

## 1. PERFORMING LABORATORY

APEX BIOSOLUTIONS  
4, rue des Grandes Pièces  
Zone EURESPACE  
25 770 SERRE LES SAPINS  
FRANCE

## 2. PRODUCT IDENTITY

| Product | Batch #   |
|---------|-----------|
| F1031V2 | 611141B01 |

Expiration date: Non communicated

Manufacturer: FRANKLAB

Manufacturing date: Non communicated

Storage conditions: as recommended by the manufacturer.

Active substances: ethanol, isopropanol, tertiary amine

Appearance of the product : liquid, colorless

Diluent recommended by the manufacturer: none, ready-to-use product

Date of receipt: 03/14/2019

Date of the study: from 03/27/2019 to 04/03/2019

## 3. EXPERIMENTAL CONDITIONS

Final concentrations of the product: 80%

Method: dilution-neutralization

Exposure time: 5 min – 10 min – 15 min

Temperature using during the assays: 20°C

Organic soil load: clean conditions, BSA 3 g/L + 3 mL/L sheep erythrocytes

Strains: *Aspergillus brasiliensis* CIP 1431.83 lot 252.09- Institut Pasteur.

Stop solution : tween 80 (30g/L) and egg yolk (5%) in distilled water.

Appearance of the product and its dilutions: clear



Diluent for fungal suspensions: trypton salt solution, sterile.

Growth conditions: MEA (Malt Extract Agar), at 30°C ± 1°.

## 4. RESULTS

The F1031V2 product is active against the fungal strains, because the reduction is greater than 4 log :

– *Aspergillus brasiliensis* R = 4,02 log

| Writer  | Supervisor  |
|---|---|
| Ms Emilie CANTREL, laboratory technician  | Ms Stephanie MOROT-BIZOT, director  |
|  |  |

## 5. CONCLUSION

**According to the EN 13624 standard (November 2013), the product F1031V2:**

- Has a fungicidal activity on the reference strains when used from the concentration 80%, for 10 min of exposure time at 20°C, in dirty conditions (BSA 3 g/L + 3 mL/L SE).

## 6. SHEETS OF RESULTS

Attached below.

For all result sheets :

Control of the methodology:

- $1,5 \times 10^7 \text{ CFU/mL} \leq N \leq 5,0 \times 10^7 \text{ CFU/mL}$
- $30 \leq N_v \leq 160 \text{ CFU/mL}$
- A, B and C  $\geq 0,5 \times N_v$
- The quotient of the weighted average counts is between 5 and 15

Caption:

Vc = counts per mL

$\bar{x}$  = average of Vc1 and Vc2

N = logarithm of CFU of the test suspension



Nv = number of CFU/mL in the suspension of validation

A = number of CFU/mL in the validation suspension of the experimental conditions

B = number of CFU/mL in the validation suspension of neutralizer toxicity

C = number of CFU/mL in the validation suspension of inactivation method by dilution-neutralization

Na = number of CFU/mL of the remaining germs after trial



| <b>Writer</b>   | <b>Supervisor</b>   |
|---|---|
| Ms Emilie CANTREL, laboratory technician  | Ms Stephanie MOROT-BIZOT, director  |
|  |  |

7. TRIAL – *Aspergillus brasiliensis*

|  |   |  |
|--|---|--|
| Standard: EN 13624<br>Product : <b>F1031V2</b><br>Batch N° : 611141B01<br>Study N° : 072D08-2019-06<br>Date of trials : 03/29/2019 | Method:<br><input checked="" type="checkbox"/> pour plating<br><input type="checkbox"/> spread plating<br><input checked="" type="checkbox"/> Number of Petri dish/mL : 2 | Neutralizer : polysorbate 80 (30g/L) + egg yolk 5%<br>Temperature: 20°C<br>Organic soil load : 3 g/L BSA + 3 mL/L SE<br>Incubation temperature : 30°C ± 1°C<br>Diluent : sterile distilled water |
|--|---|--|

| STRAIN                          | Suspension of validation Nv       |             | Suspension of validation NvB                  |                            | Validation A |                                   | Validation B |                                   | Validation C |                                   |             |
|---------------------------------|-----------------------------------|-------------|---|----------------------------|--------------|-----------------------------------|--------------|-----------------------------------|--------------|-----------------------------------|-------------|
| <i>Aspergillus brasiliensis</i> | 66                                | 63          | 1.10 <sup>-3</sup>                            | 43                         | 51           | 42                                | 44           | 50                                | 50           | 48                                | 39          |
|                                 | $\bar{x}$                         | <b>64,5</b> | $\bar{x}$                                     | <b>4,70.10<sup>4</sup></b> |              | $\bar{x}$                         | <b>43,0</b>  | $\bar{x}$                         | <b>50,0</b>  | $\bar{x}$                         | <b>43,5</b> |
|                                 | 30 ≤ Nv0 ≤ 160                    |             | 3,10 <sup>3</sup> ≤ NvB ≤ 1,6.10 <sup>5</sup> |                            |              | A ≥ 0,5 * Nv0                     |              | B ≥ 0,5 * Nv0                     |              | C ≥ 0,5 * Nv0                     |             |
|                                 | x yes <input type="checkbox"/> no |             | x yes <input type="checkbox"/> no             |                            |              | x yes <input type="checkbox"/> no |              | x yes <input type="checkbox"/> no |              | x yes <input type="checkbox"/> no |             |

| STRAIN                          | Trial suspension   |                            |      | TRIAL               |         |             | 5 min | TRIAL               |        |             | 10 min | TRIAL               |       |                 | 15 min |
|---------------------------------|--------------------|----------------------------|------|---------------------|---------|-------------|-------|---------------------|--------|-------------|--------|---------------------|-------|-----------------|--------|
| <i>Aspergillus brasiliensis</i> | 1.10 <sup>-5</sup> | >165                       | >165 | Vc                  | 121     | 133         |       | Vc                  | 18     | 19          |        | Vc                  | 2     | 2               |        |
|                                 | 1.10 <sup>-6</sup> | 41                         | 42   | Na                  | 1270,00 |             |       | Na                  | 185,00 |             |        | Na                  | <140  |                 |        |
|                                 | N                  | <b>1,88.10<sup>7</sup></b> |      | Log Na              | 3,10    |             |       | Log Na              | 2,27   |             |        | Log Na              | <2,15 |                 |        |
|                                 | Log N0             | <b>6,27</b>                |      | Log R = logN0-logNa |         | <b>3,17</b> |       | Log R = logN0-logNa |        | <b>4,00</b> |        | Log R = logN0-logNa |       | <b>&gt;4,12</b> |        |



|   |   |
|---|---|
| <b>Rédacteur</b>  | <b>Superviseur</b>  |
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**8. REPETITION - *Aspergillus brasiliensis***

|  |   |  |
|--|---|--|
| Standard: EN 13624<br>Product : <b>F1031V2</b><br>Batch N° : 611141B01<br>Study N° : 072D08-2019-06<br>Date of trials : 04/03/2019 | Method:<br><input checked="" type="checkbox"/> pour plating<br><input type="checkbox"/> spread plating<br><input checked="" type="checkbox"/> Number of Petri dish/mL : 2 | Neutralizer : polysorbate 80 (30g/L) + egg yolk 5%<br>Temperature: 20°C<br>Organic soil load : 3 g/L BSA + 3 mL/L SE<br>Incubation temperature : 30°C ± 1°C<br>Diluent : sterile distilled water |
|--|---|--|

| STRAIN                          | Suspension of validation Nv |             | Suspension of validation NvB                  |                            |    | Validation A  |             | Validation B  |             | Validation C  |             |
|---------------------------------|-----------------------------|-------------|---|----------------------------|----|---------------|-------------|---------------|-------------|---------------|-------------|
| <i>Aspergillus brasiliensis</i> | 59                          | 61          | 1.10 <sup>-3</sup>                            | 52                         | 47 | 49            | 50          | 55            | 51          | 47            | 47          |
|                                 | $\bar{x}$                   | <b>60,0</b> | $\bar{x}$                                     | <b>4,95.10<sup>4</sup></b> |    | $\bar{x}$     | <b>49,5</b> | $\bar{x}$     | <b>53,0</b> | $\bar{x}$     | <b>47,0</b> |
|                                 | 30 ≤ Nv0 ≤ 160              |             | 3,10 <sup>3</sup> ≤ NvB ≤ 1,6.10 <sup>5</sup> |                            |    | A ≥ 0,5 * Nv0 |             | B ≥ 0,5 * Nv0 |             | C ≥ 0,5 * Nv0 |             |
|                                 | × yes □ no                  |             | × yes □ no                                    |                            |    | × yes □ no    |             | × yes □ no    |             | × yes □ no    |             |

| STRAIN                          | Trial suspension   |                            |      | TRIAL               |         | 5 min       | TRIAL               |        | 10 min      | TRIAL               |       | 15 min          |
|---------------------------------|--------------------|----------------------------|------|---------------------|---------|-------------|---------------------|--------|-------------|---------------------|-------|-----------------|
| <i>Aspergillus brasiliensis</i> | 1.10 <sup>-5</sup> | >165                       | >165 | Vc                  | 135     | 149         | Vc                  | 20     | 16          | Vc                  | 3     | 4               |
|                                 | 1.10 <sup>-6</sup> | 53                         | 50   | Na                  | 1420,00 |             | Na                  | 180,00 |             | Na                  | <140  |                 |
|                                 | N                  | <b>1,97.10<sup>7</sup></b> |      | Log Na              | 3,15    |             | Log Na              | 2,26   |             | Log Na              | <2,15 |                 |
|                                 | Log N0             | <b>6,29</b>                |      | Log R = logN0-logNa |         | <b>3,14</b> | Log R = logN0-logNa |        | <b>4,03</b> | Log R = logN0-logNa |       | <b>&gt;4,14</b> |

|   |   |
|---|---|
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| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**9. TECHNICAL APPENDIX**

**MEDIA:**

MEA (Malt Extract Agar), Dominique Dutscher, ref. 777304, batch #712042

**ORGANIC SOIL LOAD :**

Albumin Serum Bovine in powder, Fraction V, Dominique Dutscher, ref. P6154, batch D1304039

Sheep erythrocytes, Analytic Lab, réf. 08449, batch n°bcbj3984V

**DILUENT**

***Trypton-Sel Solution (TS)***



Per liter of distilled water:

- a) Tryptone, Dominique Dutscher, ref. 777472, batch n° 090633 -----1,00 g/L
- b) Sodium chloride, Grosseron, ref. 9020401, batch n° FR08 085 793 ----- 8,50 g/L

**NEUTRALIZER**

Per liter of distilled water:

- Tween 80, SIGMA ALDRICH, réf. 59924, lot n° BCBJ6978V ----- 30g/L
- Egg yolk, 5% ----- 50 mL/L

|   |   |
|---|---|
| Writer  | Supervisor  |
| Ms Emilie CANTREL, laboratory technician  | Ms Stephanie MOROT-BIZOT, director  |
|  |  |

## TEST REPORT

### FUNGICIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 14562 STANDARD

Delivered to: Ms CHAKCHOUK

For : **FRANKLAB**  
3 avenue des Frênes  
78180 MONTIGNY LE BRETONNEUX  
FRANCE



Date of request: 06/23/2021

Study number: n°167D34-2021-24

#### FUNGICIDAL TESTS:

According to the European standard EN 14562 (September 2006) – Chemical disinfectants and antiseptics. Quantitative surface test for the evaluation of fungicidal or yeasticidal activity in the medical area (phase 2, step 2).

Tests using the F1031V2 product against 2 reference strains: *Candida albicans* and *Aspergillus brasiliensis*.

This test report included 7 pages.

Study completion date: 12/03/2021

Stephanie MOROT-BIZOT  
PhD in microbiology  
Study director



## SUMMARY

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4, rue des Grandes Pièces  
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25 770 SERRE LES SAPINS  
FRANCE

**2. PRODUCT IDENTITY**

| Product | Batch N° |
|---------|----------|
| F1031V2 | 7443     |

Expiration date: Non communicated

Manufacturer: FRANKLAB

Manufacturing date: Non communicated

Storage conditions: as recommended by the manufacturer.

Active substances: ethanol, propanol, alkylamine

Appearance of the product : liquid, colorless

Diluent recommended by the manufacturer: none, ready-to-use product

Date of receipt: 06/24/2021

Date of the study: from 07/16/2021 to 07/26/2021

**3. EXPERIMENTAL CONDITIONS**

Final concentrations of the product: 100%

Method: dilution-neutralization

Exposure time: 3 min - 5 min – 10 min - 15 min

Temperature using during the assays: 20°C

Organic soil load: clean conditions, BSA 0,3 g/L.

Diluent used for the microbial suspensions: trypton salt solution, sterile.

Strain : *Candida albicans*, CIP 48.72, batch 265.09 (ATCC 10231) and *Aspergillus brasiliensis* CIP 1431.83 batch n°252.09 - Institut Pasteur.

Media and growth conditions: MEA (Malt Extract Agar), at 30°C ± 1°C.

Stop solution: glass carriers in 10 ml of neutralizer [tween 80 (30g/l) and egg yolk (5%)].

**4. VALIDATIONS AND ASSAYS**

See results sheets.

– *C. albicans*, R = 4,24

– *A. brasiliensis*, R = 4,11

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## 5. CONCLUSION

**According to the EN 14562 (September 2006), the assays performed with the F1031V2 product:**

- Demonstrated a fungicidal activity when the F1031V2 product is used from the 100% concentration against the two reference strains, for an exposure time of 5 minutes at 20°C, in clean conditions (BSA 0,3 g/L)

## 6. SHEETS OF RESULTS

Attached below.

### Methodology:

- To be valid:

- $1,5 \times 10^8 \text{ CFU/mL} \leq N \leq 5 \times 10^8 \text{ CFU/mL}$
- $8,17 \leq \text{Log}N \leq 8,70$
- $1,4 \times 10^6 \text{ CFU/mL} \leq N_w \leq \lg N - 1,3$
- $R \geq 4$  for a product to be yeasticide

In the following tables:

- VC: number of CFU per ml
- $1E-XX = 1 \times 10^{-XX}$
- N: number of CFU of the fungicidal suspension per mL
- Log N: decimal logarithm of the fungicidal suspension.
- $N_w$  = water control (number of viable cells after exposure time with water).
- $N_a$ : number of viable cells after exposure time with the product.
- Log  $N_a$ : decimal logarithm of  $N_a$ .
- $R$  = logarithmic reduction of the fungicidal suspension after exposure time with the product ( $\log R = \log N_w - \log N_a$ ).

