

Clino'wipes

IMPREGNATED WIPES
WITH A DETERGENT AND DISINFECTANT
SOLUTION



Franklab Company
SteriFrance

Scientific File

Clino'wipes

SUMMARY

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REGULATORY INFORMATION

- **Clino'wipes** is a Class IIb medical device and is CE marked according to Directive 93/42/EC*.
- **Clino'wipes** is designed, manufactured and marketed by Franklab under a quality management system developed in accordance with the requirements of the international standard **ISO 13485 : 2016***.
- **Clino'wipes VIRO'Wipes** is designed, manufactured and marketed by Franklab under a quality management system developed in accordance with the requirements of the international standard **ISO 9001 : 2015***.
- **Clino'wipes** meets our **FB Ecoline*** commitment.

CERTIFICATE OF EQUIVALENCE

EQUIVALENCE CLINO'WIPES

Formula code	Designation FRANKLAB	Packaging	Commercial reference FRANKLAB
F1031V2	CLINO'WIPES	Box of 12 boxes of 150 wipes	10795

Date de création : 23/03/2023
Date de mise à jour : 23/03/2023

Disinfectant properties of CLINO'WIPES

Ready-to-use detergent and disinfectant formula

■ Bactericidal

ACTIVITY WITH MECHANICAL ACTION USING WIPES

Standards	Strains	Contact time	Temperature
EN 16615 : 2015 <i>Dirty cond.</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus hirae</i>	2 min.	20°C

ACTIVITY OF THE IMPREGNATING SOLUTION

Standards	Strains	Contact time	Temperature
EN 13727 +A1 : 2013 <i>Dirty cond.</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus hirae</i>	5 min.	20°C
EN 1276 : 2010 <i>Dirty cond.</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus hirae</i> <i>Escherichia coli</i>	5 min.	20°C
EN 13697 + A1 : 2019 <i>Dirty cond.</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus hirae</i> <i>Escherichia coli</i>	5 min.	20°C
EN 14561 : 2007 <i>Dirty cond.</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus hirae</i>	5 min.	20°C

■ Yeasticidal/ Fungicidal

ACTIVITY WITH MECHANICAL ACTION USING WIPES

Standards	Strains	Contact time	Temperature
EN 16615 : 2015 <i>Dirty cond.</i>	<i>Candida albicans</i>	2 min.	20°C

ACTIVITY OF THE IMPREGNATING SOLUTION

Standards	Strains	Contact time	Temperature
EN 13624 : 2022 <i>Dirty cond.</i>	<i>Candida albicans</i> <i>Aspergillus brasiliensis</i>	10 min.	20°C
EN 1650 : 2019 <i>Dirty cond.</i>	<i>Candida albicans</i>	5 min.	20°C
EN 13697 + A1 : 2019 <i>Dirty cond.</i>	<i>Candida albicans</i>	5 min.	20°C
EN 14562 : 2006 <i>Dirty cond.</i>	<i>Candida albicans</i> <i>Aspergillus brasiliensis</i>	10 min.	20°C

ACTIVITY OF THE IMPREGNATING SOLUTION

■ Virucidal

Standards	Strains	Contact time	Temperature
EN 14476 +A1 : 2015 <i>Dirty cond.</i>	<i>Norovirus Murin</i>	<i>5 min.</i>	<i>20°C</i>
	<i>Adénovirus</i>	<i>10 min.</i>	
	<i>Poliovirus</i>	<i>10 min.</i>	
EN 14476 +A1 : 2015 <i>Dirty cond.</i>	<i>Active on human Rotavirus</i>	<i>10 min.</i>	<i>20°C</i>

■ Mycobactericidal and/or Tuberculocidal

Standards	Strains	Contact time	Temperature
EN 14348 : 2005 <i>Dirty cond.</i>	<i>Mycobacterium terrae</i>	<i>10 min.</i>	<i>20°C</i>

■ Sporicidal

Standards	Strains	Contact time	Temperature
EN 13704 : 2018 <i>Dirty cond.</i>	<i>Clostridium difficile</i>	<i>15 min.</i>	<i>20°C</i>

RAPPORT D'ESSAI

DETERMINATION DE L'ACTIVITE BACTERICIDE DU PRODUIT F1031V2 lingettes SELON LA NORME EN 16615

Délivré à Mme CHAKCHOUK

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



Demande d'essai du : 18/10/2018

Références du dossier d'analyses : n°268D25-2018-01

ESSAIS DE BACTERICIDIE :

Selon la méthodologie de la norme européenne NF EN 16615 (Mai 2015) – antiseptiques et désinfectants chimiques – Méthode d'essai quantitative pour l'évaluation de l'activité bactéricide et levuricide sur des surfaces non poreuses, avec action mécanique à l'aide de lingettes et de lavettes dans le domaine médical (essais à 4 zones). Méthode d'essai et prescriptions (phase 2, étape 2).

Essais sur 3 souches de référence : *Staphylococcus aureus*, *Pseudomonas aeruginosa* et *Enterococcus hirae*.

Ce rapport comporte 17 pages et ne concerne que les échantillons étudiés.

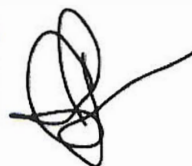
Date d'émission : 26/11/2018

Stéphanie MOROT-BIZOT
Docteur en microbiologie
Chargée de l'étude

Professeur Georges HERBEIN
Professeur des Universités Praticien Hospitalier
Expert scientifique





APEX
Mar. de confiance
APEX BIOSOLUTIONS
4, rue des grandes pièces
25770 Serre les sapins
tél 09 62 52 91 87 - info@apexlabo.com
n° SIRET 517 860 532 00012
n° TVA intra FR 2351 7860532



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

Ex. 4/16/15 - 12.2018

1. LABORATOIRE AYANT RÉALISÉ LES IDENTIFICATIONS

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

2. IDENTIFICATION DES ECHANTILLONS

Echantillon	N° lot
F1031V2 lingettes	701301

Date limite d'utilisation optimale : non communiquée

Fabricant : FRANKLAB

Date de fabrication : non communiquée

Conditions de stockage : Température ambiante et obscurité.

Composants actifs : éthanol, isopropanol, amine tertiaire

Aspect : lingettes non tissées, VH 23g/m², imprégnation 280%

Précautions d'emploi : aucune



Diluant préconisé par le fabricant : aucun, produit prêt-à-l'emploi

Date de réception au laboratoire : 24/10/2018

Période de l'étude : du 29/10/2018 au 23/11/2018

3. CONDITIONS EXPERIMENTALES

- Concentration du produit soumis à l'essai : produit pur.
- Méthode employée: dilution-neutralisation.
- Temps de contact : 1 min, 2 min et 5 min
- Température d'essai: 20°C
- Substance interférente: conditions de saleté, solution d'albumine bovine à 3g/L + 3 mL/L de sang de mouton (concentration finale).
- Diluant du produit utilisé lors des essais : solution tryptone sel stérile.
- Souches utilisées : *Staphylococcus aureus subsp. aureus* CIP 4.83 batch 15713-1d (ATCC 6538), *Pseudomonas aeruginosa* DSM 939 batch 0413 (ATCC 15442) et *Enterococcus hirae* DSM 3320 batch 0511 (ATCC 10541) - Institut Pasteur.

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- Conditions de culture: sur TSA (Gélose Tryptone Soja Agar), à 37°C ± 1°C.
- Technique d'arrêt de l'action biocide: par dilution-neutralisation, avec neutralisant à base de polysorbate 80 et de jaune d'œuf (composition en annexe).

4. RESULTATS PROPRESMENT DITS

Le produit lingettes F1031V2 est bien actif vis-à-vis des souches de référence utilisées, car la réduction obtenue est supérieure à 5 log pour les bactéries :

En conditions de saleté (moyenne des répétitions) :

- *Staphylococcus aureus*, R = 5,40 pour 2 min de contact
- *Pseudomonas aeruginosa*, R = 5,67 pour 2 min de contact
- *Enterococcus hirae*, R = 5,69 pour 2 min de contact



5. CONCLUSION

Conformément à la norme EN 16615 (Mai 2015), les essais sur le produit F1031V2 lingettes ont démontré:

- Que le produit a une activité bactéricide vis-à-vis des souches de référence *Staphylococcus aureus*, *Pseudomonas aeruginosa* et *Enterococcus hirae* en 2 min à 20°C, dans les conditions de saleté

6. FEUILLES DE RESULTATS

Voir ci-après.

