

TEST REPORT

DETERMINATION OF THE SPORICIDAL ACTIVITY OF THE F010760V2 PRODUCT ACCORDING TO THE EN 17126 STANDARD

Delivered to: **Ms CHAKCHOUK and M CHARRAT**

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Study references: #257D84-2023

SPORICIDAL TESTS:

According to the European standards EN 17126 (December 2018) – Chemical disinfectants and antiseptics - Quantitative suspension tests for the evaluation of sporicidal activity of disinfectants used in medical area (phase 2, step 1).

Tests using the F010760V2 product against the strain *Clostridium difficile*.

This test report includes 7 pages.



Study completion date: 10/19/2023

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PhD in Microbiology
Study Director



SUMMARY

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| Ms Emilie CANTREL, laboratory technician | Mrs Stephanie MOROT-BIZOT, Director |
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1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
3, rue de la terre rouge
ESPACE INDUSTRIEL DE BEAUPRE
25220 ROCHE LEZ BEAUPRE
FRANCE

2 PRODUCT IDENTITY

| Reference | Batch N° |
|-----------|----------|
| F010760V2 | 8049 |

Expiration date: non communicated

Manufacturer: FRANKLAB

Date of manufacture: non communicated

Storage conditions: room temperature and darkness

Active substances: quaternary ammoniums

Appearance of the product: liquid, green

Product diluent recommended by the manufacturer for use: tap water.

Date of delivery of the product: 07/27/2023

Date of tests: 09/15/2023 to 10/03/2023

3 EXPERIMENTAL CONDITIONS

Final concentrations of the product: 1.00% - 0.50%

Appearance of the product and its dilutions: clear

Method: dilution-neutralization

Exposure time: 15 min

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

Diluent used for the assays: hard water

Diluent used for the bacterial suspensions: sterile trypton salt solution



Bacterial strain: *Clostridium difficile* NC11209 lot 10A (R027) – HPA

Media and growth conditions: TSA (Trypton Soy Agar)

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

Product stability: limpid solution with organic soil load

Stop solution: polysorbate 80 (30 g/L), with egg yolk (5%)

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4 CONCLUSIONS

According to the EN 17126 standard (December 2018), the F010760V2 product:

- Demonstrated a sporicidal activity on the reference strain *Clostridium difficile*, when used at the concentration of 1.00%, for 15 min of contact time, at 20°C, in dirty conditions

5 VALIDATIONS AND RESULTS SHEETS

Attached below.

- *Clostridium difficile*, **R = 4,09** for 15 min of contact time (1.00%)

For all result sheets:

Methodology:

- $30 \text{ UFC/ml} < N_{v0} < 160 \text{ UFC/ml}$
- $1,5 \cdot 10^7 \text{ UFC/ml} < N < 5 \cdot 10^7 \text{ UFC/ml}$
- $6,17 \leq \lg N_0 \leq 6,70$
- $A \geq 0,5 \times N_{v0}$
- $B \geq 0,5 \times N_{v0}$
- $C \geq 0,5 \times N_{v0}$



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

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6 RESULTS SHEET- TRIAL

| TEST STRAIN | Suspension of validation (Nv0) | | Validation A | | Validation B | | Validation C | |
|------------------------------|--------------------------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|
| <i>Clostridium difficile</i> | 76 | 90 | 85 | 89 | 81 | 88 | 55 | 63 |
| | \bar{x} | 83,0 | \bar{x} | 87,0 | \bar{x} | 84,5 | \bar{x} | 59,0 |
| | 30 ≤ Nv0 ≤ 160 ? | | A ≥ 0,5 * Nv0 ? | | B ≥ 0,5 * Nv0 ? | | C ≥ 0,5 * Nv0 ? | |
| | × yes □ no | | × yes □ no | | × yes □ no | | × yes □ no | |