7. RESULTS – *Candida albicans*

|                         | Suspension of validation |       | Validation A  |       | Validation B  |      | Validation C  |      | Trial suspension   |      |     | Water control      |      |     | Concentrations (v/v) |      |       |      |        |       |        |       |     |
|-------------------------|--------------------------|-------|---------------|-------|---------------|------|---------------|------|--------------------|------|-----|--------------------|------|-----|----------------------|------|-------|------|--------|-------|--------|-------|-----|
|                         | VC1                      | VC2   | VC1           | VC2   | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 | Nw                 |      |     | 3 min                |      | 5 min |      | 10 min |       | 15 min |       |     |
|                         | VC1                      | VC2   | VC1           | VC2   | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 |                    | VC1  | VC2 |                      | VC1  | VC0   | VC1  | VC2    | VC1   | VC2    | VC1   | VC2 |
| <i>Candida albicans</i> | 102                      | 111   | 104           | 98    | 89            | 90   | 86            | 88   | 1.10 <sup>-6</sup> | 258  | 253 | 1.10 <sup>-3</sup> | 228  | 225 | 1.10 <sup>0</sup>    | 56   | 69    | 16   | 17     | 0     | 0      | 0     | 0   |
|                         | $\bar{x}$                | 106,5 | $\bar{x}$     | 101,0 | $\bar{x}$     | 89,5 | $\bar{x}$     | 87,0 | 1.10 <sup>-7</sup> | 27   | 26  | 1.10 <sup>-4</sup> | 23   | 27  | 1.10 <sup>-1</sup>   | 8    | 7     | 2    | 2      | 0     | 0      | 0     | 0   |
|                         | 30 ≤ Nv0 ≤ 160           |       | A ≥ 0,5 * Nv0 |       | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      | Log N              | 8,41 |     | Log Nw             | 6,36 |     | Log Na               | 2,80 |       | 2,22 |        | <2,15 |        | <2,15 |     |
|                         | × yes □ no               |       | × yes □ no    |       | × yes □ no    |      | × yes □ no    |      |                    |      |     |                    |      |     | Log R                | 3,56 |       | 4,14 |        | >4,21 |        | >4,21 |     |

8. REPETITION – *Candida albicans*

|                         | Suspension of validation |      | Validation A  |      | Validation B  |      | Validation C  |      | Trial suspension   |      |     | Water control      |      |     | Concentrations (v/v) |      |       |      |        |       |        |       |     |
|-------------------------|--------------------------|------|---------------|------|---------------|------|---------------|------|--------------------|------|-----|--------------------|------|-----|----------------------|------|-------|------|--------|-------|--------|-------|-----|
|                         | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 | Nw                 |      |     | 3 min                |      | 5 min |      | 10 min |       | 15 min |       |     |
|                         | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 |                    | VC1  | VC2 |                      | VC1  | VC0   | VC1  | VC2    | VC1   | VC2    | VC1   | VC2 |
| <i>Candida albicans</i> | 101                      | 96   | 91            | 93   | 92            | 90   | 85            | 79   | 1.10 <sup>-6</sup> | 243  | 251 | 1.10 <sup>-3</sup> | 260  | 253 | 1.10 <sup>0</sup>    | 64   | 49    | 23   | 20     | 3     | 3      | 0     | 0   |
|                         | $\bar{x}$                | 98,5 | $\bar{x}$     | 92,0 | $\bar{x}$     | 91,0 | $\bar{x}$     | 82,0 | 1.10 <sup>-7</sup> | 28   | 27  | 1.10 <sup>-4</sup> | 29   | 28  | 1.10 <sup>-1</sup>   | 8    | 5     | 3    | 3      | 0     | 0      | 0     | 0   |
|                         | 30 ≤ Nv0 ≤ 160           |      | A ≥ 0,5 * Nv0 |      | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      | Log N              | 8,40 |     | Log Nw             | 6,41 |     | Log Na               | 2,75 |       | 2,33 |        | <2,15 |        | <2,15 |     |
|                         | × yes □ no               |      | × yes □ no    |      | × yes □ no    |      | × yes □ no    |      |                    |      |     |                    |      |     | Log R                | 3,66 |       | 4,08 |        | >4,26 |        | >4,26 |     |

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9. RESULTS – *Aspergillus brasiliensis*

|                                 | Suspension of validation |      | Validation A  |      | Validation B  |      | Validation C  |      | Trial suspension   |      |     | Water control      |      |     | Concentrations (v/v) |       |     |       |     |        |     |        |     |
|---------------------------------|--------------------------|------|---------------|------|---------------|------|---------------|------|--------------------|------|-----|--------------------|------|-----|----------------------|-------|-----|-------|-----|--------|-----|--------|-----|
|                                 | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 |                    | VC1  | VC2 |                      | 3 min |     | 5 min |     | 10 min |     | 15 min |     |
|                                 | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2 |                    | VC1  | VC2 |                      | VC1   | VC0 | VC1   | VC2 | VC1    | VC2 | VC1    | VC2 |
| <i>Aspergillus brasiliensis</i> | 66                       | 68   | 60            | 57   | 62            | 66   | 57            | 52   | 1.10 <sup>-6</sup> | 160  | 154 | 1.10 <sup>-3</sup> | 158  | 160 | 1.10 <sup>0</sup>    | 66    | 61  | 15    | 16  | 5      | 8   | 0      | 0   |
|                                 | $\bar{x}$                | 67,0 | $\bar{x}$     | 58,5 | $\bar{x}$     | 64,0 | $\bar{x}$     | 54,5 | 1.10 <sup>-7</sup> | 19   | 16  | 1.10 <sup>-4</sup> | 18   | 17  | 1.10 <sup>-1</sup>   | 8     | 7   | 2     | 2   | 0      | 0   | 0      | 0   |
|                                 | 30 ≤ Nv0 ≤ 160           |      | A ≥ 0,5 * Nv0 |      | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      | N                  | 8,20 |     | Log Nw             | 6,21 |     | Log Na               | 2,80  |     | 2,19  |     | <2,15  |     | <2,15  |     |
|                                 | × yes □ no               |      | × yes □ no    |      | × yes □ no    |      | × yes □ no    |      |                    |      |     |                    |      |     | Log R                | 3,41  |     | 4,02  |     | >4,06  |     | >4,06  |     |

10. REPETITIONS – *Aspergillus brasiliensis*

|                                 | Suspension of validation |      | Validation A  |      | Validation B  |      | Validation C  |      | Trial suspension   |      |      | Water control      |      |      | Concentrations (v/v) |       |     |       |     |        |     |        |     |
|---------------------------------|--------------------------|------|---------------|------|---------------|------|---------------|------|--------------------|------|------|--------------------|------|------|----------------------|-------|-----|-------|-----|--------|-----|--------|-----|
|                                 | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2  |                    | VC1  | VC2  |                      | 3 min |     | 5 min |     | 10 min |     | 15 min |     |
|                                 | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1  | VC2  |                    | VC1  | VC2  |                      | VC1   | VC0 | VC1   | VC2 | VC1    | VC2 | VC1    | VC2 |
| <i>Aspergillus brasiliensis</i> | 68                       | 73   | 80            | 73   | 64            | 68   | 61            | 57   | 1.10 <sup>-6</sup> | >165 | >165 | 1.10 <sup>-3</sup> | >165 | >165 | 1.10 <sup>0</sup>    | 64    | 72  | 20    | 16  | 11     | 8   | 0      | 0   |
|                                 | $\bar{x}$                | 70,5 | $\bar{x}$     | 76,5 | $\bar{x}$     | 66,0 | $\bar{x}$     | 59,0 | 1.10 <sup>-7</sup> | 18   | 20   | 1.10 <sup>-4</sup> | 21   | 19   | 1.10 <sup>-1</sup>   | 7     | 9   | 2     | 2   | 2      | 1   | 0      | 0   |
|                                 | 30 ≤ Nv0 ≤ 160           |      | A ≥ 0,5 * Nv0 |      | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      | Log N              | 8,28 |      | Log Nw             | 6,30 |      | Log Na               | 2,83  |     | 2,26  |     | <2,15  |     | <2,15  |     |
|                                 | × yes □ no               |      | × yes □ no    |      | × yes □ no    |      | × yes □ no    |      |                    |      |      |                    |      |      | Log R                | 3,47  |     | 4,04  |     | >4,15  |     | >4,15  |     |

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## 11. TECHNICAL APPENDIX

### MEDIA

MEA (Malt Extract Agar), Dominique DUTSCHER, ref. 777304, batch n° n°712042

### DILUENT

Trypton-Salt Solution

Per liter of distilled water:

- Trypton, Dominique Dutscher, ref. 777472, batch #090633 1,00
- Sodium Chloride, Grosseron, ref. 9020401, batch #FR08 085 793 8,50

Final pH at 25°C : 7,0 ± 0,2

### NEUTRALIZER

Per liter of distilled water:

- Tween 80, Sigma Aldrich, ref 59924, batch BCBJ6978V 30 g
- Egg yolk 5 g

### ORGANIC SOIL LOAD

Bovine Albumin Sera (powder), Dominique Dutscher, ref. 871001, batch #D1304039

**GLASS CARRIERS** – blades of frosted glass 15 x 60 mm, 1 mm thick – Thermo scientific/ Menzel-Gläser  
– ref. 100 OTM, batch #01 1794389.

## TEST REPORT

|   |
|---|
| <b>DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2<br/>PRODUCT ACCORDING TO THE EN 14476:2015 STANDARD</b> |
|---|

Delivered to Ms CHAKCHOUK

For : **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**  
**FRANCE**



Date of request: 10/18/2018

Date of request: n°268D25-2018-09

### VIRUCIDAL TESTS:

According to the NF EN 14476+A2 (October 2015) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the F1031V2 product, against the *norovirus*.

This test report included 9 pages.



Study completion date: 12/14/2018

Stephanie MOROT - BIZOT  
PhD in Microbiology  
Study Director

A handwritten signature in black ink, appearing to read 'Stephanie MOROT - BIZOT'.

## SUMMARY

|   |                                     |   |
|---|-------------------------------------|---|
| 1 | PERFORMING LABORATORY .....         | 3 |
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| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 1 PERFORMING LABORATORY

APEX BIOSOLUTIONS  
4, rue des Grandes Pièces  
Zone EURESPACE  
25 770 SERRE LES SAPINS  
FRANCE

## 2 SAMPLE IDENTIFICATION

| SAMPLE  | BATCH N° |
|---------|----------|
| F1031V2 | 611141   |

Expiration date: non communicated

Manufacturer : FRANKLAB

Manufacturing date: non communicated

Storage conditions: room temperature

Active substances : ethanol, isopropanol, tertiary amine

Appearance of the product: liquid, colorless

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 10/24/2018

Date of tests: from 11/02/2018 to 12/05/2018

## 3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log TCID<sub>50</sub>

Exposure time: 30 s, 2 min and 5 min

Diluent used for the product: distilled water

Final concentrations tested: 80%

Viral strains: norovirus murin MNV-1 (IFL), grown on RAW 264.7 cells, at 37°C, under 5% CO<sub>2</sub> atmosphere

Organic soil load: 3 g/L bovine serum albumin + 3 mL/L sheep erythrocytes

Product stability: good

Stop solution: cold shock

### Viral titre:



Viral titers are expressed in log TCID<sub>50</sub> (calculated by Spearman-Kärber method) :

- For norovirus, titer = 7,125 log DICT<sub>50</sub>

## 4 VALIDATION

### a) Cytotoxicity

For norovirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**b) SENSIBILITY ASSAYS**

The viruses were titrated on cell cultures untreated with the product (indicator cell line) and titrated on cell cultures treated with the product.

According to the European standard EN 14476+A2, the F1031V2 product used at the dilution of  $10^{-2}$  does not have an effect on the viruses titration method (the difference between viral titers must be  $< 1,0$  log):

| NOROVIRUS                | Viral titer (log TCID <sub>50</sub> ) |                       |                                       |
|--------------------------|---------------------------------------|-----------------------|---------------------------------------|
|                          | Untreated cell cultures               | Treated cell cultures | Viral titer (log TCID <sub>50</sub> ) |
| PRODUCT DILUTION         |                                       |                       |                                       |
| F1031V2 10 <sup>-2</sup> | 7,125                                 | 6,750                 | <b>0,375</b>                          |

**c) VALIDATIONS OF THE COLD SHOCK METHOD** (the method is validated if the difference is  $\leq 0,5$  log):

| PRODUCT CONCENTRATION | Organic soil load     | Viral titer (log TCID <sub>50</sub> ) | Difference with the viral suspension |
|-----------------------|-----------------------|---------------------------------------|--------------------------------------|
| F1031V2               | 3 g/L BSA + 3 mL/L SE | TRIAL 1: 7,125                        | 0,000                                |
|                       |                       | TRIAL 2: 7,125                        | 0,000                                |

**d) INACTIVATION ASSAYS OF THE VIRUS WITH A CONTROL SOLUTION**

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure.

| Formaldehyde 0,7%               | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction (log TCID <sub>50</sub> ) |
|---------------------------------|---------------------------------------|---|
| <b>Viral suspension control</b> | 7,125                                 |   |
| inactivation 5 min              |                                       | 0,375   |
| inactivation 15 min             | 6,750                                 | 1,625   |
| inactivation 30 min             | 5,500                                 | <b>2,125</b>                                    |

**5 VIRUCIDAL ASSAYS**



The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0$  log.

**TRIAL 1** - The viral suspension was titrated at 7,125 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 30 s             | 20°C        | 4,000                                 | 3,125                 |
|         |               | 2 min            |             | 3,500                                 | 3,625                 |
|         |               | 5 min            |             | 3,000                                 | <b>4,125</b>          |

**TRIAL 2** - The viral suspension was titrated at 7,125 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 30 s             | 20°C        | 3,750                                 | 3,375                 |
|         |               | 2 min            |             | 3,375                                 | 3,750                 |
|         |               | 5 min            |             | 2,875                                 | <b>4,250</b>          |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |



## 6 VALIDATION OF THE METHODOLOGY



*The assays were validated as required by the European standard EN 14476+A2:*

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product: 7,125 log TCID<sub>50</sub> for norovirus
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure :
  - the reduction observed was of 2,125 log for the norovirus.
- The F1031V2 product does not have a cytotoxic effect on the RAW 264.7 cells.
- The F1031V2 product does not affect the infectious capacity of the viruses:
  - For norovirus, the differences in viral titers between the virus inoculated on RAW 264.7 cells and the virus inoculated on the RAW 264.7 cells treated with the product was  $\leq 1,0$  log (0,375 log).