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

7. *Staphylococcus aureus* - ESSAI

Vérfications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N_0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- N_{v0} est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times N_{v0}$

Légende :

Vc = dénombrement par ml

\bar{x} = moyenne de Vc1 et Vc2

N = nombre d'UFC/ml dans la suspension d'essai

Nv = nombre d'UFC/ml dans la suspension de validation

Dc0 = témoin de séchage à t0

Dct = témoin de séchage à t



B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant

C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation



Na = nombre d'UFC/ml des survivants après essai

R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 06/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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SOUCHE	Suspension d'essai		Dc0		Dct		Suspension de validation		Validation B		Validation C						
	VC1	VC2	VC1	VC2	VC1	VC2											
Staphylococcus aureus	1,00.10 ⁻⁷	290	285	1,00.10 ⁻⁵	125	133	1,00.10 ⁻⁵	130	118	80	77	80	80	69	70		
	1,00.10 ⁻⁸	32	30	1,00.10 ⁻⁶	14	14	1,00.10 ⁻⁶	12	12	\bar{x}	78,5	\bar{x}	80,0	\bar{x}	69,5		
	N	2,90.10⁹		Dc0	6,50.10⁷		Dct	6,20.10⁷		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0			
	Log N	9,46		Log DC0	7,81		Log Dct	7,79		× oui □ non		× oui □ non		× oui □ non			
Témoin eau			Essai			1 min		Essai			2 min		Essai			5 min	
	Vc1	Vc2		Vc1	Vc2		Vc1	Vc2		Vc1	Vc2		Vc1	Vc2		Vc1	Vc2
1,00.10 ⁰	201	195	Champ 1	188	235	Champ 1	100	94	Champ 1	0	2	Champ 1	0	2	Champ 1	0	2
1,00.10 ⁻¹	22	20	Na	1057,5		Na	485		Na	<70		Na	<70		Na	<70	
Nw	9,95.10 ²		Log Na	3,02		Log Na	2,69		Log Na	1,85		Log Na	1,85		Log Na	1,85	
Log Nw	3,00		Log R = log Dct - log Na	4,77		Log R = log Dct - log Na	5,10		Log R = log Dct - log Na	5,94		Log R = log Dct - log Na	5,94		Log R = log Dct - log Na	5,94	
Champ 2	21	15	Champ 2	25	20	Champ 2	5	9	Champ 2	0	0	Champ 2	0	0	Champ 2	0	0
Champ 3	3	1	Champ 3	3	2	Champ 3	0	1	Champ 3	0	0	Champ 3	0	0	Champ 3	0	0
Champ 4	0	0	Champ 4	0	1	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0
Moyenne champ 2-4	90		Moyenne champ 2-4	42,5		Moyenne champ 2-4	2,5		Moyenne champ 2-4	0		Moyenne champ 2-4	0		Moyenne champ 2-4	0	

Rédacteur	Superviseur
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8. Staphylococcus aureus - REPETITION



Vérifications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- $Nv0$ est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv0$

Légende :



- Vc = dénombrement par ml
- \bar{x} = moyenne de Vc1 et Vc2
- N = nombre d'UFC/ml dans la suspension d'essai
- Nv = nombre d'UFC/ml dans la suspension de validation
- Dc0 = témoin de séchage à t0
- Dct = témoin de séchage à t
- B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant
- C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation
- Na = nombre d'UFC/ml des survivants après essai
- R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 08/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Ex: - CMH65 - 12.2018

SOUCHE	Suspension d'essai		Dc0		Dct		Suspension de validation		Validation B		Validation C				
	VC1	VC2	VC1	VC2	VC1	VC2	VC1	VC2							
<i>Staphylococcus aureus</i>	1,00.10 ⁻⁷	312	300	1,00.10 ⁻⁵	255	247	1,00.10 ⁻⁵	237	244	101	85	92	90	99	84
	1,00.10 ⁻⁸	30	30	1,00.10 ⁻⁶	28	25	1,00.10 ⁻⁶	24	25	\bar{X}	93,0	\bar{X}	91,0	\bar{X}	91,5
	N	3,05.10 ⁹		Dc0	1,26.10 ⁸		Dct	1,20.10 ⁸		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	Log N	9,49		Log DC0	8,10		Log Dct	8,08		× oui □ non		× oui □ non		× oui □ non	
Témoins eau			Essai		1 min		Essai		2 min		Essai		5 min		
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	
1,00.10 ⁰	254	276	Champ 1	299	302	Champ 1	55	42	Champ 1	12	28	Champ 1	12	28	
1,00.10 ⁻¹	27	28	Na	1502,5		Na	242,5		Na	105		Na	105		
Nw	1,33.10 ³		Log Na	3,18		Log Na	2,39		Log Na	2,02		Log Na	2,02		
Log Nw	3,12		Log R = log Dct - log Na	4,90		Log R = log Dct - log Na	5,69		Log R = log Dct - log Na	6,06		Log R = log Dct - log Na	6,06		
Champ 2	30	24	Champ 2	27	22	Champ 2	6	4	Champ 2	2	3	Champ 2	2	3	
Champ 3	3	2	Champ 3	3	2	Champ 3	1	0	Champ 3	0	0	Champ 3	0	0	
Champ 4	0	0	Champ 4	1	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	
Moyenne champ 2-4	135		Moyenne champ 2-4	45,83		Moyenne champ 2-4	1,83		Moyenne champ 2-4	0,83		Moyenne champ 2-4	0,83		

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

9. Pseudomonas aeruginosa - ESSAI



Vérifications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- Nv0 est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv0$

Légende :



- Vc = dénombrement par ml
- \bar{x} = moyenne de Vc1 et Vc2
- N = nombre d'UFC/ml dans la suspension d'essai
- Nv = nombre d'UFC/ml dans la suspension de validation
- Dc0 = témoin de séchage à t0
- Dct = témoin de séchage à t
- B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant
- C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation
- Na = nombre d'UFC/ml des survivants après essai
- R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 13/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Ex. CN 16615, 12.2018

SOUCHE	Suspension d'essai		Dc0			Dct			Suspension de validation		Validation B		Validation C		
		VC1	VC2		VC1	VC2		VC1	VC2						
<i>Pseudomonas aeruginosa</i>	1,00.10 ⁻⁷	330	330	1,00.10 ⁻⁵	266	249	1,00.10 ⁻⁵	230	221	101	95	100	86	88	85
	1,00.10 ⁻⁸	31	34	1,00.10 ⁻⁶	26	26	1,00.10 ⁻⁶	25	22	\bar{X}	98,0	\bar{X}	93,0	\bar{X}	86,5
	N	3,30.10⁹		Dc0	1,29.10⁸		Dct	1,13.10⁸		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	Log N	9,52		Log DC0	8,11		Log Dct	8,05		× oui □ non		× oui □ non		× oui □ non	
Témoins eau			Essai			1 min	Essai			2 min	Essai			5 min	
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	
1,00.10 ⁰	268	290	Champ 1	255	302	Champ 1	52	50	Champ 1	0	0	Champ 1	0	0	
1,00.10 ⁻¹	28	30	Na	1392,5		Na	255		Na	<70		Na	<70		
Nw	1,40.10³		Log Na	3,14		Log Na	2,41		Log Na	1,85		Log Na	1,85		
Log Nw	3,15		Log R = log Dct - log Na	4,91		Log R = log Dct - log Na	5,65		Log R = log Dct - log Na	6,21		Log R = log Dct - log Na	6,21		
Champ 2	33	30	Champ 2	18	25	Champ 2	21	15	Champ 2	0	0	Champ 2	0	0	
Champ 3	5	3	Champ 3	3	2	Champ 3	3	2	Champ 3	0	0	Champ 3	0	0	
Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	
Moyenne champ 2-4	157,5		Moyenne champ 2-4	40		Moyenne champ 2-4	6,83		Moyenne champ 2-4	0		Moyenne champ 2-4	0		

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

10. Pseudomonas aeruginosa - REPETITIONVérifications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N_0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- Nv_0 est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv_0$

Légende :

Vc = dénombrement par ml

\bar{x} = moyenne de Vc1 et Vc2

N = nombre d'UFC/ml dans la suspension d'essai

Nv = nombre d'UFC/ml dans la suspension de validation

Dc0 = témoin de séchage à t0

Dct = témoin de séchage à t

B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant

C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation

Na = nombre d'UFC/ml des survivants après essai

R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 15/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur



Mme Emilie CANTREL, technicienne de laboratoire


Superviseur

Mme Stephanie MOROT-BIZOT, directrice



SOUCHE	Suspension d'essai			Dc0			Dct			Suspension de validation		Validation B		Validation C						
		VC1	VC2		VC1	VC2		VC1	VC2											
<i>Pseudomonas aeruginosa</i>	1,00.10 ⁻⁷	295	298	1,00.10 ⁻⁵	301	294	1,00.10 ⁻⁵	299	286	95	93	101	94	97	99					
	1,00.10 ⁻⁸	37	30	1,00.10 ⁻⁶	30	30	1,00.10 ⁻⁶	31	29	\bar{x}	94,0	\bar{x}	97,5	\bar{x}	98,0					
	N	3,00.10 ⁹		Dc0	1,49.10 ⁸		Dct	1,47.10 ⁸		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0						
	Log N	9,48		Log Dc0	8,17		Log Dct	8,17		× oui □ non		× oui □ non		× oui □ non						
Témoin eau			Essai			1 min			Essai			2 min			Essai			5 min		
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2			
1,00.10 ⁰	218	221	Champ 1	330	330	Champ 1	59	63	Champ 1	21	20									
1,00.10 ⁻¹	24	23	Na	1650		Na	305		Na	102,5										
Nw	1,10.10 ³		Log Na	3,22		Log Na	2,48		Log Na	2,01										
Log Nw	3,04		Log R = log Dct - log Na	4,95		Log R = log Dct - log Na	5,69		Log R = log Dct - log Na	6,16										
Champ 2	30	27	Champ 2	12	18	Champ 2	25	21	Champ 2	2	2									
Champ 3	3	3	Champ 3	3	2	Champ 3	3	1	Champ 3	0	0									
Champ 4	0	1	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0									
Moyenne champ 2-4	142,5		Moyenne champ 2-4	29,17		Moyenne champ 2-4	8,33		Moyenne champ 2-4	0,67										

