,80 R3 : 5,80 <b>R = 5,80</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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 <sup>6</sup> - 1.10 <sup>7</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>5</sup>		
<b>A. brasiliensis</b> <b>Assay Apr/14/2021</b> <b>20,1°C / RH 54%</b>	7,60.10 <sup>6</sup>	d1 : 59/76 d2 : 63/76	d1 : 30/49 d2 : 47/49	d1 : 61/76 d2 : 59/76	d1 : 6,95.10 <sup>5</sup> d2 : 6,70.10 <sup>5</sup>  T = 6,83.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,83 R2 : 5,83 R3 : 5,83 <b>R = 5,83</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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 3 mL / m<sup>3</sup> - 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 <sup>6</sup> - 5.10 <sup>6</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>5</sup>		
<i>B. subtilis</i> Assay Apr/22/21 20,4°C/RH 52%	2,65.10 <sup>6</sup>	d1 : 33/28 d2 : 34/28	d1 : 18/14 d2 : 26/14	d1 : 23/28 d2 : 24/28	d1 : 8,85.10 <sup>4</sup> d2 : 9,30.10 <sup>4</sup>  T = 9,08.10 <sup>4</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 4,96 R2 : 4,96 R3 : 4,96 <b>R = 4,96</b>
<i>C. difficile</i> Assay Apr/22/21 20,4°C/RH 52%	4,00.10 <sup>6</sup>	d1 : 33/40 d2 : 48/40	d1 : 30/37 d2 : 34/37	d1 : 44/40 d2 : 39/40	d1 : 1,12.10 <sup>5</sup> d2 : 1,22.10 <sup>5</sup>  T = 1,17.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,07 R2 : 5,07 R3 : 5,07 <b>R = 5,07</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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 3 mL / m<sup>3</sup> - 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 <sup>7</sup> - 1.10 <sup>8</sup>	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10 <sup>5</sup>		
<i>M. terrae</i> Assay Mar/16/21 19,4°C/RH 55%	1,73.10 <sup>7</sup>	d1 : 50/65 d2 : 53/65	d1 : 36/39 d2 : 42/39	d1 : 26/65 d2 : 40/65	d1 : 4,01.10 <sup>6</sup> d2 : 3,86.10 <sup>6</sup>  T = 3,94.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,59 R2 : 6,59 R3 : 6,59 <b>R = 6,59</b>
<i>M. avium</i> Assay Nov/30/20 19,1°C/RH 50%	7,25.10 <sup>7</sup>	d1 : 193/233 d2 : 175/233	d1 : 97/129 d2 : 97/129	d1 : 103/233 d2 : 132/233	d1 : 3,90.10 <sup>6</sup> d2 : 3,83.10 <sup>6</sup>  T = 3,86.10 <sup>6</sup>	d1 : 14 + 0 d2 : 16 + 0 d3 : 6 + 0	R1 : 5,44 R2 : 5,38 R3 : 5,81 <b>R = 5,54</b>

T: counting of micro-organisms on the discs.

N<sub>1</sub> : counting of test suspension by pour plate technique - N<sub>2</sub> : counting of test suspension by filtration method

n<sub>1</sub> : counting to search inhibitor effect in agar medium - n<sub>2</sub> : counting to search inhibitor effect on membrane filtration - n<sub>3</sub> : 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 3 mL / m<sup>3</sup> - 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.

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

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

## 5. Conclusion

According to the conditions of standard NF EN 17272 (April 2020), the couple device/product: NOCOSPRAY 2 serial number 172X731 / NOCOLYSE PLUS/ONE SHOT® (Batches A2910200S/1 Exp. Oct/2022 and A071220N+/1 Exp. Oct/2022), for a use in clean conditions, in medical area, led to:

- A bactericidal activity (log reduction  $\geq 5$ ) after a 3 mL/m<sup>3</sup> treatment and 60 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  $\geq 4$ ) after a 3 mL/m<sup>3</sup> treatment and 60 minutes of wait on the following strain:
  - *C. albicans* DSM 1386
  - *A. brasiliensis* CBS 733.88
- A sporicidal activity (log reduction  $\geq 4$ ) after a 3 mL/m<sup>3</sup> treatment and 60 minutes of wait on the following strain:
  - *Bacillus subtilis* CIP 52.62
  - *C. difficile* NCTCC 13366 (additional microorganism)
- A mycobactericidal activity (log reduction  $\geq 4$ ) after a 5 mL/m<sup>3</sup> treatment and 120 minutes of wait on the following strain:
  - *M. terrae* ATCC 15755
  - *M. avium* ATCC 15769
- A virucidal activity (log reduction  $\geq 4$ ) after a 3 mL/m<sup>3</sup> treatment and 120 minutes of wait on the following strains:
  - Adénovirus type 5 ATCC VR-5
  - Norovirus Murin souche S99

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