## 7 CONCLUSION

**The assays performed with the F1031V2 product, batch #611141:**

- **Demonstrated a virucidal activity on the norovirus from the concentration 80%,** as required by the European standard EN 14476+A2, following a **5 min** exposure period, at 20°C, in dirty conditions.

| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 8 TECHNICAL APPENDIX 1

**Cell line: RAW 264.7 cells (ATCC TIB-71)**

**Viral strain: norovirus murin, S99 strain (batch n° 4/200409/220409- Friedrich Loeffler Institut)**

**Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

**Reagents:**

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V
- Sheep erythrocytes, Oxoid, réf. SR 0051E, batch n° 4234000

**Inactivation solution:**

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## 9 TECHNICAL APPENDIX 2

*Table A1 – Norovirus titer, by Spaerman-Kärber method.*

Log TCID<sub>50</sub> = 7,125

| Dilution (- log)             | Results  | % positive results |
|------------------------------|----------|--------------------|
| -3                           | 44444444 | 100                |
| -4                           | 44444444 | 100                |
| -5                           | 44444444 | 100                |
| -6                           | 44444444 | 100                |
| -7                           | 11111000 | 62,5               |
| -8                           | 00000000 | 0                  |
| -9                           | 00000000 | 0                  |
| -10                          | 00000000 | 0                  |
| Sum of % of positive results |          | 462,5              |



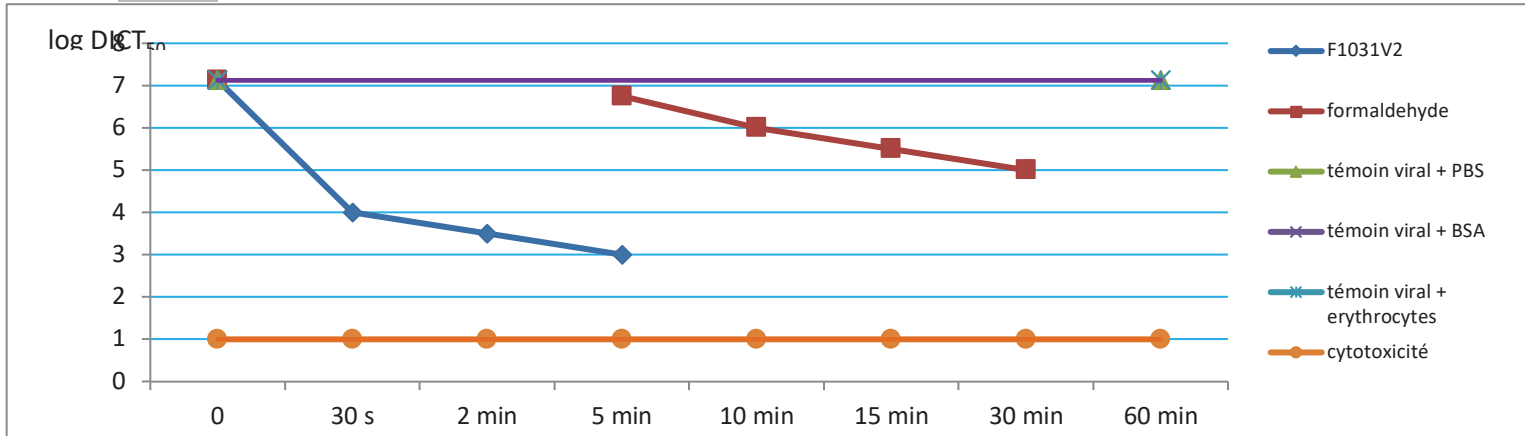
| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Chart 1 – Trials on norovirus:

TRIAL 1



TRIAL 2

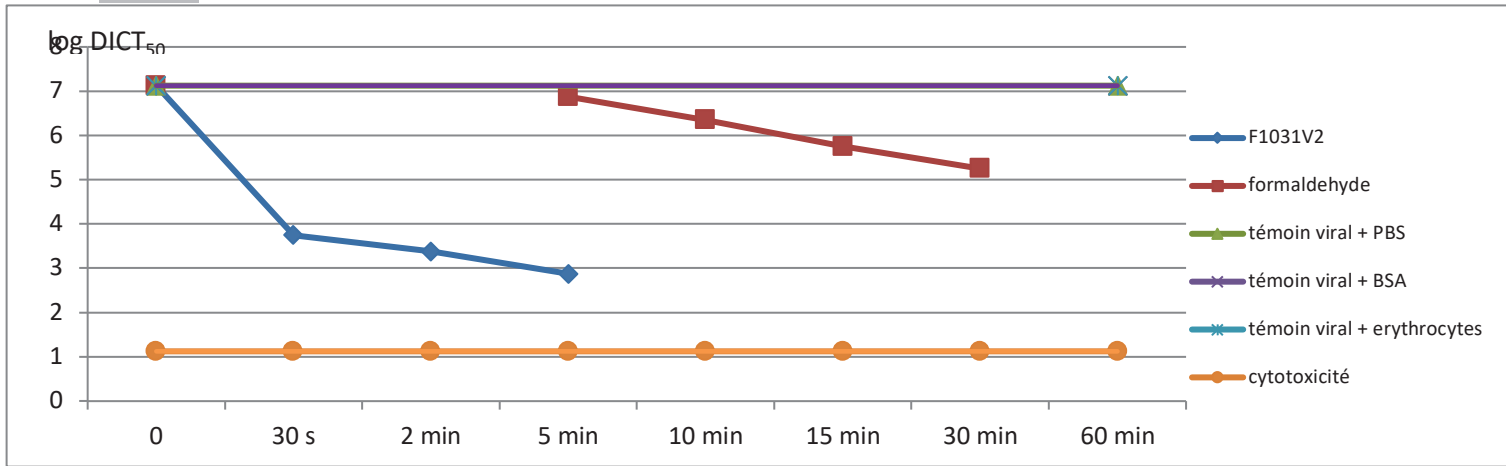


Table A2- Sensitivity of the cells to the norovirus:

| PRODUCT | DILUTION         | ORGANIC SOIL LOAD     |                 | Dilutions |      |      |      |      |      |      |      |
|---------|------------------|-----------------------|-----------------|-----------|------|------|------|------|------|------|------|
|         |                  |                       |                 | -2        | -3   | -4   | -5   | -6   | -7   | -8   | -9   |
| F1031V2 | 10 <sup>-2</sup> | 3 g/L BSA + 3 mL/L SE | Untreated cells | 4444      | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |
|         |                  |                       | Treated cells   | 4444      | 4444 | 4444 | 4444 | 4444 | 1100 | 1000 | 0000 |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |





| Rédacteur   | Superviseur   |
|---|---|
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|  |  |

Table A3 — Results on norovirus



| PRODUCT                              | Concentration    | Organic soil load     | Cytotoxicity level | Lg DICT <sub>50</sub> |      |       |       |        |        | Reduction |                    |
|--------------------------------------|------------------|-----------------------|--------------------|-----------------------|------|-------|-------|--------|--------|-----------|--------------------|
|                                      |                  |                       |                    | 0                     | 30 s | 2 min | 5 min | 15 min | 30 min |           | 60 min             |
| F1031V2 TRIAL 1                      | 100,00%          | 3 g/l BSA + 3 ml/l SE | 1                  | 7,125                 | 4    | 3,5   | 3     | N.T.   | N.T.   | N.T.      | 5 min<br>R = 4,125 |
| F1031V2 TRIAL 2                      | 100,00%          | 3 g/l BSA + 3 ml/l SE | 1,125              | 7,125                 | 3,75 | 3,375 | 2,875 | N.T.   | N.T.   | N.T.      | 5 min<br>R = 4,25  |
| Formaldehyde TRIAL 1                 | 0,70%            | PBS                   | 2,5                | 7,125                 | N.T. | N.T.  | 6,75  | 5,5    | 5      | N.T.      |                    |
| Formaldehyde TRIAL 2                 | 0,70%            | PBS                   | 2,5                | 7,125                 | N.T. | N.T.  | 6,875 | 5,75   | 5,25   | N.T.      |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | PBS                   | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA             | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA + 3 ml/l SE | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | PBS                   | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/l BSA             | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/l BSA + 3 ml/l SE | N.A.               | 7,125                 | N.T. | N.T.  | N.T.  | N.T.   | N.T.   | 7,125     |                    |
| CELL SENSITIVITY TO THE VIRUS        | 10 <sup>-2</sup> | N.A.                  | Untreated cells    | 7,125                 |      |       |       |        |        |           |                    |
|                                      |                  | N.A.                  | Treated cells      | 6,75                  |      |       |       |        |        |           |                    |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**Table A4 — Raw results**

| TRIAL 1                      | Concentration | Organic soil load   | Exposure time | Dilutions |      |      |      |      |      |      |      |      |      |
|------------------------------|---------------|---------------------|---------------|-----------|------|------|------|------|------|------|------|------|------|
|                              |               |                     |               | -1        | -2   | -3   | -4   | -5   | -6   | -7   | -8   | -9   |      |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 30 s          | 4444      | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 2 min         | 4444      | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 5 min         | 4444      | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | VIRAL CONTROL | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1110 | 0000 | 0000 | 0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444      | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444      | 4444 | 4444 | 4444 | 4444 | 1111 | 1100 | 0000 | 0000 |      |
|                              |               |                     | 15            | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 30            | 4444      | 4444 | 4444 | 1111 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444      | 4444 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |      |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |

| TRIAL 2                      | Concentration | Organic soil load   | Exposure time | Dilutions |      |      |      |      |      |      |      |      |      |
|------------------------------|---------------|---------------------|---------------|-----------|------|------|------|------|------|------|------|------|------|
|                              |               |                     |               | -1        | -2   | -3   | -4   | -5   | -6   | -7   | -8   | -9   |      |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 30 s          | 4444      | 4444 | 4444 | 4400 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 2 min         | 4444      | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 5 min         | 4444      | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | VIRAL CONTROL | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444      | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444      | 4444 | 4444 | 4444 | 4444 | 1111 | 1100 | 0000 | 0000 | 0000 |
|                              |               |                     | 15            | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 30            | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444      | 4444 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |      |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |

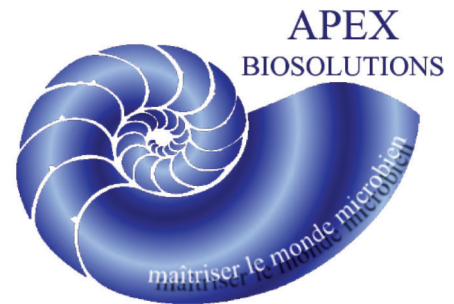
|   |   |
|---|---|
| <b>Rédacteur</b>  | <b>Superviseur</b>  |
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## TEST REPORT

|   |
|---|
| <b>DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2<br/>PRODUCT ACCORDING TO THE EN 14476:2015 STANDARD</b> |
|---|

Delivered to Ms CHAKCHOUK

For : **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**  
**FRANCE**



Date of request: 10/18/2018

Date of request: n°268D25-2018-08

### VIRUCIDAL TESTS:

According to the NF EN 14476+A2 (October 2015) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the F1031V2 product, against the *adenovirus*.

This test report included 9 pages.

Study completion date: 12/11/2018

Stephanie MOROT - BIZOT  
PhD in Microbiology  
Study Director

A handwritten signature in black ink, appearing to read 'Stephanie Morot-Bizot'.

**SUMMARY**

1 PERFORMING LABORATORY ..... 3

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| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 1 PERFORMING LABORATORY

APEX BIOSOLUTIONS  
 4, rue des Grandes Pièces  
 Zone EURESPACE  
 25 770 SERRE LES SAPINS  
 FRANCE

## 2 SAMPLE IDENTIFICATION

| SAMPLE  | BATCH N° |
|---------|----------|
| F1031V2 | 611141   |

Expiration date: non communicated

Manufacturer : FRANKLAB

Manufacturing date: non communicated

Storage conditions: room temperature

Active substances : ethanol, isopropanol, tertiary amine

Appearance of the product: liquid, colorless

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 10/24/2018

Date of tests: from 11/02/2018 to 12/05/2018

## 3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log TCID<sub>50</sub>

Exposure time: 5 min, 10 min and 30 min

Diluent used for the product: distilled water

Final concentrations tested: 80%

Viral strains: adenovirus type 5, adenoid 75 strain, grown on HEp-2 cells, at 37°C, under 5% CO<sub>2</sub> atmosphere

Organic soil load: 3 g/L bovine serum albumin + 3 mL/L sheep erythrocytes

Product stability: good

Stop solution: cold shock

### Viral titre:



Viral titers are expressed in log TCID<sub>50</sub> (calculated by Spearman-Kärber method) :

- For adenovirus, titer = 6,875 log DICT<sub>50</sub>

## 4 VALIDATION

### a) Cytotoxicity

For adenovirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |



**b) SENSIBILITY ASSAYS**

The viruses were titrated on cell cultures untreated with the product (indicator cell line) and titrated on cell cultures treated with the product.

According to the European standard EN 14476+A2, the F1031V2 product used at the dilution of  $10^{-2}$  does not have an effect on the viruses titration method (the difference between viral titers must be  $< 1,0 \log$ ):

| ADENOVIRUS               | Viral titer (log TCID <sub>50</sub> ) |                       |                                       |
|--------------------------|---------------------------------------|-----------------------|---------------------------------------|
|                          | Untreated cell cultures               | Treated cell cultures | Viral titer (log TCID <sub>50</sub> ) |
| PRODUCT DILUTION         |                                       |                       |                                       |
| F1031V2 10 <sup>-2</sup> | 6,875                                 | 6,625                 | <b>0,250</b>                          |

**c) VALIDATIONS OF THE COLD SHOCK METHOD** (the method is validated if the difference is  $\leq 0,5 \log$ ):

| PRODUCT CONCENTRATION | Organic soil load     | Viral titer (log TCID <sub>50</sub> ) | Difference with the viral suspension |
|-----------------------|-----------------------|---------------------------------------|--------------------------------------|
| F1031V2               | 3 g/L BSA + 3 mL/L SE | TRIAL 1: 6,875                        | 0,000                                |
|                       |                       | TRIAL 2: 7,000                        | 0,125                                |

**d) INACTIVATION ASSAYS OF THE VIRUS WITH A CONTROL SOLUTION**

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure.

| Formaldehyde 0,7%               | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction (log TCID <sub>50</sub> ) |
|---------------------------------|---------------------------------------|---|
| <b>Viral suspension control</b> | 6,875                                 |   |
| inactivation 5 min              |                                       | 0,375   |
| inactivation 15 min             | 6,500                                 | 1,375   |
| inactivation 30 min             | 5,500                                 | <b>1,625</b>                                    |

**5 VIRUCIDAL ASSAYS**



The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0 \log$ .

**TRIAL 1** - The viral suspension was titrated at 6,875 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 5 min            | 20°C        | 4,000                                 | 2,875                 |
|         |               | 10 min           |             | 2,875                                 | <b>4,000</b>          |
|         |               | 30 min           |             | 2,500                                 | <b>4,375</b>          |

**TRIAL 2** - The viral suspension was titrated at 7,000 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 5 min            | 20°C        | 3,375                                 | 3,625                 |
|         |               | 10 min           |             | 2,875                                 | <b>4,125</b>          |
|         |               | 30 min           |             | 2,500                                 | <b>4,500</b>          |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 6 VALIDATION OF THE METHODOLOGY



*The assays were validated as required by the European standard EN 14476+A2:*

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product: 6,875 log TCID<sub>50</sub> for adenovirus
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure :
  - the reduction observed was of 1,625 log for the adenovirus.
- The F1031V2 product does not have a cytotoxic effect on the HEp-2 cells.
- The F1031V2 product does not affect the infectious capacity of the viruses:
  - For adenovirus, the differences in viral titers between the virus inoculated on HEp-2 cells and the virus inoculated on the HEp-2 cells treated with the product was  $\leq 1,0$  log (0,250 log).