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

11. Enterococcus hirae - ESSAIVérifications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N_0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- Nv_0 est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv_0$

Légende :

Vc = dénombrement par ml

\bar{x} = moyenne de Vc1 et Vc2

N = nombre d'UFC/ml dans la suspension d'essai

Nv = nombre d'UFC/ml dans la suspension de validation

Dc0 = témoin de séchage à t0

Dct = témoin de séchage à t



B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant

C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation

Na = nombre d'UFC/ml des survivants après essai



R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 20/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

F101 - EW16665 - 12.2018

SOUCHE	Suspension d'essai		Dc0			Dct			Suspension de validation		Validation B		Validation C		
		VC1	VC2		VC1	VC2		VC1	VC2						
<i>Enterococcus hirae</i>	1,00.10 ⁻⁷	262	257	1,00.10 ⁻⁵	201	213	1,00.10 ⁻⁵	198	199	100	97	99	99	102	100
	1,00.10 ⁻⁸	29	28	1,00.10 ⁻⁶	21	22	1,00.10 ⁻⁶	25	22	\bar{x}	98,5	\bar{x}	99,0	\bar{x}	101,0
	N	2,62.10⁹		Dc0	1,04.10⁸		Dct	1,01.10⁸		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	Log N	9,42		Log DC0	8,02		Log Dct	8,00		× oui □ non		× oui □ non		× oui □ non	
Témoïn eau			Essai			1 min	Essai			2 min	Essai			5 min	
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	
1,00.10 ⁰	222	218	Champ 1	222	248	Champ 1	33	30	Champ 1	0	1	Champ 1	0	1	
1,00.10 ⁻¹	20	23	Na	1175		Na	157,5		Na	<70		Na	<70		
Nw	1,10.10³		Log Na	3,07		Log Na	2,20		Log Na	1,85		Log Na	1,85		
Log Nw	3,04		Log R = log Dct - log Na	4,93		Log R = log Dct - log Na	5,80		Log R = log Dct - log Na	6,15		Log R = log Dct - log Na	6,15		
Champ 2	18	16	Champ 2	22	16	Champ 2	20	18	Champ 2	0	0	Champ 2	0	0	
Champ 3	1	2	Champ 3	0	3	Champ 3	1	2	Champ 3	0	0	Champ 3	0	0	
Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	
Moyenne champ 2-4	85		Moyenne champ 2-4	34,17		Moyenne champ 2-4	6,83		Moyenne champ 2-4	0		Moyenne champ 2-4	0		

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

12. Enterococcus hirae - REPETITIONVérifications de la méthodologie:

- N est compris entre $1,5 \times 10^9$ UFC/ml et $5,0 \times 10^9$ UFC/ml
- $7,88 \leq \log N_0 \leq 8,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- Nv_0 est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv_0$

Légende :

Vc = dénombrement par ml

\bar{x} = moyenne de Vc1 et Vc2

N = nombre d'UFC/ml dans la suspension d'essai

Nv = nombre d'UFC/ml dans la suspension de validation

Dc0 = témoin de séchage à t0

Dct = témoin de séchage à t



B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant

C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation



Na = nombre d'UFC/ml des survivants après essai

R = réduction ($\lg R = \lg Dct - \lg Na$)

Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-01 Date des essais : 22/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

SOUCHE	Suspension d'essai		Dc0		Dct		Suspension de validation		Validation B		Validation C				
		VC1	VC2		VC1	VC2		VC1	VC2						
<i>Enterococcus hirae</i>	1,00.10 ⁻⁷	277	263	1,00.10 ⁻⁵	215	219	1,00.10 ⁻⁵	209	216	88	90	91	79	81	81
	1,00.10 ⁻⁸	27	28	1,00.10 ⁻⁶	24	22	1,00.10 ⁻⁶	22	22	\bar{X}	89,0	\bar{X}	85,0	\bar{X}	81,0
	N	2,70.10 ⁹		Dc0	1,09.10 ⁸		Dct	1,07.10 ⁸		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	Log N	9,43		Log DC0	8,04		Log Dct	8,03		× oui □ non		× oui □ non		× oui □ non	
Témoïn eau			Essai		1 min	Essai		2 min	Essai		5 min				
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	
1,00.10 ⁰	208	212	Champ 1	278	297	Champ 1	59	57	Champ 1	0	5	Champ 1	0	5	
1,00.10 ⁻¹	23	21	Na	1437,5		Na	290		Na	<70		Na	<70		
Nw	1,05.10 ³		Log Na	3,16		Log Na	2,46		Log Na	1,85		Log Na	1,85		
Log Nw	3,02		Log R = log Dct – log Na	4,87		Log R = log Dct – log Na	5,57		Log R = log Dct – log Na	6,18		Log R = log Dct – log Na	6,18		
Champ 2	12	23	Champ 2	23	20	Champ 2	15	9	Champ 2	0	1	Champ 2	0	1	
Champ 3	1	2	Champ 3	2	3	Champ 3	1	5	Champ 3	0	0	Champ 3	0	0	
Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	
Moyenne champ 2-4	115		Moyenne champ 2-4	40		Moyenne champ 2-4	5		Moyenne champ 2-4	0,17		Moyenne champ 2-4	0,17		

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

13. ANNEXE TECHNIQUE

Milieux de culture utilisés, stérilisés par autoclavage :

TSA (Gélose Tryptone Soja Agar), Dominique DUTSCHER, réf. 777410, lot n° 806051

SUBSTANCES INTERFÉRENTES :

Sérum Albumine Bovine en poudre, Fraction V, Dominique Dutscher, réf.P6154, lot D1304039
 Sang de mouton, Analytic Lab, réf. 08449, lot n°bcbj3984V.

PIECES DE LINOLEUM – linoleum en PVC, traités PUR, épaisseur 2,5 mm, 20 cm x 50 cm.

DILUANT Solution Tryptone-Sel (TS)

Ingrédients en grammes par litre d'eau distillée ou déminéralisée :

- Tryptone, Dominique Dutscher, réf. 777472, lot n° 090633 -----1,00 g/l
- Chlorure de sodium, Grosseron, ref 9020401, lot n° FR08 085 793 -----8,50 g/l

pH final après autoclavage à 25°C : 7,0 ± 0,2

NEUTRALISANT

Ingrédients par litre d'eau distillée:

Polysorbate 80, SIGMA ALDRICH, réf. 59924, lot n° BCBJ6978V ----- 30 g
 Jaune d'œuf frais ----- 50 ml

Stérilisé par filtration sur filtre 0,45 µm ; pH à 25°C : 7,4 ± 0,1

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

File - F.N 16615 12-2018

TEST REPORT

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

Delivered to Mme CHAKCHOUK

FRANKLAB
3 avenue des Frênes
78180 MONTIGNY LE BRETONNEUX
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



3, rue de la terre rouge, Espace industriel de BEAUPRE, 25 220 ROCHE LEZ BEAUPRE ▪ 09.62.52.91.87 ▪ RCS BESANÇON ▪ N° SIRET 51786053200020 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

SUMMARY

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11. TECHNICAL APPENDIX 9

Rédacteur	Superviseur
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.

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

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:

- $3 \cdot 10^2$ UFC/ml < N_v < $1,6 \cdot 10^3$ UFC/ml
- $1,5 \cdot 10^8$ UFC/ml < N < $5 \cdot 10^8$ 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

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



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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	× yes □ no		× yes □ no			× yes □ no		× yes □ no		× yes □ no	

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



8. TRIAL – RESULTS

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

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



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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 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 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160			A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	× yes □ no		× yes □ no			× yes □ no		× yes □ no		× yes □ no	

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

10. REPETITION – RESULTS

STRAIN	TRIAL SUSPENSION			TRIAL		5 min		TRIAL		2 min		TRIAL		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 ⁰	191
1.10 ⁻⁷		38	36	1.10 ⁻¹	4	2	1.10 ⁻¹	10	12	1.10 ⁻¹	21	17	1.10 ⁻¹	21	17
				1.10 ⁻²	0	0	1.10 ⁻²	1	5	1.10 ⁻²	3	2	1.10 ⁻²	3	2
N		3,70.10 ⁸		Na	245,45		Na	909,09		Na	1736,36		Na	1736,36	
log NO		7,57		log Na	2,39		log Na	2,96		log Na	3,24		log Na	3,24	
			Log R	5,18		Log R	4,61		Log R	4,33		Log R	4,33		
<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 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 ⁻¹	12	13
				1.10 ⁻²	0	0	1.10 ⁻²	1	1	1.10 ⁻²	2	3	1.10 ⁻²	2	3
	N	2,51.10 ⁸		Na	159,09		Na	640,91		Na	1236,36		Na	1236,36	
	log NO	7,40		log Na	2,20		log Na	2,81		log Na	3,09		log Na	3,09	
			Log R	5,20		Log R	4,59		Log R	4,31		Log R	4,31		
<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 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 ⁻¹	14	18
				1.10 ⁻²	0	0	1.10 ⁻²	3	5	1.10 ⁻²	2	2	1.10 ⁻²	2	2
	N	3,40.10 ⁸		Na	263,64		Na	1004,55		Na	1440,91		Na	1440,91	
	log NO	7,53		log Na	2,42		log Na	3,00		log Na	3,16		log Na	3,16	
			Log R	5,11		Log R	4,53		Log R	4,37		Log R	4,37		
<i>Escherichia coli K12</i>	1.10 ⁻⁶	265	271	1.10 ⁰	15	15	1.10 ⁰	59	51	1.10 ⁰	117	109	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 ⁻¹	17	12
				1.10 ⁻²	0	0	1.10 ⁻²	0	1	1.10 ⁻²	5	4	1.10 ⁻²	5	4
	N	2,70.10 ⁸		Na	159,09		Na	563,64		Na	1159,09		Na	1159,09	
	log NO	7,43		log Na	2,20		log Na	2,75		log Na	3,06		log Na	3,06	
			Log R	5,23		Log R	4,68		Log R	4,37		Log R	4,37		

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

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

Rédacteur

Mme Emilie CANTREL, technicienne de laboratoire

Superviseur

Mme Stephanie MOROT-BIZOT, directrice

TEST REPORT

DETERMINATION OF BACTERICIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 1276 STANDARD
--

Delivered to **Mme CHAKCHOUK**

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



Date of request: 10/18/2018

Study number: n°268D25-2018-04

BACTERICIDAL TESTS:

According to the European standard NF EN 1276 (March 2010) – antiseptics and chemical disinfectants– quantitative suspension tests for evaluation of bactericidal activity of antiseptics and chemical disinfectants used in food industrial, domestic and institutional areas (phase 2, step 1).

