

TEST REPORT

DETERMINATION OF BACTERICIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 13727 STANDARD

Delivered to Mme CHAKCHOUK

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FRANCE



Date of request: 07/21/2022

Study number: n°201D77-2022-01

BACTERICIDAL TESTS:

According to the European standard NF EN 13727 (December 2015) – antiseptics and chemical disinfectants – quantitative suspension tests for evaluation of bactericidal activity of antiseptics and chemical disinfectants used in the medical area (phase 2, step 1).

Trials on 4 reference strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae* and *Escherichia coli* K12.

This test report included 9 pages.

Study completion date: 10/25/2022

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

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

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

1. PERFORMING LABORATORY

APEX BIOSOLUTIONS
3, rue de la terre rouge
Espace industriel de BEAUPRE
25 220 ROCHE LEZ BEAUPRE
FRANCE

2. PRODUCT IDENTITY

Product	Batch N°
F1031V2	7646

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: 07/22/2022

Date of the study: from 07/25/2022 to 08/03/2022

3. EXPERIMENTAL CONDITIONS

Final concentrations of the product: 80%

Method: dilution-neutralization

Exposure time: 1 min - 2 min – 5 min

Temperature using during the assays: 20°C

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

Strains: *Staphylococcus aureus* subsp. *aureus* CIP 4.83 batch 15713-1d (ATCC 6538), *Pseudomonas aeruginosa* DSM 939 batch 0413 (ATCC 15442), *Escherichia coli* K12 DSM 498 batch n°0113 and *Enterococcus hirae* DSM 3320 batch 0511 (ATCC 10541).

Media and growth conditions: TSA (Trypton Soy Agar), at 37°C ± 1°C.

Product stability: limpid solution with organic soil load

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

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4. RESULTS

The F1031V2 product is active against the reference strains used, since the reduction obtained is greater than 5 log:

- *Staphylococcus aureus*, R = 5,15
- *Pseudomonas aeruginosa*, R = 5,10
- *Enterococcus hirae*, R = 5,16
- *Escherichia coli K12*, R = 5,20

5. CONCLUSION

According to the EN 13727 (December 2015), the F1031V2 product:

- **Demonstrated a bactericidal activity against the reference strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli K12* and *Enterococcus hirae* when used from 80%, in dirty conditions at 20°C, for 5 min of exposure time.**

6. SHEETS OF RESULTS

Attached below.

Methodology:

- 3.10^2 UFC/ml < N_v < $1,6.10^3$ UFC/ml
- $1,5.10^8$ UFC/ml < N < 5.10^8 UFC/ml
- $A \geq 0,5 \times N_{v0}$
- $B \geq 0,5 \times N_{v0}$
- $C \geq 0,5 \times N_{v0}$

Legend :

$\bar{x}$ = 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 N0 – log Na)
 VC = value counted per Petri dish
 Nv = number of CFU/mL in the suspension of validation
 A = number of CFU/mL in the control of experimental conditions
 B = number of CFU/mL in the control of neutralizer toxicity
 C = number of CFU/mL in the control of neutralization method
 Na = number of remaining germs per mL after time exposure with the product

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7. TRIAL – VALIDATIONS

STRAIN	Suspension of validation Nv		Suspension of validation NvB			Validation A		Validation B		Validation C	
<i>Staphylococcus aureus</i>	112	107	1E-03	95	99	103	108	100	96	91	90
	$\bar{x}$	109,5	$\bar{x}$	97		$\bar{x}$	105,5	$\bar{x}$	98,0	$\bar{x}$	90,5
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Enterococcus hirae</i>	85	83	1E-03	81	86	84	84	79	85	80	73
	$\bar{x}$	84,0	$\bar{x}$	83,5		$\bar{x}$	84,0	$\bar{x}$	82,0	$\bar{x}$	76,5
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Pseudomonas aeruginosa</i>	101	104	1E-03	98	93	99	99	91	94	87	89
	$\bar{x}$	102,5	$\bar{x}$	95,5		$\bar{x}$	99,0	$\bar{x}$	92,5	$\bar{x}$	88,0
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Escherichia coli K12</i>	77	76	1E-03	80	82	84	82	78	73	66	68
	$\bar{x}$	76,5	$\bar{x}$	81		$\bar{x}$	83,0	$\bar{x}$	75,5	$\bar{x}$	67,0
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	

