



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

## ASSAY REPORT N° 21-1739

### STUDY 20-2793

**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  
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FRANCE

**Assay laboratory** FONDEREPHAR  
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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 FOOD 7.9%®**

Batch : A071220FD/1

Exp.: 12/2022

Receipt : Jan/04/2021

Concentration of product: 5mL/m<sup>3</sup>

One treatment - Waiting time 60 or 120 minutes after the end of diffusion

Amount of disinfectant diffusion ≈ 162,5 mL

Time of diffusion : 9 min 45

Promotor : OXY'PHARM

Storage conditions: Ambient temperature

Period of testing: February - 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
  
- Sporocidal 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-1-250413 (passage N°1)

#### Receiving cells

Origin: ATCC  
ATCC reference: CCL-2  
Batch number ATCC: 4440136  
Internal number Batch: WCB-140613 (passage N°39)

### 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-5-100516 (passage N°5)

#### Receiving cells

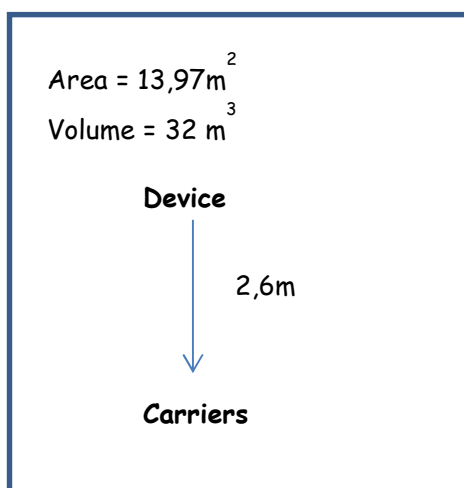
Origin : ATCC  
ATCC reference: TIB-71  
Batch number ATCC: 5822175  
Internal number Batch: WCB-210916 (passage N°26)

#### 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 62% (see results).

Initial temperatures ranging from 18,3°C to 20,2°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 367, 368, 374, 379, 382, 392)

##### 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 10096, 10154, 10192, 10201, 10241, 10254, 10270)

Recovery solution (viruses) EMEM (Internal preparation : batches N°2869, N°2870, et N°2876)

##### Filtration membranes

Nitrocellulose membranes 0,45 µm (Millipore - white / Batches FOMB14755C and F05B62670C - black / Batches FOKB98880C and F9HA42174)

##### Culture media

Malt Extract agar (Internal preparation - Batches 10242 Exp. Apr/22/21 and 10252 Exp. Apr/24/21)

Trypcase soy agar (Biomérieux - Batch 1008444040 Exp. June/09/2022)

Middlebrook agar + OADC (Internal preparation - Batch 10147 Exp. Mar/15/2021)

BHIYT-L Agar (Counting of *Clostridium difficile*) (Internal preparation - Batch 10253 Exp. Apr/29/2021)

EMEM (Internal preparation - batches N°2869, N°2870, et 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 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  $2 \cdot 10^5$  cell/ml.
- Incubate for 72 hours at  $36 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$  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

- 5 mL / m<sup>3</sup> - waiting 60 minutes - Batch A071220FD/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>S. aureus</i> * Assay Mar/10/2021 18,3°C / HR 50%	4,05.10 <sup>8</sup>	d1 : 60/41 d2 : 59/41	d1 : 42/47 d2 : 47/47	d1 : 45/41 d2 : 45/41	d1 : 1,40.10 <sup>7</sup> d2 : 1,57.10 <sup>7</sup>  T = 1,49.10 <sup>7</sup>	d1 : 1 + 0 d2 : 0 + 0 d3 : 1 + 0	R1 : 7,17 R2 : 7,17 R3 : 7,17 <b>R = 7,17</b>
<i>A. baumannii</i> * Assay Mar/10/2021 18,3°C / HR 50%	7,50.10 <sup>8</sup>	d1 : 66/75 d2 : 79/75	d1 : 67/65 d2 : 70/65	d1 : 66/75 d2 : 52/75	d1 : 2,85.10 <sup>6</sup> d2 : 2,40.10 <sup>6</sup>  T = 2,63.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,42 R2 : 6,42 R3 : 6,42 <b>R = 6,42</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>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/13/2021 19,8°C / RH 53%	6,15.10 <sup>9</sup>	d1 : 59/62 d2 : 53/62	d1 : 42/38 d2 : 41/38	d1 : 49/62 d2 : 51/62	d1 : 2,19.10 <sup>6</sup> d2 : 2,05.10 <sup>6</sup>  T = 2,12.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 14 + 0	R1 : 6,33 R2 : 6,33 R3 : 5,18 <b>R = 5,95</b>
<i>E. hirae</i> * Assay Mar/24/2021 19,7°C / RH 50%	2,85.10 <sup>8</sup>	d1 : 28/27 d2 : 30/27	d1 : 33/32 d2 : 36/32	d1 : 21/27 d2 : 19/27	d1 : 3,00.10 <sup>6</sup> d2 : 1,26.10 <sup>7</sup>  T = 7,80.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,89 R2 : 6,89 R3 : 6,89 <b>R = 6,89</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 5 mL / m<sup>3</sup> - waiting 60 minutes - Batch A071220FD/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 Mar/31/2021 19,0°C / RH 50%	5,90.10 <sup>7</sup>	d1 : 55/59 d2 : 56/59	d1 : 58/56 d2 : 57/56	d1 : 50/59 d2 : 45/59	d1 : 5,80.10 <sup>5</sup> d2 : 5,05.10 <sup>5</sup>  T = 5,43.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,73 R2 : 5,73 R3 : 5,73 <b>R = 5,73</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>		
<i>A. brasiliensis</i> Assay Mar/31/2021 19,0°C / RH 50%	9,95.10 <sup>6</sup>	d1 : 55/47 d2 : 43/47	d1 : 33/29 d2 : 23/29	d1 : 27/47 d2 : 29/47	d1 : 1,21.10 <sup>6</sup> d2 : 1,19.10 <sup>6</sup>  T = 1,20.10 <sup>6</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,08 R2 : 6,08 R3 : 6,08 <b>R = 6,08</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. Sporicidal activity