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

This test report included 9 pages.

Study completion date: 11/24/2018



Stephanie MOROT-BIZOT
PhD in microbiology
Study director

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

4, rue des Grandes Pièces, zone Eurespace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51
▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪
info@apexlabo.com

SUMMARY

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Rédacteur	Superviseur
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

Product	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 11/08/2018 to 11/20/2018

3. EXPERIMENTAL CONDITIONS

- Final concentrations: 80%.
- Method: EN 1276
- Exposure time: 2 min – 5 min – 10 min
- Trial temperature: 20°C
- Organic soil load: dirty conditions (BSA 3 g/L)
- Strains: *Staphylococcus aureus* subsp. *aureus* CIP 4.83 batch 15713-1d (ATCC 6538), *Pseudomonas aeruginosa* DSM 939 batch 0413 (ATCC 15442), *Enterococcus hirae* DSM 3320 batch 0511 (ATCC 10541) and *Escherichia coli* DSM682 batch 1112 (ATCC 10536).
- 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) and egg yolk (5%) in distilled water.

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

4. RESULTS

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

- *Staphylococcus aureus*, R = 5,22
- *Pseudomonas aeruginosa*, R = 5,24
- *Enterococcus hirae*, R > 5,30
- *Escherichia coli*, R > 5,32

5. CONCLUSION

According to the EN 1276 (March 2010), the F1031V2 product:

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

6. SHEETS OF RESULTS



Attached below.

Methodology:

- $3 \cdot 10^2$ UFC/ml < N_v < $1,6 \cdot 10^3$ UFC/ml
- $1,5 \cdot 10^8$ UFC/ml < N < $5 \cdot 10^8$ 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

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



7. TRIAL – VALIDATIONS

STRAIN	Suspension of validation		Validation A		Validation B		Validation C	
<i>Staphylococcus aureus</i>	83	85	90	88	87	95	90	84
	\bar{x}	84,0	\bar{x}	89,0	\bar{x}	91,0	\bar{x}	87,0
	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	
<i>Pseudomonas aeruginosa</i>	100	88	83	85	81	80	89	75
	\bar{x}	94,0	\bar{x}	84,0	\bar{x}	80,5	\bar{x}	82,0
	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	
<i>Enterococcus hirae</i>	73	78	81	70	75	74	68	73
	\bar{x}	75,5	\bar{x}	75,5	\bar{x}	74,5	\bar{x}	70,5
	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	
<i>Escherichia coli</i>	80	89	85	85	86	79	81	82
	\bar{x}	84,5	\bar{x}	85,0	\bar{x}	82,5	\bar{x}	81,5
	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	

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



8. TRIAL – RESULTS

STRAIN	TRIAL SUSPENSION			TRIAL			TRIAL			TRIAL		
					2 min			5 min			10 min	
<i>Staphylococcus aureus</i>	1.10 ⁻⁶	>330	>330	Vc	>330	>330	Vc	25	20	Vc	0	2
	1.10 ⁻⁷	28	32	Na	>3300		Na	225,00		Na	<140	
	N	3,00.10 ⁸		Log Na	>3,52		Log Na	2,35		Log Na	<2,15	
	Log N ₀	7,48		Log R = log N ₀ -log Na	<3,96		Log R = log N ₀ -log Na	5,13		Log R = log N ₀ -log Na	>5,33	
<i>Pseudomonas aeruginosa</i>	1.10 ⁻⁶	>330	>330	Vc	>330	>330	Vc	22	26	Vc	3	0
	1.10 ⁻⁷	40	37	Na	>3300		Na	240,00		Na	<140	
	N	3,85.10 ⁸		Log Na	>3,52		Log Na	2,38		Log Na	<2,15	
	Log N ₀	7,59		Log R = log N ₀ -log Na	<4,07		Log R = log N ₀ -log Na	5,21		Log R = log N ₀ -log Na	>5,44	
<i>Enterococcus hirae</i>	1.10 ⁻⁶	258	273	Vc	212	178	Vc	0	3	Vc	0	0
	1.10 ⁻⁷	24	28	Na	1950,00		Na	<140		Na	<140	
	N	2,65.10 ⁸		Log Na	3,29		Log Na	<2,15		Log Na	<2,15	
	Log N ₀	7,42		Log R = log N ₀ -log Na	4,13		Log R = log N ₀ -log Na	>5,27		Log R = log N ₀ -log Na	>5,27	
<i>Escherichia coli</i>	1.10 ⁻⁶	294	291	Vc	139	148	Vc	10	2	Vc	0	0
	1.10 ⁻⁷	33	30	Na	1435,00		Na	<140		Na	<140	
	N	2,95.10 ⁸		Log Na	3,16		Log Na	<2,15		Log Na	<2,15	
	Log N ₀	7,47		Log R = log N ₀ -log Na	4,31		Log R = log N ₀ -log Na	>5,32		Log R = log N ₀ -log Na	>5,32	

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



9. REPETITION - VALIDATIONS

STRAIN	Suspension of validation		Validation A		Validation B		Validation C	
<i>Staphylococcus aureus</i>	90	94	82	86	93	89	83	83
	\bar{x}	92,0	\bar{x}	84,0	\bar{x}	91,0	\bar{x}	83,0
	30 ≤ Nv0 ≤ 160 × yes <input type="checkbox"/> no		A ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		B ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		C ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no	
<i>Pseudomonas aeruginosa</i>	99	101	94	95	97	92	93	86
	\bar{x}	100,0	\bar{x}	94,5	\bar{x}	94,5	\bar{x}	89,5
	30 ≤ Nv0 ≤ 160 × oui <input type="checkbox"/> non		A ≥ 0,5 * Nv0 × oui <input type="checkbox"/> non		B ≥ 0,5 * Nv0 × oui <input type="checkbox"/> non		C ≥ 0,5 * Nv0 × oui <input type="checkbox"/> non	
<i>Enterococcus hirae</i>	77	78	78	72	75	82	61	70
	\bar{x}	77,5	\bar{x}	75,0	\bar{x}	78,5	\bar{x}	65,5
	30 ≤ Nv0 ≤ 160 × yes <input type="checkbox"/> no		A ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		B ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		C ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no	
<i>Escherichia coli</i>	89	84	90	88	75	92	81	76
	\bar{x}	86,5	\bar{x}	89,0	\bar{x}	83,5	\bar{x}	78,5
	30 ≤ Nv0 ≤ 160 × yes <input type="checkbox"/> no		A ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		B ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no		C ≥ 0,5 * Nv0 × yes <input type="checkbox"/> no	

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

10. REPETITION – RESULTS

STRAIN	TRIAL SUSPENSION			TRIAL			TRIAL			TRIAL								
						2 min			5 min			10 min						
<i>Staphylococcus aureus</i>	1.10 ⁻⁶	>330	>330	Vc	>330	>330	Vc	18	16	Vc	2	4						
	1.10 ⁻⁷	33	35	Na	>3300			Na	170,00			Na	<140					
	N	3,40.10 ⁸			Log Na	>3,52			Log Na	2,23			Log Na	<2,15				
	Log N ₀	7,53			Log R = log N ₀ -log Na			<4,01			Log R = log N ₀ -log Na			5,30		Log R = log N ₀ -log Na		>5,38
<i>Pseudomonas aeruginosa</i>	1.10 ⁻⁶	>330	>330	Vc	>330	277	Vc	20	20	Vc	5	0						
	1.10 ⁻⁷	37	37	Na	3035,00			Na	200,00			Na	<140					
	N	3,70.10 ⁸			Log Na	3,48			Log Na	2,30			Log Na	<2,15				
	Log N ₀	7,57			Log R = log N ₀ -log Na			4,09			Log R = log N ₀ -log Na			5,27		Log R = log N ₀ -log Na		>5,42
<i>Enterococcus hirae</i>	1.10 ⁻⁶	285	294	Vc	121	115	Vc	4	8	Vc	0	0						
	1.10 ⁻⁷	30	33	Na	1180,00			Na	<140			Na	<140					
	N	2,92.10 ⁸			Log Na	3,07			Log Na	<2,15			Log Na	<2,15				
	Log N ₀	7,47			Log R = log N ₀ -log Na			4,40			Log R = log N ₀ -log Na			>5,32		Log R = log N ₀ -log Na		>5,32
<i>Escherichia coli</i>	1.10 ⁻⁶	288	288	Vc	155	94	Vc	1	0	Vc	0	0						
	1.10 ⁻⁷	28	31	Na	1245,00			Na	<140			Na	<140					
	N	2,89.10 ⁸			Log Na	3,10			Log Na	<2,15			Log Na	<2,15				
	Log N ₀	7,46			Log R = log N ₀ -log Na			4,36			Log R = log N ₀ -log Na			>5,31		Log R = log N ₀ -log Na		>5,31