## 7 CONCLUSION

**The assays performed with the F1031V2 product, batch #611141:**

- **Demonstrated a virucidal activity on the adenovirus from the concentration 80%**, as required by the European standard EN 14476+A2, following a **10 min** exposure period, at 20°C, in dirty conditions.

| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 8 TECHNICAL APPENDIX 1

**Cell line: HEp-2 cells (RD-Biotech réf. 84011, batch n°110315-118)**

**Viral strain: adenovirus type 5, adenoïd 75 strain (ATCC réf. VR-5, batch n°3679877)**

**Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

**Reagents:**

- Albumine bovine sera, Dominique DUTSCHER, ref. 871001, batch D1304039
- Sheep erythrocytes, Oxoid, réf. SR 0051E, batch n° 4234000

**Inactivation solution:**

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## 9 TECHNICAL APPENDIX 2

*Table A1 – Adenovirus titer, by Spaerman-Kärber method.*

Log TCID<sub>50</sub> = 6,875

| Dilution (- log)             | Results  | % positive results |
|------------------------------|----------|--------------------|
| -3                           | 44444444 | 100                |
| -4                           | 44444444 | 100                |
| -5                           | 44444444 | 100                |
| -6                           | 44444444 | 100                |
| -7                           | 44400000 | 37,5               |
| -8                           | 00000000 | 0                  |
| -9                           | 00000000 | 0                  |
| -10                          | 00000000 | 0                  |
| Sum of % of positive results |          | 437,5              |



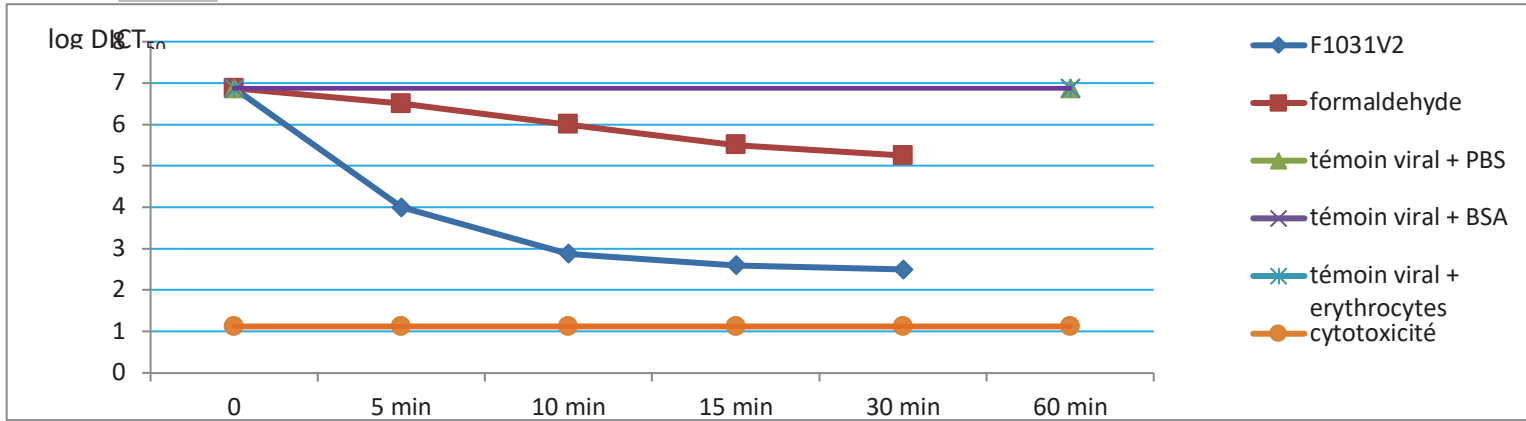
| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Chart 1 – Trials on adenovirus:

TRIAL 1



TRIAL 2

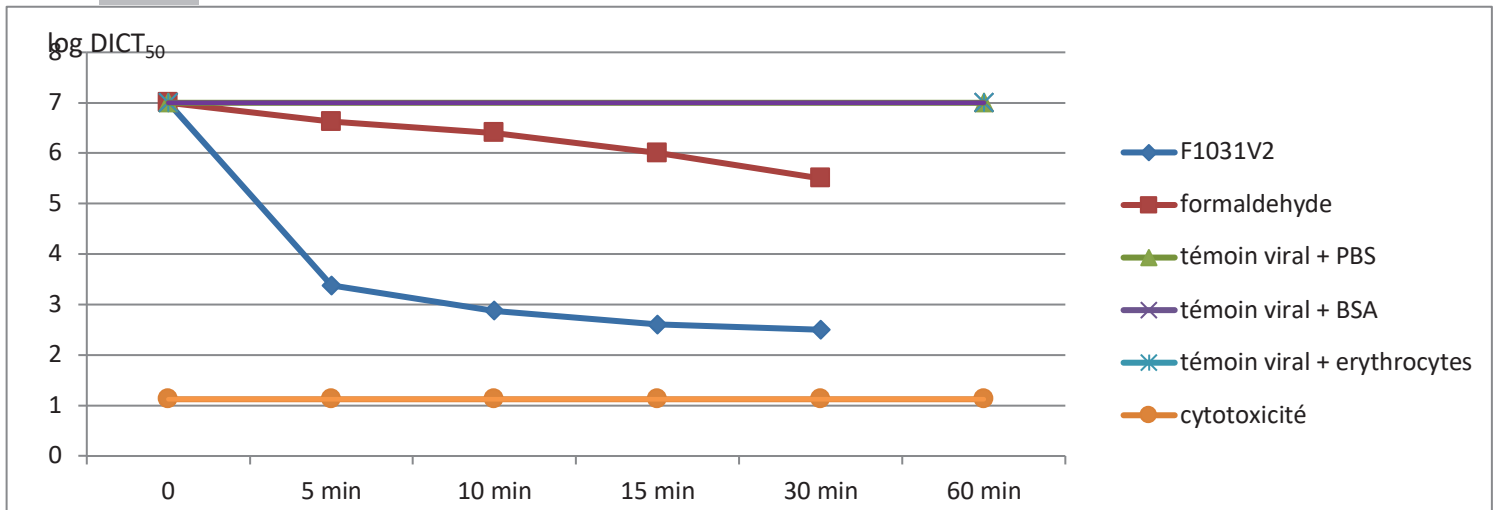


Table A2- Sensitivity of the cells to the adenovirus:

| PRODUCT | DILUTION         | ORGANIC SOIL LOAD     |                 | Dilutions |      |      |      |      |      |      |      |  |
|---------|------------------|-----------------------|-----------------|-----------|------|------|------|------|------|------|------|--|
|         |                  |                       |                 | -2        | -3   | -4   | -5   | -6   | -7   | -8   | -9   |  |
| F1031V2 | 10 <sup>-2</sup> | 3 g/L BSA + 3 mL/L SE | Untreated cells | 4444      | 4444 | 4444 | 4444 | 1111 | 1110 | 0000 | 0000 |  |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 |  |
|         |                  |                       | Treated cells   | 4444      | 4444 | 4444 | 4444 | 1111 | 1000 | 1000 | 0000 |  |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 |  |



| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Table A3 — Results on adenovirus

| PRODUCT                              | Concentration    | Organic soil load     | Cytotoxicity level | Lg DICT <sub>50</sub> |       |        |        |        |        | Reduction           |
|--------------------------------------|------------------|-----------------------|--------------------|-----------------------|-------|--------|--------|--------|--------|---------------------|
|                                      |                  |                       |                    | 0                     | 5 min | 10 min | 15 min | 30 min | 60 min |                     |
| F1031V2 TRIAL 1                      | 80,00%           | 3 g/l BSA + 3 ml/l SE | 1,125              | 6,875                 | 4,000 | 2,875  | N.T.   | 2,500  | N.T.   | 10 min<br>R = 4,000 |
| F1031V2 TRIAL 2                      | 80,00%           | 3 g/l BSA + 3 ml/l SE | 1,000              | 7,000                 | 3,375 | 2,875  | N.T.   | 2,500  | N.T.   | 10 min<br>R = 4,125 |
| Formaldehyde TRIAL 1                 | 0,70%            | PBS                   | 2,375              | 6,875                 | 6,500 | N.T.   | 5,500  | 5,250  | N.T.   |                     |
| Formaldehyde TRIAL 2                 | 0,70%            | PBS                   | 2,000              | 7,000                 | 6,625 | N.T.   | 6,000  | 5,50   | N.T.   |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | PBS                   | N.A.               | 6,875                 | N.T.  | N.T.   | N.T.   | N.T.   | 6,875  |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA             | N.A.               | 6,875                 | N.T.  | N.T.   | N.T.   | N.T.   | 6,875  |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA + 3 ml/L SE | N.A.               | 6,875                 | N.T.  | N.T.   | N.T.   | N.T.   | 6,875  |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | PBS                   | N.A.               | 7,000                 | N.T.  | N.T.   | N.T.   | N.T.   | 7,000  |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/L BSA             | N.A.               | 7,000                 | N.T.  | N.T.   | N.T.   | N.T.   | 7,000  |                     |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/L BSA + 3 ml/L SE | N.A.               | 7,000                 | N.T.  | N.T.   | N.T.   | N.T.   | 7,000  |                     |
| CELL SENSITIVITY TO THE VIRUS        | 10 <sup>-2</sup> | N.A.                  | Untreated cells    | 6,875                 |       |        |        |        |        |                     |
|                                      |                  | N.A.                  | Treated cells      | 6,625                 |       |        |        |        |        |                     |





| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Table A4 — Raw results

| TRIAL 1                      | Concentration | Organic soil load   | Exposure time | Dilutions    |              |              |              |              |              |              |              |              |              |              |
|------------------------------|---------------|---------------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|                              |               |                     |               | -1           | -2           | -3           | -4           | -5           | -6           | -7           | -8           | -9           |              |              |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 5 min         | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 10 min        | 4444<br>4444 | 4444<br>4444 | 4440<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 30 min        | 4444<br>4444 | 4444<br>4444 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
|                              |               |                     | VIRAL CONTROL | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444<br>4000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>1111 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 15            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 30            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>1100 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444<br>4444 | 4444<br>4440 | 0000<br>0000 | 0000<br>0000 | N.T.<br>N.T. | N.T.<br>N.T. | N.T.<br>N.T. | N.T.<br>N.T. | N.T.<br>N.T. |              |              |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1110<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |

| TRIAL 2                      | Concentration | Organic soil load   | Exposure time | Dilutions    |              |              |              |              |              |              |              |              |              |
|------------------------------|---------------|---------------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|                              |               |                     |               | -1           | -2           | -3           | -4           | -5           | -6           | -7           | -8           | -9           |              |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 5 min         | 4444<br>4444 | 4444<br>4444 | 4444<br>4440 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
|                              |               |                     | 10 min        | 4444<br>4444 | 4444<br>4444 | 4440<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
|                              |               |                     | 30 min        | 4444<br>4444 | 4444<br>4444 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
|                              |               |                     | VIRAL CONTROL | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>1111 | 1000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |
|                              |               |                     | 15            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 30            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444<br>4444 | 4444<br>0000 | 0000<br>0000 | 0000<br>0000 | N.T.<br>N.T. | N.T.<br>N.T. | N.T.<br>N.T. | N.T.<br>N.T. |              |              |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |
|                              |               |                     | 60            | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 4444<br>4444 | 1111<br>1111 | 1111<br>0000 | 0000<br>0000 | 0000<br>0000 |              |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## TEST REPORT

**DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2  
WIPES PRODUCT ACCORDING TO THE EN 14476:2019 STANDARD**

Delivered to Ms CHAKCHOUK

For : **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**  
**FRANCE**



Date of request: 10/18/2018

Date of request: n°268D25-2018-23

### VIRUCIDAL TESTS:

According to the NF EN 14476+A2 (July 2019) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the F1031V2 wipes product, against the *Poliovirus*.

This test report included 10 pages.

Study completion date: 12/31/2018

Stephanie MOROT - BIZOT  
PhD in Microbiology  
Study Director



**SUMMARY**

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| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |



**1 PERFORMING LABORATORY**

APEX BIOSOLUTIONS  
 4, rue des Grandes pièces  
 Zone EURESPACE  
 25770 SERRE LES SAPINS  
 France

**2 SAMPLE IDENTIFICATION**

| SAMPLE        | BATCH N° |
|---------------|----------|
| F1031V2 WIPES | 701301   |

Expiration date: non communicated

Manufacturer: FRANKLAB

Date of manufacture: non communicated

Storage conditions: room temperature and darkness

Active substances: ethanol, isopropanol, tertiary amine

Appearance of the product: non-woven wipes, VH 23g/m<sup>2</sup>, 280% impregnation.

Product diluent recommended by the manufacturer for use: none, ready-to-use for product.

Date of delivery of the product: 10/24/2018

Date of tests: 10/29/2018 to 11/23/2018

**3 EXPERIMENTAL CONDITIONS**

Temperature used during the assays: 20°C ± 1°C

Titration units: UFP/mL (Poliovirus)

Exposure time: 5 min, 10 min and 30 min

Diluent used for the product: distilled water

Final concentrations tested: 80%. Extraction of the product by manual spinning

Viral strains: Poliovirus type 1, LSc-2ab strain (IFL), grown on VERO cells, at 37°C, under 5% CO<sub>2</sub> atmosphere

Organic soil load: 3 g/L BSA + 3 mL sheep erythrocytes



Product stability: good

Stop solution: cold shock

**Viral titre:**

Viral titers are expressed in log TCID<sub>50</sub> (calculated by Spearman-Kärber method) :

- Poliovirus = 6,59 log PFU/mL.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

#### 4 VALIDATION

##### a) Cytotoxicity

The cell toxicity was observed:

- until to the dilution  $10^{-1}$  on VERO cells

##### b) SENSIBILITY ASSAYS

The viruses were titrated on cell cultures untreated with the product (indicator cell line) and titrated on cell cultures treated with the product. According to the European standard EN 14476+A2, the F1031V2 WIPES product used at the dilution of  $10^{-2}$  does not have an effect on the viruses titration method (the difference between viral titers must be  $< 1,0$  log):

| POLIOVIRUS       |           | Viral titer (log PFU/mL) |                       |  |
|------------------|-----------|--------------------------|-----------------------|--|
| PRODUCT DILUTION |           | Untreated cell cultures  | Treated cell cultures | Difference of viral titer (log PFU/mL <sub>0</sub> ) |
| F1031V2          | $10^{-1}$ | 6,60                     | 6,42                  | <b>0,18</b>  |

##### c) VALIDATIONS OF THE COLD SHOCK METHOD (the method is validated if the difference is $\leq 0,5$ log):

###### POLIOVIRUS

| PRODUCT CONCENTRATION | Organic soil load     | Viral titer (log PFU/mL) | Difference of viral titer |
|-----------------------|-----------------------|--------------------------|---------------------------|
| F1031V2 80%           | 3 g/L BSA + 3 mL/L SE | TRIAL 1: 6,59            | 0,00                      |
|                       |                       | TRIAL 2: 6,59            | 0,00                      |

##### a) INACTIVATION ASSAYS OF THE VIRUS WITH A CONTROL SOLUTION

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure.