| TEST STRAIN | Trial suspension | | | TRIAL | | | TRIAL | | |
|------------------------------|-----------------------|----------------------|-----|--------------------|--------|----|--------------------|--------------------|----|
| | | | | | 1.00% | | | 0.50% | |
| <i>Clostridium difficile</i> | 1.10 ⁻⁵ | 249 | 257 | Vc | | | Vc | | |
| | 1.10 ⁻⁶ | 26 | 30 | 1.10 ⁰ | 16 | 20 | 1.10 ⁰ | 101 | 93 |
| | N | 2,55.10 ⁷ | | 1.10 ⁻¹ | 2 | 2 | 1.10 ⁻¹ | 13 | 11 |
| | log N0 | 6,41 | | Na | 180,00 | | Na | 970,00 | |
| | 6,17 ≤ lg N0 ≤ 6,70 ? | | | log Na | 2,26 | | log Na | 2,99 | |
| | × yes □ no | | | Lg R = logN0-logNa | | | 4,15 | Lg R = logN0-logNa | |
| | | | | | | | 3,42 | | |

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7 RESULTS SHEET – REPETITION

| TEST STRAIN | Suspension of validation (Nv0) | | Validation A | | Validation B | | Validation C | |
|------------------------------|-----------------------------------|-------------|-----------------------------------|-------------|-----------------------------------|-------------|-----------------------------------|-------------|
| <i>Clostridium difficile</i> | 65 | 62 | 73 | 76 | 79 | 82 | 60 | 56 |
| | \bar{x} | 63,5 | \bar{x} | 74,5 | \bar{x} | 80,5 | \bar{x} | 58,0 |
| | 30 ≤ Nv0 ≤ 160 ? | | A ≥ 0,5 * Nv0 ? | | B ≥ 0,5 * Nv0 ? | | C ≥ 0,5 * Nv0 ? | |
| | × yes <input type="checkbox"/> no | | × yes <input type="checkbox"/> no | | × yes <input type="checkbox"/> no | | × yes <input type="checkbox"/> no | |

| TEST STRAIN | Trial suspension | | | TRIAL 1.00% | | | TRIAL 0.50% | | |
|------------------------------|-----------------------------------|----------------------|-----|--------------------|-------------|----|--------------------|-------------|----|
| <i>Clostridium difficile</i> | 1.10 ⁻⁵ | 236 | 225 | Vc | | | Vc | | |
| | 1.10 ⁻⁶ | 25 | 25 | 1.10 ⁰ | 22 | 22 | 1.10 ⁰ | 99 | 82 |
| | N | 2,32.10 ⁷ | | 1.10 ⁻¹ | 3 | 1 | 1.10 ⁻¹ | 12 | 10 |
| | Log N0 | 6,37 | | Na | 220,00 | | Na | 905,00 | |
| | 6,17 ≤ lg N0 ≤ 6,70 ? | | | log Na | 2,34 | | log Na | 2,96 | |
| | × yes <input type="checkbox"/> no | | | Lg R = logN0-logNa | 4,03 | | Lg R = logN0-logNa | 3,41 | |

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8 TECHNICAL APPENDIX

Media:

BHIYT-L (Broth Heart Infusion Yeast Taurocholate L cystein), Dominique Dutscher, ref. 994057, batch 712123

ORGANIC SOIL LOAD:

Bovine serum albumin powder, Dominique Dutscher, Ref. 871001, batch D1304039

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

Diluent

Trypton-Sel Solution (TS)

Ingredients in grams per litre of distilled water:

- Trypton, Dominique Dutscher, ref. 777472, batch n ° 090633 -----1,00 g/l
- 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

Stop solution

Ingredients per liter of distilled water:

- Tween 80, Sigma Aldrich, ref 59924, batch BCBJ6978V----- 30 g
- Egg yolk, ----- 50 mL



HARD WATER

Solution A: -MgCl₂ anhydrous, ref. M8266, batch n° 108K0068, SIGMA ALDRICH

- CaCl₂ Anhydrous, Ref. C1016, batch n° 059K0030, SIGMA ALDRICH

Solution B: - NaHCO₃, Ref. S6014, batch n°059K0052, SIGMA ALDRICH

pH after filtration: 7.0 ± 0.2 at 25 °C

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