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8. TRIAL – RESULTS

STRAIN	TRIAL SUSPENSION			TRIAL			TRIAL			TRIAL		
					5 min			2 min			1 min	
Staphylococcus aureus	1.10 ⁻⁶	>330	>330	1.10 ⁰	22	25	1.10 ⁰	86	79	1.10 ⁰	208	237
	1.10 ⁻⁷	33	32	1.10 ⁻¹	3	4	1.10 ⁻¹	10	11	1.10 ⁻¹	22	24
				1.10 ⁻²	0	0	1.10 ⁻²	1	1	1.10 ⁻²	3	3
	N	3,25.10 ⁸		Na	245,45		Na	845,45		Na	2231,82	
	log NO	7,51		log Na	2,39		log Na	2,93		log Na	3,35	
				Log R	5,12		Log R	4,58		Log R	4,16	
Enterococcus hirae	1.10 ⁻⁶	270	261	1.10 ⁰	19	21	1.10 ⁰	67	72	1.10 ⁰	138	127
	1.10 ⁻⁷	27	28	1.10 ⁻¹	2	3	1.10 ⁻¹	8	9	1.10 ⁻¹	15	14
				1.10 ⁻²	0	0	1.10 ⁻²	1	0	1.10 ⁻²	2	2
	N	2,66.10 ⁸		Na	204,55		Na	709,09		Na	1336,36	
	log NO	7,43		log Na	2,31		log Na	2,85		log Na	3,13	
				Log R	5,12		Log R	4,58		Log R	4,30	
Pseudomonas aeruginosa	1.10 ⁻⁶	>330	>330	1.10 ⁰	28	25	1.10 ⁰	78	92	1.10 ⁰	146	155
	1.10 ⁻⁷	33	35	1.10 ⁻¹	4	4	1.10 ⁻¹	11	12	1.10 ⁻¹	15	18
				1.10 ⁻²	0	0	1.10 ⁻²	2	2	1.10 ⁻²	2	2
	N	3,40.10 ⁸		Na	277,27		Na	877,27		Na	1518,18	
	log NO	7,53		log Na	2,44		log Na	2,94		log Na	3,18	
				Log R	5,09		Log R	4,59		Log R	4,35	
Escherichia coli K12	1.10 ⁻⁶	263	258	1.10 ⁰	16	19	1.10 ⁰	59	64	1.10 ⁰	122	136
	1.10 ⁻⁷	29	28	1.10 ⁻¹	2	2	1.10 ⁻¹	7	7	1.10 ⁻¹	13	15
				1.10 ⁻²	0	0	1.10 ⁻²	0	1	1.10 ⁻²	2	2
	N	2,63.10 ⁸		Na	177,27		Na	622,73		Na	1300,00	
	log NO	7,42		log Na	2,25		log Na	2,79		log Na	3,11	
				Log R	5,17		Log R	4,63		Log R	4,31	