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

11. TECHNICAL APPENDIX

MEDIA:

TSA (Trypton Soy Agar), Dominique DUTSCHER, ref. 777410, batch n° 806051

ORGANIC SOIL LOAD :

Albumin Serum Bovine in powder, Fraction V, Dominique Dutscher, ref. 871001, batch D1304039

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, batch n° FR08 085 793 ----- 8,50 g/L

NEUTRALIZER

Per liter of distilled water:

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

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

TEST REPORT

**DETERMINATION OF THE BACTERICIDAL ACTIVITY OF THE
F1031V2 PRODUCT ACCORDING TO THE EN 13697 STANDARD**

Delivered to **Mme CHAKCHOUK**

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



Date of request: 10/18/2018

Study number: n°268D25-2018-06

BACTERICIDAL AND FUNGICIDAL TESTS:

Tests based on European standard NF EN 13697 (June 2015) – chemical antiseptics and disinfectants – quantitative non-porous surface tests for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in the field of food industries, in domestic areas and communities.

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

This test report includes 7 pages.

Study completion date: 12/07/2018



Stephanie MOROT - BIZOT
PhD in Microbiology
Study Director



4, rue des Grandes Pièces, zone Eurespace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51
▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪
info@apexlabo.com

SUMMARY

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Editor	Supervisor
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
4, RUE DES GRANDES PIECES
25 770 SERRE LES SAPINS
FRANCE

2 PRODUCT IDENTITY

Product	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: tap water

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

Date of tests: from 11/18/2018 to 12/05/2018

3 EXPERIMENTAL CONDITIONS

Concentrations of the product: 100%

Appearance of the product and its dilutions: clear

Method used: dilution-neutralization

Contact time: 2 min - 5 min – 10 min

Temperature test: 20°C

Diluent of the product used in the tests: sterile distilled water

Diluent of microbial suspensions: solution tryptone-sterile salt



Strains: *Staphylococcus aureus subsp. aureus* CIP 4.83 batch 15713-1d (ATCC 6538), *Escherichia coli* DSM682 batch 1112 (ATCC 10536), *Pseudomonas aeruginosa* DSM 939 batch 0413 (ATCC 15442) et *Enterococcus hirae* DSM 3320 batch 0511 (ATCC 10541) - Institut Pasteur.

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

Organic soil load: dirty conditions, BSA 3 g/L

Product stability: stable

Stop solution: Transferring the disc into 10 mL neutralizer.

Editor	Supervisor
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	

4 VALIDATIONS AND RESULTS

- *Staphylococcus aureus*, R = 4,03
- *Pseudomonas aeruginosa*, R = 4,04
- *Enterococcus hirae*, R > 4,14
- *Escherichia coli*, R > 4,12

5 CONCLUSIONS

According to the EN 13697 standard (June 2015), the F1031V2 product:

- **Demonstrated a bactericidal activity on the reference strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus hirae* and *Escherichia coli*, when used at the concentration of 100%, for 5 min of contact time, at 20 °C, in dirty conditions**

6 RESULTS SHEETS

Attached below.



For all result sheets :

Methodology:



- $6.57 \leq N \leq 7.10$ for bacteria
- $Nc \geq 6.27$ for bacteria
- $NC > 0,5 * Nc ; NT > 0,5 * Nc$
- $5,57 \leq \log N \leq 6,10$ for fungi
- $\text{Log } Nc \geq 5,27$ for fungi

Legend :

Nc = logarithm of the number of cfu per test area for the water control
 ND = logarithm of the number of cfu per test surface for testing with disinfectant
 N = logarithm of the number of cfu of the microbial test suspension
 NC = logarithm of the number of cfu per test area for the neutralization control
 NT = logarithm of the number of cfu per test area for the neutralizer control
 Nts = number of cfu remaining on the test surface.
 D = dilution factor for the dilution considered.
 R = logarithmic reduction obtained.
 VC = value counted per Petri dish



<u>Editor</u>	<u>Supervisor</u>
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	

	TRIAL SUSPENSION			VALIDATIONS						WATER CONTROL			CONCENTRATIONS (v/v)						
				NT			NC			Nc			2 min		5 min		10 min		
		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	VC1	VC2	VC1	VC2
<i>Pseudomonas aeruginosa</i>	1.10 ⁻⁶	302	297	1.10 ⁻³	199	190	1.10 ⁻³	201	194	1.10 ⁻³	188	185	1.10 ⁰	78	89	18	19	0	3
	1.10 ⁻⁷	30	30	1.10 ⁻⁴	20	19	1.10 ⁻⁴	22	20	1.10 ⁻⁴	22	19	1.10 ⁻¹	10	10	2	2	0	0
	Log N	6,87		Log NT	6,29		Log NC	6,30		Log Nc	6,27		Log Nd	2,92		2,27		<2,15	
										Nts	8		Nts	1		1		0	
									Drying time: 47 min			Log R	3,35	4,02	>4,12				
<i>Escherichia coli</i>	1.10 ⁻⁶	269	270	1.10 ⁻³	190	188	1.10 ⁻³	195	199	1.10 ⁻³	200	191	1.10 ⁰	70	66	10	15	0	0
	1.10 ⁻⁷	28	29	1.10 ⁻⁴	22	19	1.10 ⁻⁴	23	22	1.10 ⁻⁴	20	20	1.10 ⁻¹	9	7	1	2	0	0
	Log N	6,83		Log NT	6,28		Log NC	6,30		Log Nc	6,29		Log Nd	2,83		2,18		<2,15	
										Nts	12		Nts	5		0		0	
									Drying time: 43 min			Log R	3,46	4,11	>4,14				
<i>Staphylococcus aureus</i>	1.10 ⁻⁶	>330	>330	1.10 ⁻³	211	208	1.10 ⁻³	200	198	1.10 ⁻³	229	206	1.10 ⁰	61	60	21	19	0	5
	1.10 ⁻⁷	38	39	1.10 ⁻⁴	23	22	1.10 ⁻⁴	22	22	1.10 ⁻⁴	23	21	1.10 ⁻¹	7	6	3	2	0	0
	Log N	6,92		Log NT	6,32		Log NC	6,30		Log Nc	6,34		Log Nd	2,78		2,30		<2,15	
										Nts	8		Nts	12		3		0	
									Drying time: 39 min			Log R	3,56	4,04	>4,19				
<i>Enterococcus hirae</i>	1.10 ⁻⁶	255	258	1.10 ⁻³	198	223	1.10 ⁻³	220	215	1.10 ⁻³	201	203	1.10 ⁰	97	94	3	7	0	1
	1.10 ⁻⁷	27	26	1.10 ⁻⁴	22	23	1.10 ⁻⁴	24	23	1.10 ⁻⁴	22	20	1.10 ⁻¹	11	10	0	0	0	0
	Log N	6,81		Log NT	6,33		Log NC	6,34		Log Nc	6,31		Log Nd	2,98		<2,15		<2,15	
										Nts	0		Nts	29		1		0	
									Drying time: 49 min			Log R	3,33	>4,16	>4,16				

Editor	Supervisor
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	

8 RESULTS SHEET – REPETITIONS

	TRIAL SUSPENSION			VALIDATIONS						WATER CONTROL			CONCENTRATIONS (v/v)						
				NT			NC			Nc			2 min		5 min		10 min		
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	VC1	VC2	VC1	VC2	
<i>Pseudomonas aeruginosa</i>	1.10 ⁻⁶	299	283	1.10 ⁻³	201	193	1.10 ⁻³	188	195	1.10 ⁻³	190	197	1.10 ⁰	180	195	18	17	15	16
	1.10 ⁻⁷	30	32	1.10 ⁻⁴	21	22	1.10 ⁻⁴	20	21	1.10 ⁻⁴	20	23	1.10 ⁻¹	19	20	2	2	2	2
	Log N	6,86		Log NT	6,30		Log NC	6,28		Log Nc	6,29		Log Nd	3,27		2,24		2,19	
											Nts	4		Nts	5		0		0
										Drying time: 41 min			Log R	3,02	4,05	4,10			
<i>Escherichia coli</i>	1.10 ⁻⁶	277	273	1.10 ⁻³	175	173	1.10 ⁻³	155	169	1.10 ⁻³	185	183	1.10 ⁰	116	123	10	12	0	0
	1.10 ⁻⁷	28	28	1.10 ⁻⁴	19	17	1.10 ⁻⁴	17	18	1.10 ⁻⁴	23	21	1.10 ⁻¹	13	12	1	1	0	0
	Log N	6,84		Log NT	6,24		Log NC	6,21		Log Nc	6,27		Log Nd	3,08		<2,15		<2,15	
											Nts	10		Nts	1		0		0
										Drying time: 48 min			Log R	3,19	>4,12	>4,12			
<i>Staphylococcus aureus</i>	1.10 ⁻⁶	>330	>330	1.10 ⁻³	225	228	1.10 ⁻³	208	212	1.10 ⁻³	199	207	1.10 ⁰	79	91	20	20	0	3
	1.10 ⁻⁷	36	34	1.10 ⁻⁴	24	23	1.10 ⁻⁴	23	21	1.10 ⁻⁴	20	22	1.10 ⁻¹	12	9	0	2	0	0
	Log N	6,92		Log NT	6,36		Log NC	6,32		Log Nc	6,31		Log Nd	2,93		2,30		<2,15	
											Nts	0		Nts	15		0		2
										Drying time: 42 min			Log R	3,38	4,01	>4,16			
<i>Enterococcus hirae</i>	1.10 ⁻⁶	279	274	1.10 ⁻³	250	226	1.10 ⁻³	200	209	1.10 ⁻³	186	189	1.10 ⁰	55	50	3	0	0	0
	1.10 ⁻⁷	30	28	1.10 ⁻⁴	28	24	1.10 ⁻⁴	25	21	1.10 ⁻⁴	19	20	1.10 ⁻¹	8	5	0	0	0	0
	Log N	6,84		Log NT	6,38		Log NC	6,32		Log Nc	6,27		Log Nd	2,72		<2,15		<2,15	
											Nts	3		Nts	23		0		0
										Drying time: 53 min			Log R	3,55	>4,12	>4,12			