- Treatment 5 mL / m<sup>3</sup> - waiting 60 minutes - Batch A071220FD/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 Mar/24/21 19,7°C/RH 50%	3,55.10 <sup>6</sup>	d1 : 29/36 d2 : 37/36	d1 : 24/20 d2 : 28/20	d1 : 30/36 d2 : 30/36	d1 : 1,45.10 <sup>5</sup> d2 : 1,30.10 <sup>5</sup>  T = 1,38.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,14 R2 : 5,14 R3 : 5,14 <b>R = 5,14</b>
<i>C. difficile</i> * Assay Mar/31/21 19°C/RH 50%	2,28.10 <sup>6</sup>	d1 : 22/22 d2 : 20/22	d1 : 19/21 d2 : 18/21	d1 : 12/22 d2 : 15/22	d1 : 0,71.10 <sup>5</sup> d2 : 0,70.10 <sup>5</sup>  T = 0,71.10 <sup>5</sup>	d1 : 0 + 0 d2 : 1 + 0 d3 : 0 + 0	R1 : 4,85 R2 : 4,85 R3 : 4,85 <b>R = 4,85</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 5 mL / m<sup>3</sup> - waiting 120 minutes - Batch A071220FD/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			
	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 Feb/23/21 20,2°C/RH 56%	2,76.10 <sup>7</sup>	d1 : 92/103 d2 : 87/103	d1 : 87/82 d2 : 81/82	d1 : 79/103 d2 : 73/103	d1 : 4,93.10 <sup>6</sup> d2 : 3,44.10 <sup>6</sup>  T = 4,19.10 <sup>6</sup>	d1 : 20 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,32 R2 : 6,62 R3 : 6,62 <b>R = 6,19</b>
<i>M. avium</i> Assay Feb/16/21 20,1°C/RH 62%	4,10.10 <sup>7</sup>	d1 : 39/42 d2 : 22/42	d1 : 25/34 d2 : 26/34	d1 : 27/42 d2 : 31/42	d1 : 5,03.10 <sup>5</sup> d2 : 1,01.10 <sup>6</sup>  T = 7,57.10 <sup>5</sup>	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 5,88 R2 : 5,88 R3 : 5,88 <b>R = 5,88</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 5 mL / m<sup>3</sup> - waiting 60 minutes - Batch A071220FD/1**

**- Adenovirus type 5**

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

<b>Assay June/07/2021 18,6°C/RH 54%</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	7.00	
Average	6.88	Difference <1 lg.
<b>- Without traitement (S2)</b>	6.94	
Carrier 1	7.13	
<b>Efficiency for suppression of disinfectant activity</b>		
<b>- With treatment (D1)</b>	6.63	
Carrier1	6.88	
Carrier 2	6.76	Difference <0,5 lg.
Average		
<b>- Without traitement (D2)</b>		
Carrier 1	7.00	
<b>Test control</b>		
Carrier1	6.50	
Carrier 2	6.75	
Average	6.63	
<b>Assay</b>		
Support 1	2.50	
Support 2	2.38	<b>4.13</b>
Support 3	2.63	
Average	2.50	

- **Murine Norovirus**

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

Essai du June/10/2021 20,1°C/RH 54%	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
<b>Sensitivity of cells to virus</b>		
- <b>With treatment (S1)</b>		
Carrier 1	7.38	Difference <1 lg.
Carrier 2	7.13	
Average	7.25	
- <b>Without traitement (S2)</b>		
Carrier 1	7.50	
<b>Efficiency for suppression of disinfectant activity</b>		
- <b>With treatment (D1)</b>		
Carrier1	7.13	Difference <0,5 lg.
Carrier 2	7.00	
Average	7.07	
- <b>Without traitement (D2)</b>		
Carrier 1	7.13	
<b>Test control</b>		
Carrier1	6.13	
Carrier 2	6.25	
Average	6.19	
<b>Assay</b>		
Support 1	1.50	<b>4.48</b>
Support 2	2.00	
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 FOOD 7,9%® (Batch A071220FD/1 Exp. 12/2022), for a use in clean conditions, in medical area, led to:

- A **bactericidal** activity (log reduction  $\geq 5$ ) after a 5 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 5 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 5 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 5 mL/m<sup>3</sup> treatment and 60 minutes of wait on the following strains:
  - 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.