###### POLIOVIRUS

| Formaldehyde 0,7%               | Viral titer (log PFU/mL) | Viral titer reduction (log PFU/mL) |
|---------------------------------|--------------------------|------------------------------------|
| <b>Viral suspension control</b> | 6,59                     |                                    |
| inactivation 5 min              | 6,51                     | 0,08                               |
| inactivation 15 min             | 5,56                     | 1,03                               |
| inactivation 30 min             | 4,48                     | <b>2,11</b>                        |



#### 5 VIRUCIDAL ASSAYS

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0$  log.

###### POLIOVIRUS

**TRIAL 1** - The viral suspension was titrated at **6,59 log PFU/mL**.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log UFP/mL) | Viral titer reduction |
|---------|---------------|------------------|-------------|--------------------------|-----------------------|
| F1031V2 | 80%           | 5 min            | 20°C        | 2,88                     | 3,71                  |
|         |               | 10 min           |             | 2,43                     | <b>4,16</b>           |
|         |               | 30 min           |             | 1,96                     | <b>4,63</b>           |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**TRIAL 2** - The viral suspension was titrated at **6,59 log PFU/mL**.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log PFU/mL) | Viral titer reduction |
|---------|---------------|------------------|-------------|--------------------------|-----------------------|
| F1031V2 | 80%           | 5 min            | 20°C        | 2,96                     | 3,63                  |
|         |               | 10 min           |             | 2,39                     | <b>4,20</b>           |
|         |               | 30 min           |             | 1,86                     | <b>4,73</b>           |

## 6 VALIDATION OF THE METHODOLOGY



The assays were validated as required by the European standard EN 14476+A2:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product:
  - 6,59 log PFU/mL for the Poliovirus
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure :
  - the reduction observed was of 2,11 log for the poliovirus.
- The F1031V2 product does not have a cytotoxic effect on the cells.
- The F1031V2 product does not affect the infectious capacity of the viruses:
  - For poliovirus, the differences in viral titers between the virus inoculated on VERO cells and the virus inoculated on the VERO cells treated with the product was  $\leq 1,0$  log (0,18 log).

## 7 CONCLUSION

**The assays performed with the F1031V2 product, batch #701301:**

- **Demonstrated a virucidal activity on the Poliovirus, from the concentration 80%**, as required by the European standard EN 14476+A2, following a **10 min** exposure period, at 20°C, in dirty conditions.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 8 TECHNICAL APPENDIX 1

**Cell line:** VERO cells (RD-Biotech ref. 84009, batch n°110118-110V)

**Viral strain:** Poliovirus type 1, LSc-2ab strain (ref RVB-1260 - batch n° 2/10121998- Friedrich Loeffler Institut)

**Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

**Reagents:**

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V
- Sheep erythrocytes, Oxoïd, ref. SR 0051E, batch n° 4234000

**Inactivation solution:**



- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## 9 TECHNICAL APPENDIX 2

*Table A1 – poliovirus titer, by Spaerman-Kärber method:*

| Dilution (- log) | WELL 1 | WELL 2 | WELL 3 | TOTAL PER DILUTION |
|------------------|--------|--------|--------|--------------------|
| -4               | 385,00 | 385,00 | 398,00 | 1168,00            |
| -5               | 43,00  | 39,00  | 34,00  | 116,00             |
| -6               | 4,00   | 5,00   | 3,00   | 12,00              |
| TOTAL            |        |        |        | 1296,00            |

log UFP/mL = 6,59

| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

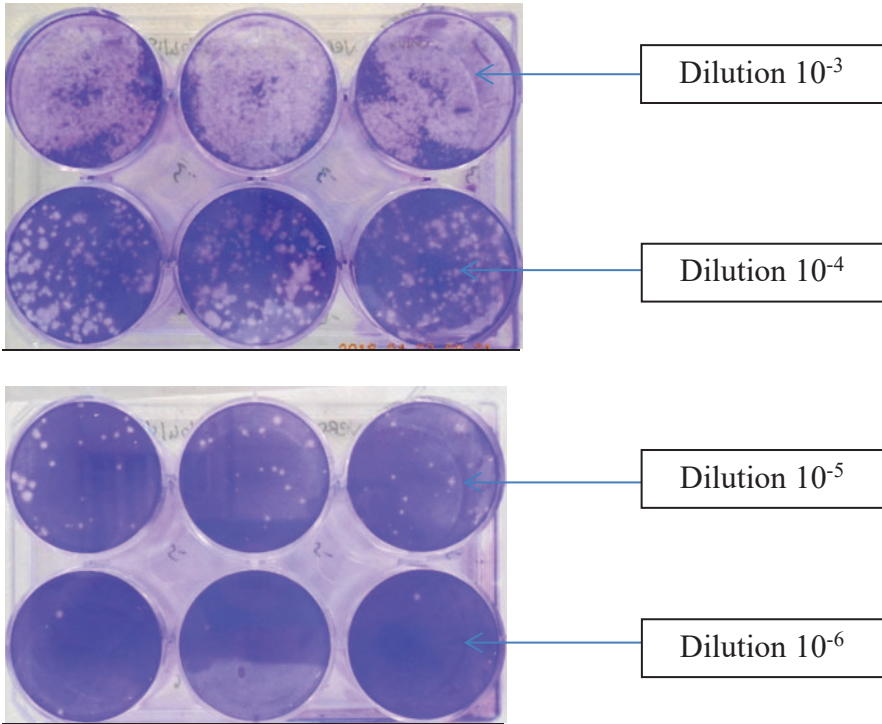
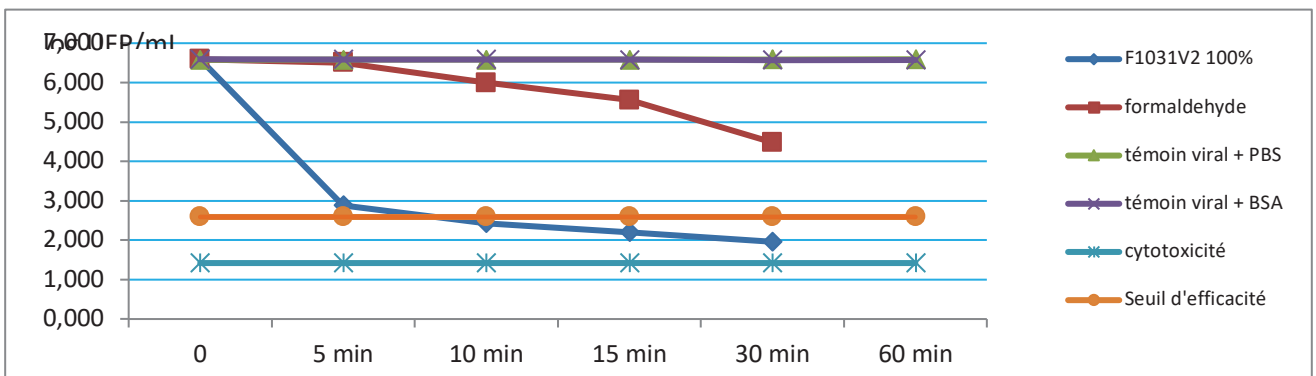
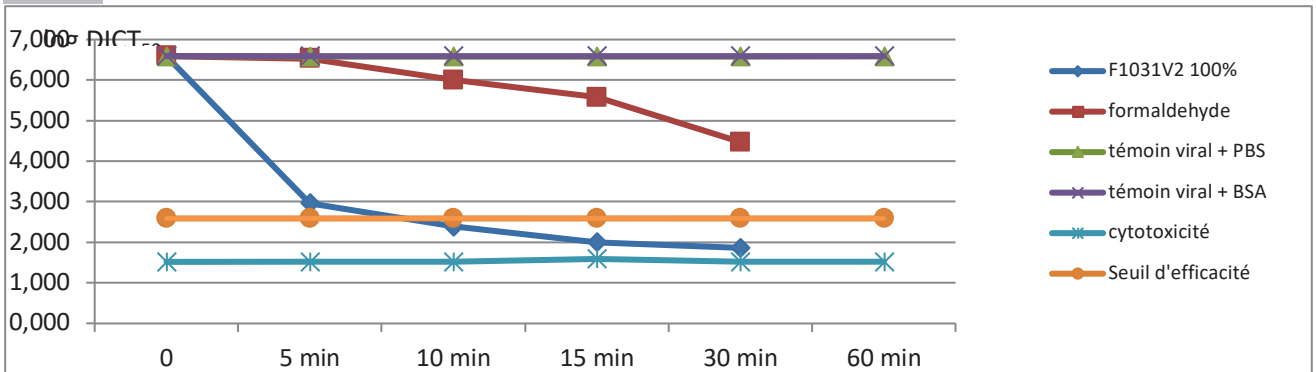


Chart 1 – Trials on poliovirus:

**TRIAL 1**



**TRIAL 2**



| Rédacteur                                       | Superviseur                           |
|---|---------------------------------------|
| Mme Emilie CANTREL, technicienne de laboratoire | Mme Stephanie MOROT-BIZOT, directrice |
|   |                                       |

Table A2- Sensitivity of the cells to the poliovirus:

| Product | Dilution         | Organic soil load       |                 | Dilutions |    |     |     |    |     | Total plaques | Log  | Log R |
|---------|------------------|-------------------------|-----------------|-----------|----|-----|-----|----|-----|---------------|------|-------|
|         |                  |                         |                 | -1        | -2 | -3  | -4  | -5 | -6  |               |      |       |
| F1031V2 | 10 <sup>-1</sup> | 3 g/l BSA+<br>3 mL/L SE | Untreated cells |           |    |     | 390 | 40 | 4   | 1324          | 6,60 | 0,18  |
|         |                  |                         |                 |           |    |     | 395 | 42 | 4   |               |      |       |
|         |                  |                         |                 |           |    |     | 399 | 45 | 5   |               |      |       |
|         |                  |                         | Treated cells   |           |    | 259 | 26  | 3  | 879 | 6,42          |      |       |
|         |                  |                         |                 |           |    | 249 | 25  | 3  |     |               |      |       |
|         |                  |                         |                 |           |    | 282 | 29  | 3  |     |               |      |       |

Table A3 — Results on poliovirus

| PRODUCT                              | Concentration    | Organic soil load        | Cytotoxicity level | Lg PFU/mL |       |        |        |        |        | Reduction          |
|--------------------------------------|------------------|--------------------------|--------------------|-----------|-------|--------|--------|--------|--------|--------------------|
|                                      |                  |                          |                    | 0         | 5 min | 10 min | 15 min | 30 min | 60 min |                    |
| F1031V2 TRIAL 1                      | 100,00%          | 3 g/l BSA +<br>3 mL/L SE | 1,42               | 6,59      | 2,88  | 2,43   | N.T.   | 1,96   | N.T.   | 10 min<br>R = 4,16 |
| F1031V2 TRIAL 2                      | 100,00%          | 3 g/l BSA +<br>3 mL/L SE | 1,52               | 6,59      | 2,96  | 2,39   | N.T.   | 1,86   | N.T.   | 10 min<br>R = 4,20 |
| Formaldehyde TRIAL 1                 | 0,70%            |                          | 0,36               | 6,59      | 6,51  | N.T.   | 5,56   | 4,48   | N.T.   |                    |
| Formaldehyde TRIAL 2                 | 0,70%            |                          | 0,34               | 6,59      | 6,53  | N.T.   | 5,58   | 4,47   | N.T.   |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | PBS                      | N.A.               | 6,58      | N.T.  | N.T.   | N.T.   | N.T.   | 6,59   |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA +<br>3 mL/L SE | N.A.               | 6,59      | N.T.  | N.T.   | N.T.   | N.T.   | 6,57   |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | PBS                      | N.A.               | 6,59      | N.T.  | N.T.   | N.T.   | N.T.   | 6,59   |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/l BSA +<br>3 mL/L SE | N.A.               | 6,59      | N.T.  | N.T.   | N.T.   | N.T.   | 6,59   |                    |
| CELL SENSITIVITY TO THE VIRUS        | 10 <sup>-2</sup> | N.A.                     | Untreated cells    | 6,60      |       |        |        |        |        |                    |
|                                      |                  | N.A.                     | Treated cells      | 6,42      |       |        |        |        |        |                    |







| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Table A4 — Raw results

| TRIAL 1                      | Concentration | Organic soil load     | Exposure time | Dilutions |    |     |     |    |    |
|------------------------------|---------------|-----------------------|---------------|-----------|----|-----|-----|----|----|
|                              |               |                       |               | -1        | -2 | -3  | -4  | -5 | -6 |
| F1031V2 TRIAL 1              | 80,00%        | 3 g/l BSA + 3 mL/L SE | 5 min         | 85        | 8  | 1   |     |    |    |
|                              |               |                       |               | 77        | 6  | 0   |     |    |    |
|                              |               |                       |               | 69        | 7  | 0   |     |    |    |
|                              |               |                       | 10 min        | 24        | 3  | 0   |     |    |    |
|                              |               |                       |               | 30        | 2  | 0   |     |    |    |
| 30 min                       | 26            | 4                     | 0             |           |    |     |     |    |    |
|                              | 12            | 1                     | 0             |           |    |     |     |    |    |
|                              |               |                       | 8             | 0         | 0  |     |     |    |    |
|                              |               |                       | 9             | 0         | 0  |     |     |    |    |
|                              |               |                       | VIRAL CONTROL | 6,59      |    |     |     |    |    |
| F1031V2 TRIAL 1 cytotoxicity | 80,00%        | 3 g/l BSA + 3 mL/L SE | N.A.          | 25        | 2  |     |     |    |    |
|                              |               |                       |               | 28        | 0  |     |     |    |    |
|                              |               |                       |               | 24        | 0  |     |     |    |    |
| Formaldehyde                 | 0,70%         |                       | 5             |           |    |     | 301 | 30 | 4  |
|                              |               |                       |               |           |    |     | 332 | 28 | 2  |
|                              |               |                       |               |           |    |     | 341 | 34 | 4  |
|                              |               |                       | 15            |           |    | 359 | 44  | 4  | 0  |
|                              |               |                       |               |           |    | 360 | 42  | 8  | 0  |
|                              |               |                       |               |           |    | 347 | 35  | 4  | 1  |
|                              |               |                       | 30            |           |    | 285 | 33  | 3  | 0  |
|                              |               |                       |               |           |    | 274 | 29  | 4  | 0  |
|                              |               |                       |               |           |    | 269 | 29  | 3  | 0  |
| Formaldehyde (cytotoxicity)  | 0,70%         |                       | N.A.          | 22        | 2  |     |     |    |    |
|                              |               |                       |               | 22        | 0  |     |     |    |    |
|                              |               |                       |               | 26        | 3  |     |     |    |    |
| viral control of infectivity | N.A.          | PBS                   | 0             |           |    |     | 370 | 38 | 4  |
|                              |               |                       |               |           |    |     | 375 | 41 | 5  |
|                              |               |                       | 60            |           |    |     | 379 | 39 | 4  |
|                              |               |                       |               |           |    |     | 379 | 43 | 6  |
|                              |               |                       |               |           |    |     | 388 | 45 | 4  |
|                              |               |                       |               |           |    |     | 395 | 40 | 4  |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 mL/L SE | 0             |           |    |     | 395 | 44 | 5  |
|                              |               |                       |               |           |    |     | 384 | 40 | 4  |
|                              |               |                       | 60            |           |    |     | 389 | 39 | 5  |
|                              |               |                       |               |           |    |     | 390 | 38 | 7  |
|                              |               |                       |               |           |    |     | 346 | 38 | 4  |
|                              |               |                       |               |           |    |     | 376 | 43 | 4  |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