Editor	Supervisor
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	

9 TECHNICAL APPENDIX

Media:

TSA (Trypton Soy Agar), Dominique DUTSCHER, ref. 777410, batch n°806051

ORGANIC SOIL LOAD:

Bovine serum albumin powder, Dominique Dutscher, Ref. P6154, batch D1304039

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 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/L
- Egg yolk, 5%----- 50 g/L

Sterilised by autoclaving (without egg yolk)

STAINLESS STEEL CARRIERS – Discs 2 cm diameter in stainless steel 1.4301, grade 2B (MECAPOL).

<u>Editor</u>	<u>Supervisor</u>
Ms Emilie CANTREL, laboratory technician	Mrs Stephanie MOROT-BIZOT, Director
	



Instituto Valenciano de Microbiología

Masía El Romeral
Ctra. Bétera – San Antonio de Benagéber, Km 0,3
46117 Bétera (Valencia)
Tel. 96 169 17 02
Fax 96 169 16 37
e-mail: ivami@ivami.com
www.ivami.com
CIF B-96337217



Test with the certificate of GLPs
(Good Laboratory Practices)
No. 2/21-C.VAL. General Directorate of
Pharmacy and Medical Devices of the Health
Department of the Valencian Region. Spain

Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area (phase 2, step 2), with the product F1031V2. (EN 14561: 2007 Standard)

Report

Registration No.: D/22/B0550.

- 1. **Laboratory identification** Instituto Valenciano de Microbiología.
- 2. **Client identification** FRANKLAB.
Address 3 Avenue des Frênes.
 78180 Montigny le Bretonneux, France.
- 3. **Sample identification** (information provided by the client)
 - Product name **F1031V2.**
 - Batch number 7833.
 - Expiration date 22/08/2024.
 - Manufacturer (supplier) FRANKLAB.
 - Store conditions 5-25°C.
 - Conditions of use Instruments.
 - Solvent of the product recommended by the manufacturer Not applicable.
 - Active(s) Substance(s) and its concentration (s) Not indicated.
 - Concentrations ordered for the assay Pure (100%).

IVAMI is not responsible for client-supplied information.

F2V1 FN 14561 10 22 - EN

4. Information about sample reception

- Date of reception of the sample 2022/09/02.
- Date of reception of order with test conditions 2022/09/01.
- Aspect of the received sample Transparent liquid in plastic package.

5. Method of assay and its validation

EN 14561: 2007 Standard.

- Method Dilution-neutralization.
- Neutralizer Tryptone 5 g/L, yeast extract, 2.5 g/L, dextrose 10 g/L, sodium thioglycolate 1 g/L, sodium thiosulfate 1 g/L, sodium bisulphite 2.5 g/L, soya lecithin 7 g/L, polysorbate-80 5 g/L, glycine 1 g/L, l-histidine 1 g/L and saponin 30 g/L.

6. Experimental conditions

- Assay period 2022/09/25 to 2022/09/29.
- Solvent of the product used in the assay .. Sterile distilled water.
- Product concentrations for the assay Pure (100%), 50% and 0.1%.
- Aspect of the dilutions of the product Transparent liquid.
- Contact time 5 minutes.
- Assay temperature +20°C ± 1°C.
- Interfering substance Bovine serum albumin 3 g/L + 3 mL/L erythrocytes.
- Stability of the mixture (product diluted in sterile distilled water) Stable.
- Incubation temperature +36°C ± 1°C
- Drying time of the slides *P.aeruginosa*: 22 minutes.
S.aureus: 23 minutes.
E.hirae: 21 minutes.
- Identification of the strains used:
 - *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442).
 - *Staphylococcus aureus* (CECT 239 = ATCC 6538).
 - *Enterococcus hirae* (CECT 4081 = ATCC 10541).

7. Results of the assay

- Assay of validation See tables 1, 2, 3, 5, 6, 7, 9, 10 and 11.
- Evaluation of bactericidal activity See tables 4, 8 and 12.
- Number of replicates per assay organism ... 1.

8. Special remarks

- All controls and validation were between the basic limits.
- At least one concentration of the sample showed a log reduction lower than 5 log.
- At least one concentration of the sample showed a log reduction higher than 5 log.
- There was not any precipitation during the assay procedure (the assay mixtures were homogeneous).

9. Conclusion

The product **F1031V2**, batch 7833, when it is pure (100%), **shows bactericidal activity** after 5 minutes at 20°C ±1°C, under dirty conditions (bovine serum albumin 3 g/L + 3 mL/L erythrocytes.), for the reference strains *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442), *Staphylococcus aureus* (CECT 239 = ATCC 6538) and *Enterococcus hirae* (CECT 4081 = ATCC 10541), when tested as required by **EN 14561: 2007 Standard**.

Note: The results obtained correspond to the sample received in the laboratory.

Use of the ENAC mark: The ENAC “mark” can only be used by the holder of the accreditation. Its use in packaging, installations, shop windows, advertising or other documentation format other than that issued by the accredited entity (IVAMI) is not allowed.

Bétera (Valencia), September 30, 2022.

HUGUET LOPEZ,
ALVARO (FIRMA)

Signed. Álvaro Huguet
Responsible Technician
(Investigator)

Quality Assurance Review:

The assay development and the results obtained have been supervised by the Director of the study.

The Quality Assurance Director has inspected the development of the assay, proving that has been realized following the proper procedure and using the adequate media, materials and reagents, following the Good Laboratory Practices (GLPs) as well and the final report contains the primary data obtained.

TORRELLAS MARCÓ,
MAX (FIRMA)

Signed. Max Torrellas
Responsible for the Laboratory Area
(Study Director)

ESTEBAN BERMUDEZ,
ENCARNACION PILAR
(FIRMA)

Signed. Encarnación Esteban
Technical Director
(Quality Assurance Director)

Reference

- **EN 14561 : 2007.** Chemical disinfectants and antiseptics. Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area. Test method and requirements (phase 2, step2). AENOR.

DESIN-1032-b //EN 14561: 2007 Version 3 (2019-10-02)
Registration No.: D/22/B0550

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Instituto Valenciano de Microbiología

Results of the assay with *Pseudomonas aeruginosa* (CECT 116 = ATCC 15442).

Seeding: Pour plates. No. of plates: 1/mL. Drying time of the slide: 22 minutes.

Table 1.-Validation and controls.

Suspension of validation (N_{v0})				Control of experimental conditions (A)				Control of the neutralizer (B)				Validation of the method (C) Sample concentration: Pure (100%)			
Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2
52	56	52	56	49	46	49	46	39	42	39	42	47	49	47	49
$30 \leq X$ of $N_{v0} \leq 160$? $X = 54$				X of A is $\geq 0.5 \times X$ of N_{v0} ? $X = 47.5$				X of B is $\geq 0.5 \times X$ of N_{v0} ? $X = 40.5$				X of C is $\geq 0.5 \times X$ of N_{v0} ? $X = 48$			
Yes				Yes				Yes				Yes			

Table 2.-Suspension of the assay.

Suspension of assay (N)	N	Counts per plate		Vc_1	Vc_2	$X_{wm} = 2.23 \times 10^9$ $\lg N = 9.35$ $9.17 \leq \lg N \leq 9.7?$ Yes
	10^{-7}	229	216	229	216	
	10^{-8}	22	23	22	23	

Table 3.-Water control.

Water control (N_w)	N_w	Counts per plate		Vc_1	Vc_2	$X_{wm} \times 10 = 9.60 \times 10^7$ $\lg N_w = 7.98$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)?$ Yes
	10^{-5}	93	99	93	99	

Table 4.-Results of the activity assays with the sample.

Sample concentration	Dilution	Counts per plate		Vc_1	Vc_2	$Lg Na = \lg (X_o / X_{wm}) + 1$	$Lg R$ ($\lg N_w = 7.98$)	Time of contact (min)
Pure (100%)	10^0	0	0	<14	<14	<2.15	>5.83	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
50%	10^0	0	0	<14	<14	<2.15	>5.83	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
0.1%	10^0	>330	>330	>330	>330	> 6.52	<1.46	5
	10^{-1}	>330	>330	>330	>330			
	10^{-2}	>330	>330	>330	>330			
	10^{-3}	>330	>330	>330	>330			

Results of the assay with *Staphylococcus aureus* (CECT 239 = ATCC 6538).