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9. REPETITION - VALIDATIONS

STRAIN	Suspension of validation Nv		Suspension of validation NvB			Validation A		Validation B		Validation C	
<i>Staphylococcus aureus</i>	116	108	1E-03	98	102	98	111	95	97	91	93
	$\bar{x}$	112,0	$\bar{x}$	100		$\bar{x}$	104,5	$\bar{x}$	96,0	$\bar{x}$	92,0
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Enterococcus hirae</i>	73	79	1E-03	77	77	69	70	78	76	65	73
	$\bar{x}$	76,0	$\bar{x}$	77		$\bar{x}$	69,5	$\bar{x}$	77,0	$\bar{x}$	69,0
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Pseudomonas aeruginosa</i>	93	96	1E-03	99	92	90	92	96	98	82	89
	$\bar{x}$	94,5	$\bar{x}$	95,5		$\bar{x}$	91,0	$\bar{x}$	97,0	$\bar{x}$	85,5
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	
<i>Escherichia coli K12</i>	70	71	1E-03	78	74	80	72	66	63	55	61
	$\bar{x}$	70,5	$\bar{x}$	76		$\bar{x}$	76,0	$\bar{x}$	64,5	$\bar{x}$	58,0
	$30 \leq Nv0 \leq 160$		$30 \leq Nv0 \leq 160$			$A \geq 0,5 * Nv0$		$B \geq 0,5 * Nv0$		$C \geq 0,5 * Nv0$	
	× yes ☐ no		× yes ☐ no			× yes ☐ no		× yes ☐ no		× yes ☐ no	

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10. REPETITION – RESULTS

STRAIN	TRIAL SUSPENSION			TRIAL			TRIAL			TRIAL		
					5 min			2 min			1 min	
<i>Staphylococcus aureus</i>	1.10 ⁻⁶	>330	>330	1.10 ⁰	26	22	1.10 ⁰	85	93	1.10 ⁰	191	153
	1.10 ⁻⁷	38	36	1.10 ⁻¹	4	2	1.10 ⁻¹	10	12	1.10 ⁻¹	21	17
				1.10 ⁻²	0	0	1.10 ⁻²	1	5	1.10 ⁻²	3	2
	N	3,70.10 ⁸		Na	245,45		Na	909,09		Na	1736,36	
	log NO	7,57		log Na	2,39		log Na	2,96		log Na	3,24	
<i>Enterococcus hirae</i>	1.10 ⁻⁶	247	253	1.10 ⁰	14	17	1.10 ⁰	60	65	1.10 ⁰	120	127
	1.10 ⁻⁷	25	28	1.10 ⁻¹	2	2	1.10 ⁻¹	9	7	1.10 ⁻¹	12	13
				1.10 ⁻²	0	0	1.10 ⁻²	1	1	1.10 ⁻²	2	3
	N	2,51.10 ⁸		Na	159,09		Na	640,91		Na	1236,36	
	log NO	7,40		log Na	2,20		log Na	2,81		log Na	3,09	
<i>Pseudomonas aeruginosa</i>	1.10 ⁻⁶	>330	>330	1.10 ⁰	23	25	1.10 ⁰	99	92	1.10 ⁰	131	154
	1.10 ⁻⁷	35	33	1.10 ⁻¹	6	4	1.10 ⁻¹	16	14	1.10 ⁻¹	14	18
				1.10 ⁻²	0	0	1.10 ⁻²	3	5	1.10 ⁻²	2	2
	N	3,40.10 ⁸		Na	263,64		Na	1004,55		Na	1440,91	
	log NO	7,53		log Na	2,42		log Na	3,00		log Na	3,16	
<i>Escherichia coli</i> K12	1.10 ⁻⁶	265	271	1.10 ⁰	15	15	1.10 ⁰	59	51	1.10 ⁰	117	109
	1.10 ⁻⁷	28	29	1.10 ⁻¹	3	2	1.10 ⁻¹	6	8	1.10 ⁻¹	17	12
				1.10 ⁻²	0	0	1.10 ⁻²	0	1	1.10 ⁻²	5	4
	N	2,70.10 ⁸		Na	159,09		Na	563,64		Na	1159,09	
	log NO	7,43		log Na	2,20		log Na	2,75		log Na	3,06	

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11. TECHNICAL APPENDIX

MEDIA:

TSA (Trypton Soy Agar), Dominique DUTSCHER, ref. 777410, batch n°906172

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) Trypton, Dominique Dutscher, ref. 777472, batch n° 090633 -----1,00 g/L
- b) Sodium chloride, Grosseron, ref 9020401, lot 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
- Saponin, Analytic Lab, réf. 84510, lot n° BCBL6449V ----- 30 g
- Egg yolk, 5% ----- 50 mL/L

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