| TRIAL 2                      | Concentration | Organic soil load     | Exposure time | Dilutions |    |    |     |    |    |
|------------------------------|---------------|-----------------------|---------------|-----------|----|----|-----|----|----|
|                              |               |                       |               | -1        | -2 | -3 | -4  | -5 | -6 |
| F1031V2 TRIAL 2              | 80,00%        | 3 g/l BSA + 3 mL/L SE | 5 min         | 90        | 10 | 1  |     |    |    |
|                              |               |                       |               | 94        | 10 | 1  |     |    |    |
|                              |               |                       |               | 86        | 9  | 2  |     |    |    |
|                              |               |                       | 10 min        | 25        | 4  | 0  |     |    |    |
| 21                           | 2             | 0                     |               |           |    |    |     |    |    |
| 26                           | 3             | 0                     |               |           |    |    |     |    |    |
| 30 min                       | 8             | 1                     | 0             |           |    |    |     |    |    |
|                              | 8             | 1                     | 0             |           |    |    |     |    |    |
|                              |               |                       | 6             | 0         | 0  |    |     |    |    |
|                              |               |                       | VIRAL CONTROL | 6,59      |    |    |     |    |    |
| F1031V2 TRIAL 2 cytotoxicity | 80,00%        | 3 g/l BSA + 3 mL/L SE | N.A.          | 30        | 2  |    |     |    |    |
|                              |               |                       |               | 28        | 3  |    |     |    |    |
|                              |               |                       |               | 33        | 3  |    |     |    |    |
| Formaldehyde                 | 0,70%         |                       | 5             |           |    |    | 325 | 34 | 5  |
|                              |               |                       |               |           |    |    | 344 | 38 | 4  |
|                              |               |                       |               |           |    |    | 337 | 39 | 4  |
| 15                           |               |                       |               | 380       | 39 | 4  | 0   |    |    |
|                              |               |                       |               | 376       | 42 | 5  | 0   |    |    |
|                              |               |                       |               | 371       | 39 | 4  | 0   |    |    |
| 30                           |               |                       |               | 301       | 30 | 3  | 0   |    |    |
|                              |               |                       |               | 296       | 31 | 2  | 0   |    |    |
|                              |               |                       |               | 279       | 30 | 3  | 0   |    |    |
| Formaldehyde (cytotoxicity)  | 0,70%         |                       | N.A.          | 20        | 2  |    |     |    |    |
|                              |               |                       |               | 20        | 2  |    |     |    |    |
|                              |               |                       |               | 26        | 3  |    |     |    |    |
| viral control of infectivity | N.A.          | PBS                   | 0             |           |    |    | 385 | 38 | 4  |
|                              |               |                       |               |           |    |    | 381 | 41 | 5  |
| 60                           |               |                       |               |           |    |    | 390 | 39 | 4  |
|                              |               |                       |               |           |    |    | 378 | 40 | 4  |
|                              |               |                       |               |           |    |    | 389 | 39 | 4  |
|                              |               |                       |               |           |    |    | 389 | 41 | 6  |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 mL/L SE | 0             |           |    |    | 401 | 40 | 4  |
|                              |               |                       |               |           |    |    | 395 | 42 | 5  |
| 60                           |               |                       |               |           |    |    | 378 | 39 | 4  |
|                              |               |                       |               |           |    |    | 392 | 39 | 4  |
|                              |               |                       |               |           |    |    | 393 | 41 | 7  |
|                              |               |                       |               |           |    |    | 379 | 44 | 5  |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |



## TEST REPORT

|   |
|---|
| <b>DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2<br/>PRODUCT ACCORDING TO THE EN 14476:2015 STANDARD</b> |
|---|

Delivered to Ms CHAKCHOUK

For : **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**  
**FRANCE**



Date of request: 10/18/2018

Date of request: n°268D25-2018-10

### VIRUCIDAL TESTS:

According to the NF EN 14476+A2 (October 2015) standard – chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

Tests using the F1031V2 product, against the *rotavirus*.

This test report included 9 pages.



Study completion date: 12/14/2018

Stephanie MOROT - BIZOT  
PhD in Microbiology  
Study Director



## SUMMARY

|   |                                     |   |
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| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 1 PERFORMING LABORATORY

APEX BIOSOLUTIONS  
4, rue des Grandes Pièces  
Zone EURESPACE  
25 770 SERRE LES SAPINS  
FRANCE

## 2 SAMPLE IDENTIFICATION

| SAMPLE  | BATCH N° |
|---------|----------|
| F1031V2 | 611141   |

Expiration date: non communicated

Manufacturer : FRANKLAB

Manufacturing date: non communicated

Storage conditions: room temperature

Active substances : ethanol, isopropanol, tertiary amine

Appearance of the product: liquid, colorless

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 10/24/2018

Date of tests: from 11/02/2018 to 12/05/2018

## 3 EXPERIMENTAL CONDITIONS

Temperature used during the assays: 20°C ± 1°C

Titration units: log TCID<sub>50</sub>

Exposure time: 2 min, 5 min and 10 min

Diluent used for the product: distilled water

Final concentrations tested: 80%

Viral strains: human rotavirus(ATCC VR-2551), grown on MA-104 cells, at 37°C, under 5% CO<sub>2</sub> atmosphere

Organic soil load: 3 g/L bovine serum albumin + 3 mL/L sheep erythrocytes

Product stability: good

Stop solution: cold shock

### Viral titre:



Viral titers are expressed in log TCID<sub>50</sub> (calculated by Spearman-Kärber method) :

- For rotavirus, titer = 6,625 log DICT<sub>50</sub>

## 4 VALIDATION

### a) Cytotoxicity

For rotavirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

**b) SENSIBILITY ASSAYS**

The viruses were titrated on cell cultures untreated with the product (indicator cell line) and titrated on cell cultures treated with the product.

According to the European standard EN 14476+A2, the F1031V2 product used at the dilution of  $10^{-2}$  does not have an effect on the viruses titration method (the difference between viral titers must be  $< 1,0$  log):

| ROTAVIRUS                | Viral titer (log TCID <sub>50</sub> ) |                       |                                       |
|--------------------------|---------------------------------------|-----------------------|---------------------------------------|
|                          | Untreated cell cultures               | Treated cell cultures | Viral titer (log TCID <sub>50</sub> ) |
| PRODUCT DILUTION         |                                       |                       |                                       |
| F1031V2 10 <sup>-2</sup> | 6,625                                 | 6,500                 | <b>0,125</b>                          |

**c) VALIDATIONS OF THE COLD SHOCK METHOD** (the method is validated if the difference is  $\leq 0,5$  log):

| PRODUCT CONCENTRATION | Organic soil load     | Viral titer (log TCID <sub>50</sub> ) | Difference with the viral suspension |
|-----------------------|-----------------------|---------------------------------------|--------------------------------------|
| F1031V2               | 3 g/L BSA + 3 mL/L SE | TRIAL 1: 6,625                        | 0,000                                |
|                       |                       | TRIAL 2: 6,625                        | 0,000                                |

**d) INACTIVATION ASSAYS OF THE VIRUS WITH A CONTROL SOLUTION**

The viral titer reduction (difference between the titers of the viral suspension treated with 0,7 % formaldehyde and the viral suspension control) must be between -0,5 and -2,5 log after 30 min of exposure.

| Formaldehyde 0,7%               | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction (log TCID <sub>50</sub> ) |
|---------------------------------|---------------------------------------|---|
| <b>Viral suspension control</b> | 6,625                                 |   |
| inactivation 5 min              |                                       | 0,375   |
| inactivation 15 min             | 6,500                                 | 1,625   |
| inactivation 30 min             | 5,375                                 | <b>1,750</b>                                    |

**5 VIRUCIDAL ASSAYS**



The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0$  log.

**TRIAL 1** - The viral suspension was titrated at 6,625 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 2 min            | 20°C        | 3,500                                 | 3,125                 |
|         |               | 5 min            |             | 2,750                                 | 3,875                 |
|         |               | 10 min           |             | 2,375                                 | <b>4,250</b>          |

**TRIAL 2** - The viral suspension was titrated at 6,625 log TCID<sub>50</sub>.

| PRODUCT | Concentration | Time of exposure | Temperature | Viral titer (log TCID <sub>50</sub> ) | Viral titer reduction |
|---------|---------------|------------------|-------------|---------------------------------------|-----------------------|
| F1031V2 | 80%           | 2 min            | 20°C        | 3,500                                 | 3,125                 |
|         |               | 5 min            |             | 3,125                                 | 3,500                 |
|         |               | 10 min           |             | 2,375                                 | <b>4,250</b>          |

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 6 VALIDATION OF THE METHODOLOGY



*The assays were validated as required by the European standard EN 14476+A2:*

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product: 6,625 log TCID<sub>50</sub> for rotavirus
- The virus was inactivated with the control solution of 0,7 % formaldehyde after 30 min of exposure :
  - the reduction observed was of 1,750 log for the rotavirus.
- The F1031V2 product does not have a cytotoxic effect on the MA-104 cells.
- The F1031V2 product does not affect the infectious capacity of the viruses:
  - For rotavirus, the differences in viral titers between the virus inoculated on MA-104 cells and the virus inoculated on the MA-104 cells treated with the product was  $\leq 1,0$  log (0,125 log).

## 7 CONCLUSION

**The assays performed with the F1031V2 product, batch #611141:**

- **Demonstrated a virucidal activity on the rotavirus from the concentration 80%,** as required by the European standard EN 14476+A2, following a **10 min** exposure period, at 20°C, in dirty conditions.

| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 8 TECHNICAL APPENDIX 1

**Cell line: MA-104 cells (HPA réf. 85102918, batch n° 11H030)**

**Viral strain: Rotavirus humain, ATCC ref. VR-2551**

**Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

**Reagents:**

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V
- Sheep erythrocytes, Oxoid, réf. SR 0051E, batch n° 4234000

**Inactivation solution:**

- formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## 9 TECHNICAL APPENDIX 2

*Table A1 – Rotavirus titer, by Spaerman-Kärber method:*

Log TCID<sub>50</sub> = 6,625

| Dilution (- log)             | Results  | % positive results |
|------------------------------|----------|--------------------|
| -3                           | 44444444 | 100                |
| -4                           | 44444444 | 100                |
| -5                           | 44444444 | 100                |
| -6                           | 44444444 | 100                |
| -7                           | 10000000 | 12,5               |
| -8                           | 00000000 | 0                  |
| -9                           | 00000000 | 0                  |
| -10                          | 00000000 | 0                  |
| Sum of % of positive results |          | 412,5              |



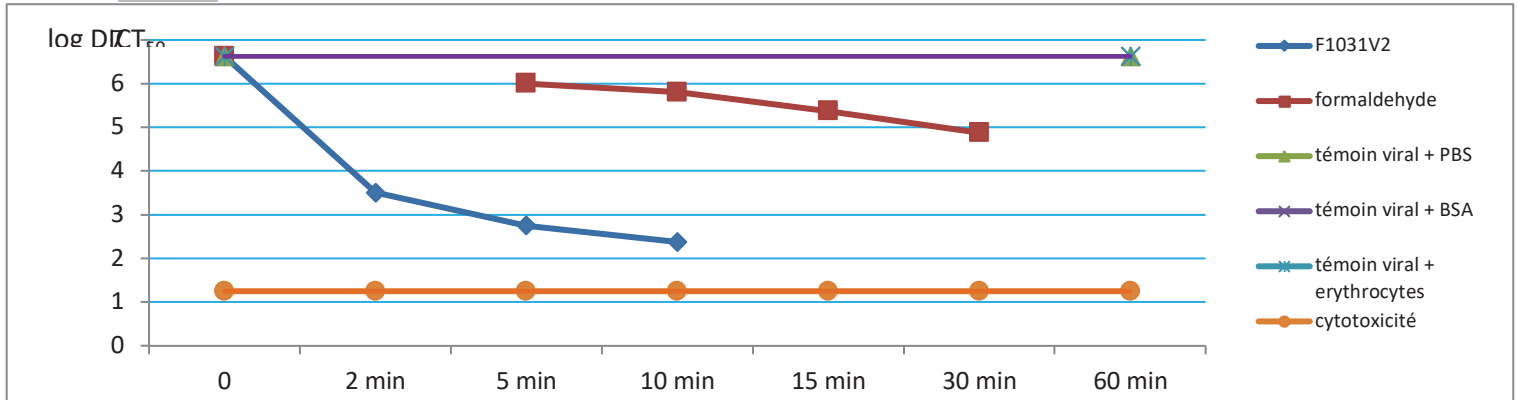
| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Chart 1 – Trials on rotavirus:

TRIAL 1



TRIAL 2

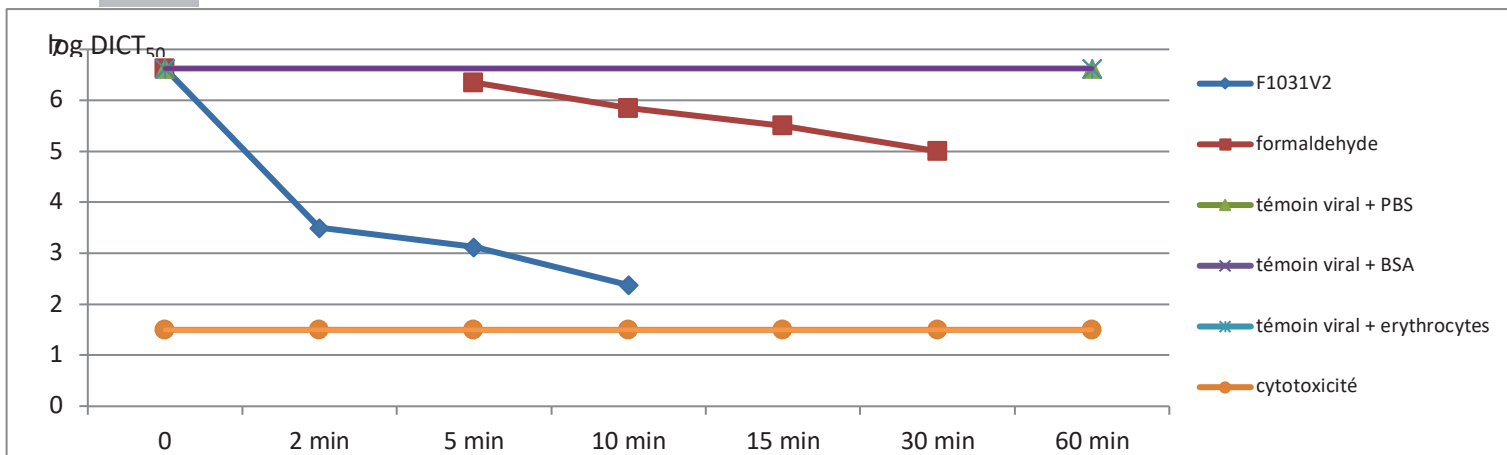


Table A2- Sensitivity of the cells to the rotavirus:

| PRODUCT | DILUTION         | ORGANIC SOIL LOAD     |                 | Dilutions |      |      |      |      |      |      |      |
|---------|------------------|-----------------------|-----------------|-----------|------|------|------|------|------|------|------|
|         |                  |                       |                 | -2        | -3   | -4   | -5   | -6   | -7   | -8   | -9   |
| F1031V2 | 10 <sup>-2</sup> | 3 g/L BSA + 3 mL/L SE | Untreated cells | 4444      | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
|         |                  |                       | Treated cells   | 4444      | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |
|         |                  |                       |                 | 4444      | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |





| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

Table A3 — Results on rotavirus

| PRODUCT                              | Concentration    | Organic soil load     | Cytotoxicity level | Lg DICT <sub>50</sub> |       |       |        |        |        |        | Reduction          |
|--------------------------------------|------------------|-----------------------|--------------------|-----------------------|-------|-------|--------|--------|--------|--------|--------------------|
|                                      |                  |                       |                    | 0                     | 2 min | 5 min | 10 min | 15 min | 30 min | 60 min |                    |
| F1031V2 TRIAL 1                      | 80,00%           | 3 g/l BSA + 3 ml/l SE | 1,25               | 6,625                 | 3,5   | 2,75  | 2,375  | N.T.   | N.T.   | N.T.   | 10 min<br>R = 4,25 |
| F1031V2 TRIAL 2                      | 80,00%           | 3 g/l BSA + 3 ml/l SE | 1,5                | 6,625                 | 3,5   | 3,125 | 2,375  | N.T.   | N.T.   | N.T.   | 10 min<br>R = 4,25 |
| Formaldehyde TRIAL 1                 | 0,70%            | PBS                   | 2,375              | 6,625                 | N.T.  | 6,5   | N.T.   | 5,375  | 4,875  | N.T.   |                    |
| Formaldehyde TRIAL 2                 | 0,70%            | PBS                   | 2,5                | 6,625                 | N.T.  | 6,5   | N.T.   | 5,5    | 5      | N.T.   |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | PBS                   | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA             | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 1 | N.A.             | 3 g/l BSA + 3 ml/l SE | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | PBS                   | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/l BSA             | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| VIRAL CONTROL OF INFECTIVITY TRIAL 2 | N.A.             | 3 g/l BSA + 3 ml/l SE | N.A.               | 6,625                 | N.T.  | N.T.  | N.T.   | N.T.   | N.T.   | 6,625  |                    |
| CELL SENSITIVITY TO THE VIRUS        | 10 <sup>-2</sup> | N.A.                  | Untreated cells    | 6,625                 |       |       |        |        |        |        |                    |
|                                      |                  | N.A.                  | Treated cells      | 6,500                 |       |       |        |        |        |        |                    |



| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |



**Table A4 — Raw results**

| TRIAL 1                      | Concentration | Organic soil load   | Exposure time | Dilutions |      |      |      |      |      |      |      |      |      |
|------------------------------|---------------|---------------------|---------------|-----------|------|------|------|------|------|------|------|------|------|
|                              |               |                     |               | -1        | -2   | -3   | -4   | -5   | -6   | -7   | -8   | -9   |      |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 2 min         | 4444      | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 5 min         | 4444      | 4444 | 4400 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 10 min        | 4444      | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | VIRAL CONTROL | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 | 0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444      | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444      | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 15            | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 30            | 4444      | 4444 | 4444 | 1111 | 1110 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444      | 4444 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |      |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 0001 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |

| TRIAL 2                      | Concentration | Organic soil load   | Exposure time | Dilutions |      |      |      |      |      |      |      |      |      |
|------------------------------|---------------|---------------------|---------------|-----------|------|------|------|------|------|------|------|------|------|
|                              |               |                     |               | -1        | -2   | -3   | -4   | -5   | -6   | -7   | -8   | -9   |      |
| F1031V2                      | 80,00%        | 3 g/l BSA + 3 ml SE | 2 min         | 4444      | 4444 | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 5 min         | 4444      | 4444 | 4440 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | 10 min        | 4444      | 4444 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |
|                              |               |                     | VIRAL CONTROL | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 | 0000 |
| F1031V2 cytotoxicity         | 80,00%        | 3 g/l BSA + 3 ml SE | N.A.          | 4444      | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde                 | 0,70%         | PBS                 | 5             | 4444      | 4444 | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 15            | 4444      | 4444 | 4444 | 4444 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
|                              |               |                     | 30            | 4444      | 4444 | 4444 | 1111 | 1111 | 0000 | 0000 | 0000 | 0000 |      |
| Formaldehyde (cytotoxicity)  | 0,70%         | PBS                 | N.A.          | 4444      | 4444 | 0000 | 0000 | N.T. | N.T. | N.T. | N.T. | N.T. |      |
| viral control of infectivity | N.A.          | PBS                 | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 0000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA           | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
| viral control of infectivity | N.A.          | 3 g/l BSA + 3 ml SE | 0             | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |
|                              |               |                     | 60            | 4444      | 4444 | 4444 | 4444 | 4444 | 4444 | 1000 | 0000 | 0000 |      |

|   |   |
|---|---|
| <b>Rédacteur</b>  | <b>Superviseur</b>  |
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## TEST REPORT

### TUBERCULOCIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 14348 STANDARD

Delivered to Ms CHAKCHOUK

For: **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**



Date of request: 10/18/2018

Study number: n°268D25-2018-24

#### MYCOBACTERICIDAL TESTS:

According to the European standard NF EN 14348 (June 2005) – Chemical disinfectants and antiseptics - Test suspension for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area -  
Test method and requirements (phase 2, step 2)

Tests using the F1031V2 LINGETTES product against 1 reference strain: *Mycobacterium terrae*.

This test report included 7 pages.

Study completion date: 01/25/2019

Stephanie MOROT-BIZOT  
PhD in microbiology  
Study director



## SUMMARY

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Sponsor : FRANKLAB

## 1 PERFORMING LABORATORY

APEX BIOSOLUTIONS  
4, rue des Grandes Pièces  
Zone EURESPACE  
25 770 SERRE LES SAPINS  
FRANCE

## 2 PRODUCT IDENTITY

| Product           | Batch N° |
|-------------------|----------|
| F1031V2 lingettes | 701301   |

Expiration date: non communicated

Manufacturer : FRANKLAB

Manufacturing date: non communicated

Storage conditions: room temperature

Active substances : ethanol, isopropanol, tertiary amine

Appearance of the product: non-woven wipes, VH 23g/m<sup>2</sup>, 280% impregnation

Product diluent recommended by the manufacturer for use: none, ready-to-use product

Date of delivery of the product: 11/09/2018

Date of tests: from 10/29/2018 to 12/30/2018

## 3 EXPERIMENTAL CONDITIONS

Final concentrations of the product: 80% - Extraction of the product by manual spinning.

Appearance of the product and its dilutions: clear.

Method: dilution-neutralization.

Exposure time: 5 min, 10 min and 60 min

Temperature using during the assays: 20°C ± 1°C

Diluent used for the assays: distilled water.

Diluent used for the mycobacterial suspensions: sterile trypton salt solution.

Organic soil load: dirty conditions, BSA 3 g/L + sheep erythrocytes 3 mL/L

Product stability: limpid solution with organic soil load

Neutralizer: tween 80 and egg yolk

Strains: *Mycobacterium terrae* CIP 104321, batch n°16308 (Institut Pasteur).

Media: Middlebrook 7H9 ADC 10% broth, Middlebrook 7H10 OADC 10% media, 37°C ± 1°C.

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#### 4 VALIDATIONS AND ASSAYS

See results sheets.

The tuberculocidal activity is demonstrated if the reduction of the population is  $\geq 4$  log.

– *Mycobacterium terrae*, R = 4,89 (10 min)

#### 5 CONCLUSION

**According to the EN 14348 standard (June 2005), the F1031V2 lingettes product:**

**- demonstrated a tuberculocidal activity against *Mycobacterium terrae* strain in 10 min at 20°C, under dirty conditions, when used pure**

#### 6 SHEETS OF RESULTS

See below.

Methodology controls:

- $1,5 \times 10^9$  UFC/ml  $\leq N \leq 5,0 \times 10^9$  UFC/ml
- $3 \times 10^2$  UFC/ml  $\leq N_v \leq 1,6 \times 10^3$
- $30 \leq N_v0 \leq 160$  UFC/ml
- A, B and C  $\geq 0,5 \times N_v0$

Vc = number of colonies on Petri dish

Nv0 = number of colonies / ml in A, B and C solutions

N = number of CFU/ml of the test suspension

Na = number of survivor per ml after time exposure with the product

A = number of CFU/ml in validation assay of experimental conditions

B = number of CFU/ml in validation assay of non-toxicity of the Neutralizer

C = number of CFU/ml in validation assay of the dilution-neutralization method

$\bar{x}$  = average of Vc1 and Vc2

LOG R = reduction ( $\lg R = \lg N0 - \lg Na$ )

Study No: 268D25-2018-24 F1031V2 lingettes  
 Sponsor : FRANKLAB

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### 7 *Mycobacterium terrae* - trial

|   |   |   |
|---|---|---|
| Standard: EN 14348<br>Product : <b>F1031V2 LINGETTES</b><br>Batch N° : 701301<br>Study N° : 268D25-2018-24<br>Date of trials : 11/19/2018 | Method:<br><input checked="" type="checkbox"/> Pour plating<br><input type="checkbox"/> Spread plating<br><input checked="" type="checkbox"/> Number of petri dishes: 2 petri dish / ml | Neutralizer : tween 80 + egg yolk 5%<br>Temperature of trials : 20°C<br>Organic soil load : 3 g/L BSA + 3 mL/L SE<br>Incubation temperature : 37°C ± 1°C<br>Diluent : sterile distilled water |
|---|---|---|

|                             | Suspension of validation |      | Validation A  |      | Validation B  |      | Validation C  |      | Trial suspension   |                       | Concentrations (v/v) |                    |                      |                      |     |                      |    |    |
|-----------------------------|--------------------------|------|---------------|------|---------------|------|---------------|------|--------------------|-----------------------|----------------------|--------------------|----------------------|----------------------|-----|----------------------|----|----|
|                             | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1                   | VC2                  | 5 min              |                      | 10 min               |     | 60 min               |    |    |
| <i>Mycobacterium terrae</i> | 66                       | 57   | 61            | 66   | 53            | 58   | 50            | 47   | 1.10 <sup>-7</sup> | 227                   | 217                  | 1.10 <sup>0</sup>  | >660                 | >660                 | 301 | 298                  | 46 | 35 |
|                             | $\bar{X}$                | 61,5 | $\bar{X}$     | 64,0 | $\bar{X}$     | 55,5 | $\bar{X}$     | 48,5 | 1.10 <sup>-8</sup> | 25                    | 22                   | 1.10 <sup>-1</sup> | 266                  | 248                  | 34  | 30                   | 8  | 5  |
|                             | 30 ≤ Nv0 ≤ 160           |      | A ≥ 0,5 * Nv0 |      | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      |                    |                       |                      | 1.10 <sup>-2</sup> | 30                   | 28                   | 5   | 4                    | 2  | 0  |
|                             | x yes □ no               |      | x oui □ non   |      | x oui □ non   |      | x oui □ non   |      |                    |                       |                      | 1.10 <sup>-3</sup> | 4                    | 3                    | 0   | 1                    | 0  | 0  |
|                             |                          |      |               |      |               |      |               |      |                    | Log N                 | 9,35                 | Na                 | 2,60.10 <sup>4</sup> | 3,01.10 <sup>3</sup> |     | 4,05.10 <sup>2</sup> |    |    |
|                             |                          |      |               |      |               |      |               |      |                    | Log N0                | 8,35                 | Log Na             | 4,41                 | 3,48                 |     | 2,61                 |    |    |
|                             |                          |      |               |      |               |      |               |      |                    | 8,17 ≤ log N0 ≤ 8,70? |                      | Log R              | 3,94                 | 4,87                 |     | 5,74                 |    |    |
|                             |                          |      |               |      |               |      |               |      | x yes □ no         |                       |                      |                    |                      |                      |     |                      |    |    |

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Study No: 268D25-2018-24 F1031V2 lingettes

6/7

Sponsor : FRANKLAB

**8 *Mycobacterium terrae* - repetition**

|   |   |   |
|---|---|---|
| Standard: EN 14348<br>Product : <b>F1031V2 LINGETTES</b><br>Batch N° : 701301<br>Study N° : 268D25-2018-24<br>Date of trials : 11/20/2018 | Method:<br><br><input checked="" type="checkbox"/> Pour plating<br><input type="checkbox"/> Spread plating<br><input checked="" type="checkbox"/> Number of petri dishes: 2 petri dish / ml | Neutralizer : tween 80 + egg yolk 5%<br>Temperature of trials : 20°C<br>Organic soil load : 3 g/L BSA + 3 mL/L SE<br>Incubation temperature : 37°C ± 1°C<br>Diluent : sterile distilled water |
|---|---|---|

|                             | Suspension of validation |      | Validation A  |      | Validation B  |      | Validation C  |      | Trial suspension   |                       |       | Concentrations (v/v) |                      |                      |     |                      |     |    |
|-----------------------------|--------------------------|------|---------------|------|---------------|------|---------------|------|--------------------|-----------------------|-------|----------------------|----------------------|----------------------|-----|----------------------|-----|----|
|                             | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1                   | VC2   | 5 min                |                      | 10 min               |     | 60 min               |     |    |
|                             | VC1                      | VC2  | VC1           | VC2  | VC1           | VC2  | VC1           | VC2  |                    | VC1                   | VC2   | VC1                  | VC2                  | VC1                  | VC2 | VC1                  | VC2 |    |
| <i>Mycobacterium terrae</i> | 48                       | 53   | 56            | 63   | 61            | 60   | 34            | 41   | 1.10 <sup>-7</sup> | 165                   | 171   | 1.10 <sup>0</sup>    | 662                  | 662                  | 223 | 191                  | 49  | 50 |
|                             | $\bar{x}$                | 50,5 | $\bar{x}$     | 59,5 | $\bar{x}$     | 60,5 | $\bar{x}$     | 37,5 | 1.10 <sup>-8</sup> | 19                    | 20    | 1.10 <sup>-1</sup>   | 401                  | 389                  | 26  | 31                   | 6   | 6  |
|                             | 30 ≤ Nv0 ≤ 160           |      | A ≥ 0,5 * Nv0 |      | B ≥ 0,5 * Nv0 |      | C ≥ 0,5 * Nv0 |      |                    |                       |       | 1.10 <sup>-2</sup>   | 55                   | 46                   | 2   | 0                    | 1   | 1  |
|                             | x yes □ no               |      | x yes □ no    |      | x yes □ no    |      | x yes □ no    |      |                    |                       |       | 1.10 <sup>-3</sup>   | 6                    | 10                   | 0   | 0                    | 0   | 0  |
|                             |                          |      |               |      |               |      |               |      |                    | Log N                 | 9,23  | Na                   | 4,05.10 <sup>4</sup> | 2,14.10 <sup>3</sup> |     | 4,95.10 <sup>2</sup> |     |    |
|                             |                          |      |               |      |               |      |               |      |                    | Log NO                | 8,23  | Log Na               | 4,61                 | 3,33                 |     | 2,69                 |     |    |
|                             |                          |      |               |      |               |      |               |      |                    | 8,17 ≤ log NO ≤ 8,70? | Log R | 3,62                 | 4,90                 | 5,54                 |     |                      |     |    |
|                             |                          |      |               |      |               |      |               |      | x yes □ no         |                       |       |                      |                      |                      |     |                      |     |    |