Seeding: Pour plates. No. of plates: 1/mL. Drying time of the slide: 23 minutes.

Table 5.-Validation and controls.

Suspension of validation (N_{v0})				Control of experimental conditions (A)				Control of the neutralizer (B)				Validation of the method (C) Sample concentration: Pure (100%).			
Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2	Counts per plate		Vc_1	Vc_2
109	112	109	112	93	98	93	98	97	105	97	105	89	96	89	96
$30 \leq X \text{ of } N_{v0} \leq 160?$ $X = 110.5$				$X \text{ of } A \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 95.5$				$X \text{ of } B \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 101$				$X \text{ of } C \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 92.5$			
Yes				Yes				Yes				Yes			

Table 6.-Suspension of the assay.

Suspension of assay (N)	N	Counts per plate		Vc_1	Vc_2	$X_{wm} = 4.45 \times 10^9$ $\lg N = 9.65$ $9.17 \leq \lg N \leq 9.7?$ Yes
	10^{-7}	>330	>330	>330	>330	
	10^{-8}	46	43	46	43	

Table 7.-Water control.

Water control (N_w)	N_w	Counts per plate		Vc_1	Vc_2	$X_{wm} \times 10 = 3.90 \times 10^7$ $\lg N_w = 7.59$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)?$ Yes
	10^{-5}	38	40	38	40	

Table 8.-Results of the activity assays with the sample.

Sample concentration (%)	Dilution	Counts per plate		Vc_1	Vc_2	$\lg N_a = \lg(X \text{ o } X_{wm}) + 1$	$\lg R (\lg N_w = 7.59)$	Time of contact (min)
Pure (100%)	10^0	0	0	<14	<14	<2.15	>5.44	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
50%	10^0	0	0	<14	<14	<2.15	>5.44	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
0.1%	10^0	>330	>330	>330	>330	> 6.52	<1.07	5
	10^{-1}	>330	>330	>330	>330			
	10^{-2}	>330	>330	>330	>330			
	10^{-3}	>330	>330	>330	>330			

Results of the assay with *Enterococcus hirae* (CECT 4081 = ATCC 10541).

Seeding: Pour plates. No. of plates: 1/mL. Drying time of the slide: 21 minutes.

Table 9.-Validation and controls.

Suspension of validation (N_{v0})				Control of experimental conditions (A)				Control of the neutralizer (B)				Validation of the method (C) Sample concentration: Pure (100%)			
Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}	Counts per plate		V_{c1}	V_{c2}
46	50	46	50	39	37	39	37	40	38	40	38	35	38	35	38
$30 \leq X \text{ of } N_{v0} \leq 160?$ $X = 48$				$X \text{ of } A \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 38$				$X \text{ of } B \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 39$				$X \text{ of } C \text{ is } \geq 0.5 \times X \text{ of } N_{v0}?$ $X = 36.5$			
Yes				Yes				Yes				Yes			

Table 10.-Suspension of the assay.

Suspension of assay (N)	N	Counts per plate		V_{c1}	V_{c2}	$X_{wm} = 1.97 \times 10^9$ $\lg N = 9.29$ $9.17 \leq \lg N \leq 9.7?$ Yes
	10^{-7}	181	176	181	176	
	10^{-8}	18	18	18	18	

Table 11.-Water control.

Water control (N_w)	N_w	Counts per plate		V_{c1}	V_{c2}	$X_{wm} \times 10 = 8.60 \times 10^7$ $\lg N_w = 7.94$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)?$ Yes
	10^{-5}	82	90	82	90	

Table 12.-Results of the activity assays with the sample.

Sample concentration (%)	Dilution	Counts per plate		V_{c1}	V_{c2}	$\lg Na = \lg (X_0 / X_{wm}) + 1$	$\lg R (\lg N_w = 7.94)$	Time of contact (min)
Pure (100%)	10^0	0	0	<14	<14	< 2.15	>5.79	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
50%	10^0	0	0	<14	<14	< 2.15	>5.79	5
	10^{-1}	0	0	<14	<14			
	10^{-2}	0	0	<14	<14			
	10^{-3}	0	0	<14	<14			
0.1%	10^0	>330	>330	>330	>330	> 6.52	< 1.42	5
	10^{-1}	>330	>330	>330	>330			
	10^{-2}	>330	>330	>330	>330			
	10^{-3}	>330	>330	>330	>330			

F2.V1 - EN 14561 - 10.12 - CR

Explanations:

Vc = Count per mL (one or more plates).

X = mean of Vc_1 and Vc_2 .

X_{wm} = ponderated mean of X ;

R (reduction) = ($\lg R = \log N_w - \log N_a$).

If $N_a < 140$, $\log R = \lceil \log N_w - 2,15 \rceil$

FDV1 - EN 14561 - 10.22.20

RAPPORT D'ESSAI

DETERMINATION DE L'ACTIVITE LEVURICIDE DU PRODUIT F1031V2 lingettes SELON LA NORME EN 16615

Délivré à Mme CHAKCHOUK

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



Demande d'essai du : 18/10/2018

Références du dossier d'analyses : n°268D25-2018-02

ESSAIS DE LEVURICIDIE :

Selon la méthodologie de la norme européenne NF EN 16615 (Mai 2015) – antiseptiques et désinfectants chimiques – Méthode d'essai quantitative pour l'évaluation de l'activité bactéricide et levuricide sur des surfaces non poreuses, avec action mécanique à l'aide de lingettes et de lavettes dans le domaine médical (essais à 4 zones). Méthode d'essai et prescriptions (phase 2, étape 2).

Essais sur 1 souche de référence : *Candida albicans*.

Ce rapport comporte 9 pages et ne concerne que les échantillons étudiés.

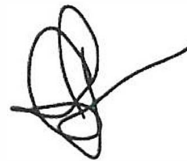
Date d'émission : 29/11/2018

Stéphanie MOROT-BIZOT
Docteur en microbiologie
Chargée de l'étude

Professeur Georges HERBEIN
Professeur des Universités Praticien Hospitalier
Expert scientifique





APEX
APEX BIOSOLUTIONS
4, rue des grandes pièces
25770 Serre les sapins
tél 03 82 52 91 87 - info@apexlabo.com
n° SIRET 517 860 532 00012
n° TVA intra FR 2351 7860532



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

F1031-V1-16615-10-2018

1. LABORATOIRE AYANT RÉALISÉ LES IDENTIFICATIONS

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

2. IDENTIFICATION DES ECHANTILLONS

Echantillon	N° lot
F1031V2 <u>lingettes</u>	701301

Date limite d'utilisation optimale : non communiquée

Fabricant : FRANKLAB

Date de fabrication : non communiquée

Conditions de stockage : Température ambiante et obscurité.

Composants actifs : éthanol, isopropanol, amine tertiaire

Aspect : lingettes non tissées, VH 23g/m², imprégnation 280%

Précautions d'emploi : aucune



Diluant préconisé par le fabricant : aucun, produit prêt-à-l'emploi

Date de réception au laboratoire : 24/10/2018

Période de l'étude : du 09/11/2018 au 28/11/2018

3. CONDITIONS EXPERIMENTALES

- Concentration du produit soumis à l'essai : produit pur.
- Méthode employée: dilution-neutralisation.
- Temps de contact : 1 min, 2 min et 5 min
- Température d'essai: 20°C
- Substance interférente: conditions de saleté, solution d'albumine bovine à 3g/L + 3 mL/L de sang de mouton (concentration finale).
- Diluant du produit utilisé lors des essais : solution tryptone sel stérile.
- Souches utilisées : *Candida albicans* CIP 48.72 lot 265.09 - Institut Pasteur.
- Conditions de culture: sur GEM (Gélose à l'Extrait de Malt), à 30°C ± 1°C.

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

- Technique d'arrêt de l'action biocide: par dilution-neutralisation, avec neutralisant à base de polysorbate 80 et de jaune d'œuf (composition en annexe).

4. RESULTATS PROPREMENT DITS

Le produit lingettes F1031V2 est bien actif vis-à-vis des souches de référence utilisées, car la réduction obtenue est supérieure à 4 log pour les levures :

En conditions de saleté (moyenne des répétitions) :

- *Candida albicans*, R = 4,37 pour 2 min de contact



5. CONCLUSION

Conformément à la norme EN 16615 (Mai 2015), les essais sur le produit F1031V2 lingettes ont démontré:

- Que le produit a une activité levuricide vis-à-vis de la souche de référence *Candida albicans* en 2 min à 20°C, dans les conditions de saleté

6. FEUILLES DE RESULTATS

Voir ci-après.