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## 9 TECHNICAL APPENDIX

### MEDIA

- Middlebrook broth 7H9, FLUKA, ref. 100957898, batch n° BCBC4788
- ADC 10%, FLUKA, ref. 101007527, batch n° BCBD4192
- Middlebrook media and Cohn 7H10 SIGMA-ALDRICH, ref. M0303, batch n°1405662
- OADC 10%, FLUKA, ref. 100962567, batch n° BCBC5497

### DILUENT

#### Trypton-Salt Solution Per liter of distilled water:

- Trypton, Dominique Dutscher, ref. 777472, batch n° 090633 1,00 g
- Sodium Chloride, Grosseron, ref. 9020401, batch n° FR08 085 793 8,50 g

Final pH at 25°C : 7,0 ± 0,2

### NEUTRALIZER

#### Ingredients per liter of distilled water:

- Tween 80, Sigma Aldrich, ref 59924, batch BCBJ6978V 30 g
- Egg yolk 5 g

Sterilised by autoclaving

### ORGANIC SOIL LOAD

Albumin Serum Bovine in powder, Fraction V, Dominique Dutscher, ref. P6154, batch D1304039



## TEST REPORT

### DETERMINATION OF THE SPORICIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE ON 13704 STANDARD

Delivered to Mme CHAKCHOUK

For : **FRANKLAB**  
**3 avenue des Frênes**  
**78180 MONTIGNY LE BRETONNEUX**  
**FRANCE**



Date of request:: 10/18/2018

Study n°: n°268D25-2018-11

#### SPORICIDAL TESTS :

According to the European standards EN 13704 (April 2002) – Chemical disinfectants and antiseptics - Quantitative suspension tests for the evaluation of sporicidal activity of disinfectants used in food, industrial, domestic and institutional areas (phase 2, step 1).

Tests using the F1031V2 product against the strain *Clostridium difficile*.

This test report includes 7 pages.



Study completion date: 12/24/2018

Stephanie MOROT - BIZOT  
PhD in Microbiology  
Study Director



## SUMMARY

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| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 1 PERFORMING LABORATORY

APEX BIOSOLUTIONS  
4, rue des Grandes Pièces  
Zone EURESPACE  
25 770 SERRE LES SAPINS  
FRANCE

## 2 PRODUCT IDENTITY

| SAMPLE  | BATCH  |
|---------|--------|
| F1031V2 | 611141 |

Expiration date: non communicated

Manufacturer: FRANKLAB

Date of manufacture: non communicated

Storage conditions: room temperature and darkness

Active substances: tertiary amine, ethanol, isopropanol

Appearance of the product: clear, colorless.

Product diluent recommended by the manufacturer for use: ready-to-use product.

Date of delivery of the product: 10/24/2018

Date of tests: 11/09/2018 to 12/05/2018

## 3 EXPERIMENTAL CONDITIONS

Final concentrations of the product: 100%

Appearance of the product and its dilutions: clear

Method: dilution-neutralization

Exposure time: 5 min, 10 min, 15 min and 60 min

Temperature using during the assays: 20°C ± 1°C

Diluent used for the assays: sterile distilled water

Diluent used for the bacterial suspensions: sterile trypton salt solution



Bacterial strains: *Clostridium difficile* NC11209 batch 10A – HPA

Media and growth conditions: RCM (Reinforced Clostridial Medium) at 37°C ± 1°C.

Organic soil load: clean conditions (BSA 0,3 g/L) and dirty conditions (BSA 3 g/L)

Product stability: limpid solution with organic soil load

Stop solution: Tween 80 and egg yolk.

| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

#### 4 RESULTS

The F1031V2 product is active because the reduction is  $> 3 \log$  :

See results sheets

- *Clostridium difficile* (15 min) : R = 3,15 log

#### 5 CONCLUSION

**According to the EN 13704 standard (April 2002), the F1031V2 product:**

- Demonstrated a sporicidal activity on the reference strain *Clostridium difficile* when used at the concentration of 100%, for 15 min of contact time, at 20 °C, in dirty conditions (3 g/L BSA)

#### 6 SHEETS OF RESULTS

See above.

For all result sheets :

Methodology:

- $30 \text{ UFC/ml} < N_{v0} < 160 \text{ UFC/ml}$
- $1,5 \cdot 10^6 \text{ UFC/ml} < N < 5 \cdot 10^6 \text{ UFC/ml}$
- $5,17 \leq \lg N_0 \leq 5,70$
- $A \geq 0,5 \times N_{v0}$
- $B \geq 0,5 \times N_{v0}$
- $C \geq 0,5 \times N_{v0}$



Legend :

Na = average of the number of cfu counted on Vc1 and Vc2

Log N = logarithm of the number of cfu of the microbial test suspension

Log R = logarithmic reduction obtained ( $\log R = \log N_0 - \log N_a$ )

VC = value counted per Petri dish



| Rédacteur   | Superviseur   |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

7 *Clostridium difficile* - TRIAL

|   |   |  |
|---|---|--|
| Standard: EN 13704<br>Product : <b>F1031V2</b><br>Batch N° : 611141<br>Study N° : 268D25-2018-11<br>Date of trials : 11/19/2018 | Method:<br><br><input checked="" type="checkbox"/> pour plating<br><input type="checkbox"/> spread plating<br><input checked="" type="checkbox"/> Number of Petri dish/mL : 1 | Neutralizer : polysorbate 80 (30g/L) + egg yolk 5%<br>Temperature: 20°C<br>Organic soil load : 3 g/L BSA<br>Incubation temperature : 37°C ± 1°C<br>Diluent : sterile distilled water |
|---|---|--|

| STRAIN          | Suspension of validation     |               | Validation A |               | Validation B |             | Validation C |       |
|-----------------|------------------------------|---------------|--------------|---------------|--------------|-------------|--------------|-------|
|                 | <i>Clostridium difficile</i> | 73            | 85           | 80            | 82           | 77          | 79           | 68    |
| $\bar{x}$       |                              | 79,0          | $\bar{x}$    | 81,0          | $\bar{x}$    | 78,0        | $\bar{x}$    | 73,5  |
| Nv              |                              | 790,0         | A            | 810,0         | B            | 780,0       | C            | 735,0 |
| 600 ≤ Nv ≤ 3000 |                              | A ≥ 0,05 * Nv |              | B ≥ 0,05 * Nv |              | C ≥ 0,5 * B |              |       |
| × yes □ no      |                              | × yes □ no    |              | × yes □ no    |              | × yes □ no  |              |       |

| STRAIN             | Suspension of validation     |                      |     | TRIAL                 |         | 5 min |                       | TRIAL  |    |                       | 10 min |    | TRIAL                 |        |    | 15 min |  |
|--------------------|------------------------------|----------------------|-----|-----------------------|---------|-------|-----------------------|--------|----|-----------------------|--------|----|-----------------------|--------|----|--------|--|
|                    | <i>Clostridium difficile</i> | 1.10 <sup>-4</sup>   | 238 | 241                   | Vc      | 107   | 95                    | Vc     | 50 | 48                    | Vc     | 15 | 18                    | Vc     | 15 | 18     |  |
| 1.10 <sup>-5</sup> |                              | 25                   | 25  | Na                    | 1010,00 |       | Na                    | 490,00 |    | Na                    | 165,00 |    | Na                    | 165,00 |    |        |  |
| N                  |                              | 2,40.10 <sup>6</sup> |     | Log Na                | 3,00    |       | Log Na                | 2,69   |    | Log Na                | 2,22   |    | Log Na                | 2,22   |    |        |  |
| Log N0             |                              | 5,38                 |     | Log R = log N0-log Na | 2,38    |       | Log R = log N0-log Na | 2,69   |    | Log R = log N0-log Na | 3,16   |    | Log R = log N0-log Na | 3,16   |    |        |  |



|   |   |
|---|---|
| <b>Rédacteur</b>  | <b>Superviseur</b>  |
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

8 *Clostridium difficile* – REPETITION

|   |   |  |
|---|---|--|
| Standard: EN 13704<br>Product : <b>F1031V2</b><br>Batch N° : 611141<br>Study N° : 268D25-2018-11<br>Date of trials : 11/29/2018 | Method:<br><input checked="" type="checkbox"/> pour plating<br><input type="checkbox"/> spread plating<br><input checked="" type="checkbox"/> Number of Petri dish/mL : 1 | Neutralizer : polysorbate 80 (30g/L) + egg yolk 5%<br>Temperature: 20°C<br>Organic soil load : 3 g/L BSA<br>Incubation temperature : 37°C ± 1°C<br>Diluent : sterile distilled water |
|---|---|--|

| STRAIN                       | Suspension of validation          |              | Validation A                      |              | Validation B                      |              | Validation C                      |              |
|------------------------------|-----------------------------------|--------------|-----------------------------------|--------------|-----------------------------------|--------------|-----------------------------------|--------------|
| <i>Clostridium difficile</i> | 73                                | 85           | 80                                | 82           | 77                                | 79           | 68                                | 79           |
|                              | $\bar{x}$                         | <b>79,0</b>  | $\bar{x}$                         | <b>81,0</b>  | $\bar{x}$                         | <b>78,0</b>  | $\bar{x}$                         | <b>73,5</b>  |
|                              | Nv                                | <b>790,0</b> | A                                 | <b>810,0</b> | B                                 | <b>780,0</b> | C                                 | <b>735,0</b> |
|                              | 600 ≤ Nv ≤ 3000                   |              | A ≥ 0,05 * Nv                     |              | B ≥ 0,05 * Nv                     |              | C ≥ 0,5 * B                       |              |
|                              | × yes <input type="checkbox"/> no |              | × yes <input type="checkbox"/> no |              | × yes <input type="checkbox"/> no |              | × yes <input type="checkbox"/> no |              |

| STRAIN                       | Suspension of validation |                      |     | TRIAL                 |         | 5 min       | TRIAL                 |        | 10 min      | TRIAL                 |        | 15 min      |
|------------------------------|--------------------------|----------------------|-----|-----------------------|---------|-------------|-----------------------|--------|-------------|-----------------------|--------|-------------|
| <i>Clostridium difficile</i> | 1.10 <sup>-4</sup>       | 238                  | 241 | Vc                    | 107     | 95          | Vc                    | 50     | 48          | Vc                    | 15     | 18          |
|                              | 1.10 <sup>-5</sup>       | 25                   | 25  | Na                    | 1010,00 |             | Na                    | 490,00 |             | Na                    | 165,00 |             |
|                              | N                        | 2,40.10 <sup>6</sup> |     | Log Na                | 3,00    |             | Log Na                | 2,69   |             | Log Na                | 2,22   |             |
|                              | Log N0                   | <b>5,38</b>          |     | Log R = log N0-log Na |         | <b>2,38</b> | Log R = log N0-log Na |        | <b>2,69</b> | Log R = log N0-log Na |        | <b>3,16</b> |

| <u>Rédacteur</u>  | <u>Superviseur</u>  |
|---|---|
| Mme Emilie CANTREL, technicienne de laboratoire                                     | Mme Stephanie MOROT-BIZOT, directrice   |
|  |  |

## 9 TECHNICAL APPENDIX

### MEDIA:

TSA (Trypton Soy Agar), D. DUTSCHER, réf. 777410, batch n° 707171

**RCM** (Reinforced Clostridial Medium), D. DUTSCHER, réf. 1007, batch n° 709203

**ANAEROGEN BAGS**, THERMOFISHER, réf. AN0035A, batch n° 6323ZJ

### DILUENT Trypton-Salt Solution (TS)

#### Ingredients in grams per litre of distilled water:

- a) Trypton, Dominique Dutscher, ref. 777472, batch n° 090633 -----1,00 g/l  
 b) Sodium chloride, Grosseron, ref. n° 9020401, batch n° FR08 085 793 -----8,50 g/l

pH after autoclaving at 25 °C: 7.0 ± 0.2

### ORGANIC SOIL LOAD:



Bovine serum albumin powder, ESTER TECHNOPOLE, réf.1000-70, batch D1304039

### Stop solution

#### Ingredients per liter of distilled water:

- Tween 80, SIGMA ALDRICH, réf. 59924, lot n° BCBJ6978V ----- 30 g/L  
 - Egg yolk ----- 50 mL

Sterilised by autoclaving

| <b>Rédacteur</b>  | <b>Superviseur</b>  |
|---|---|
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|  |  |

## MATERIAL COMPATIBILITY

*Simple immersion method*

### ■ Materials compatible with Clino'wipes:

- ✓ *Stainless steel*
- ✓ *Anodised aluminium*
- ✓ *Chrome*
- ✓ *Endoscope sheath*
- ✓ *Brass*
- ✓ *Epoxy steel*
- ✓ *HDPE*
- ✓ *Polymethylmetacrylate*
- ✓ *Polypropylene*
- ✓ *PVC*
- ✓ *Silicone*
- ✓ *Viton*
- ✓ *Neoprene*
- ✓ *PVC coated fabric*
- ✓ *Polycarbonate*
- ✓ *Natural rubber*
- ✓ *Synthetic rubber*
- ✓ *Corian*
- ✓ *Linoleum*
- ✓ *Polyphenylsulfone*
- ✓ *Polyurethane*
- ✓ *PVC floor covering*
- ✓ *Raw aluminium*
- ✓ *Kerrock*
- ✓ *ABS*
- ✓ *Polysulfone*



# Clino'wipes



Product for professional use - follow the precautions for use. Before use, read the label and product information. Class IIb medical device.

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