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

7. Candida albicans - ESSAI



Vérifications de la méthodologie:

- N est compris entre $1,5 \times 10^8$ UFC/ml et $5,0 \times 10^8$ UFC/ml
- $6,88 \leq \log N0 \leq 7,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- $Nv0$ est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv0$

Légende :

- Vc = dénombrement par ml
- \bar{x} = moyenne de Vc1 et Vc2
- N = nombre d'UFC/ml dans la suspension d'essai
- Nv = nombre d'UFC/ml dans la suspension de validation
- Dc0 = témoin de séchage à t0
- Dct = témoin de séchage à t
- B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant
- C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation
- Na = nombre d'UFC/ml des survivants après essai
- R = réduction ($\lg R = \lg Dct - \lg Na$)



Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-02 Date des essais : 13/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL /L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

F200 - EN 16615 - 12-2018

2018-11-15-1615-17-2018

SOUCHE	Suspension d'essai		Dc0		Dct		Suspension de validation		Validation B		Validation C				
		VC1	VC2		VC1	VC2		VC1	VC2						
<i>Candida albicans</i>	1,00.10 ⁻⁶	253	257	1,00.10 ⁻⁴	188	179	1,00.10 ⁻⁴	176	166	55	81	76	73	73	68
	1,00.10 ⁻⁷	27	26	1,00.10 ⁻⁵	20	21	1,00.10 ⁻⁵	20	18	\bar{x}	68,0	\bar{x}	74,5	\bar{x}	70,5
	N	2,56.10⁸		Dc0	9,27.10⁶		Dct	8,64.10⁶		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	Log N	8,41		Log DC0	6,97		Log Dct	6,94		x oui □ non		x oui □ non		x oui □ non	
Témoin eau			Essai			1 min	Essai			2 min	Essai			5 min	
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	
1,00.10 ⁰	208	212	Champ 1	239	215	Champ 1	112	108	Champ 1	18	24	Champ 1	18	24	
1,00.10 ⁻¹	23	22	Na	1135		Na	550		Na	105		Na	105		
Nw	1,06.10 ³		Log Na	3,06		Log Na	2,74		Log Na	2,02		Log Na	2,02		
Log Nw	3,02		Log R = log Dct – log Na	3,88		Log R = log Dct – log Na	4,20		Log R = log Dct – log Na	4,92		Log R = log Dct – log Na	4,92		
Champ 2	50	32	Champ 2	12	33	Champ 2	5	10	Champ 2	1	0	Champ 2	1	0	
Champ 3	4	3	Champ 3	1	3	Champ 3	1	1	Champ 3	0	0	Champ 3	0	0	
Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	
Moyenne champ 2-4	205,00		Moyenne champ 2-4	40,83		Moyenne champ 2-4	2,83		Moyenne champ 2-4	0,17		Moyenne champ 2-4	0,17		

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

8. Candida albicans - REPETITION



Vérifications de la méthodologie:

- N est compris entre $1,5 \times 10^8$ UFC/ml et $5,0 \times 10^8$ UFC/ml
- $6,88 \leq \log N0 \leq 7,40$
- $NW > 100$ UFC/25 cm² sur les champs 2 à 4
- $Nv0$ est compris entre 3×10^1 UFC/ml et $1,6 \times 10^2$ UFC/ml
- B et C sont égaux ou supérieurs à $0,5 \times Nv0$

Légende :

- Vc = dénombrement par ml
- \bar{x} = moyenne de Vc1 et Vc2
- N = nombre d'UFC/ml dans la suspension d'essai
- Nv = nombre d'UFC/ml dans la suspension de validation
- Dc0 = témoin de séchage à t0
- Dct = témoin de séchage à t
- B = nombre d'UFC/ml dans le mélange d'essai de validation de la toxicité du neutralisant
- C = nombre d'UFC/ml dans le mélange d'essai de validation de l'inactivation par dilution-neutralisation
- Na = nombre d'UFC/ml des survivants après essai
- R = réduction ($\lg R = \lg Dct - \lg Na$)



Norme: EN 16615 Produit : F1031V2 lingettes Lot N° : 701301 Etude N° : 268D25-2018-02 Date des essais : 20/11/2018	Méthode: <input checked="" type="checkbox"/> Ensemencement dans la masse <input type="checkbox"/> Ensemencement en surface <input checked="" type="checkbox"/> Nombre de boîtes de Pétri/mL : 2	Neutralisant : polysorbate 80 (30g/L) + jaune d'œuf 5% Température des essais : 20°C Substance interférente : 3 g/L BSA + 3 mL/L érythrocytes de mouton Température d'incubation : 37°C ± 1°C Diluant : eau distillée stérile
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Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

F268D25-2018-02-12-2018

F1031 - CN/16615 - 12-2018

SOUICHE	suspension d'essai		Dc0			Dct			Suspension de validation		Validation B		Validation C				
		VC1	VC2		VC1	VC2		VC1	VC2								
<i>Candida albicans</i>	1,00.10 ⁻⁶	263	279	1,00.10 ⁻⁴	200	197	1,00.10 ⁻⁴	149	156	88	93	78	71	70	66		
	1,00.10 ⁻⁷	30	28	1,00.10 ⁻⁵	23	21	1,00.10 ⁻⁵	16	18	\bar{x}	90,5	\bar{x}	74,5	\bar{x}	68,0		
	N	2,73.10⁸		Dc0	1,00.10⁷		Dct	7,70.10⁶		30 ≤ Nv0 ≤ 160		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0			
	Log N	8,44		Log DC0	7,00		Log Dct	6,89		× oui □ non		× oui □ non		× oui □ non			
	Témoin eau			Essai			1 min		Essai			2 min		Essai			5 min
	Vc1	Vc2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2
1,00.10 ⁰	216	221	Champ 1	181	179	Champ 1	48	42	Champ 1	15	16	Champ 1	15	16	Champ 1	15	16
1,00.10 ⁻¹	22	22	Na	900		Na	225		Na	77,5		Na	77,5		Na	77,5	
Nw	1,09.10 ³		Log Na	2,95		Log Na	2,35		Log Na	1,89		Log Na	1,89		Log Na	1,89	
Log Nw	3,04		Réduction	3,94		Réduction	4,54		Réduction	5,00		Réduction	5,00		Réduction	5,00	
Champ 2	28	25	Champ 2	27	20	Champ 2	23	22	Champ 2	5	11	Champ 2	5	11	Champ 2	5	11
Champ 3	2	3	Champ 3	3	2	Champ 3	2	0	Champ 3	1	1	Champ 3	1	1	Champ 3	1	1
Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0	Champ 4	0	0
Moyenne champ 2-4	132,50		Moyenne champ 2-4	43,33		Moyenne champ 2-4	7,83		Moyenne champ 2-4	3,00		Moyenne champ 2-4	3,00		Moyenne champ 2-4	3,00	

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

9. ANNEXE TECHNIQUE

Milieux de culture utilisés, stérilisés par autoclavage :

GEM (Gélose à l'extrait de Malt), Dominique DUTSCHER, réf. 777304, lot n° 712042

SUBSTANCES INTERFÉRENTES :

Sérum Albumine Bovine en poudre, Fraction V, Dominique Dutscher, réf.P6154, lot D1304039

Sang de mouton, Analytic Lab, réf. 08449, lot n°bcbj3984V.

PIECES DE LINOLEUM – linoleum en PVC, traités PUR, épaisseur 2,5 mm, 20 cm x 50 cm.

DILUANT Solution Tryptone-Sel (TS)

Ingrédients en grammes par litre d'eau distillée ou déminéralisée :

- Tryptone, Dominique Dutscher, réf. 777472, lot n° 090633 -----1,00 g/l
- Chlorure de sodium, Grosseron, ref 9020401, lot n° FR08 085 793 -----8,50 g/l

pH final après autoclavage à 25°C : 7,0 ± 0,2



NEUTRALISANT

Ingrédients par litre d'eau distillée:

Polysorbate 80, SIGMA ALDRICH, réf. 59924, lot n° BCBJ6978V ----- 30 g

Jaune d'œuf frais ----- 50 ml

Stérilisé par filtration sur filtre 0,45 µm ; pH à 25°C : 7,4 ± 0,1

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

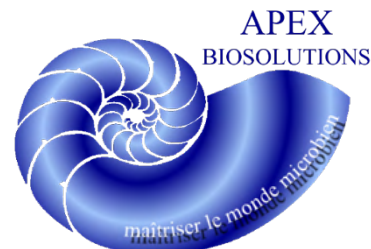
F241-DV16615-12-2018

TEST REPORT

**DETERMINATION OF YEASTICIDAL ACTIVITY OF THE F1031V2
PRODUCT ACCORDING TO THE EN 13624 STANDARD**

Delivered to Ms CHAKCHOUK

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



Date of request: 10/18/2018

Study number: n°268D25-2018-03

YEASTICIDAL TESTS :

According to the European standard NF EN 13624 (November 2013) – antiseptics and chemical disinfectants– quantitative suspension tests for evaluation of fungicidal activity of antiseptics and chemical disinfectants used in the medical area (phase 2, step 1).

Trials on 1 strain : *Candida albicans*.

This test report included 7 pages.

Study completion date: 11/24/2018

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

A handwritten signature in black ink, appearing to read 'Stephanie Morot-Bizot', is located below the printed name and title.

SUMMARY

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

1. PERFORMING LABORATORY

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

2. PRODUCT IDENTITY

Product	Batch #
F1031V2	611141

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: 10/24/2018

Date of the study: from 11/05/2018 to 11/20/2018

3. EXPERIMENTAL CONDITIONS

Final concentrations of the product: 80%

Method: dilution-neutralization

Exposure time: 2 min – 5 min - 10 min

Temperature using during the assays: 20°C

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

Strain: *Candida albicans* CIP 48.72 lot 265.09

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 :

– *Candida albicans* R = 4,10 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 yeasticidal activity on the reference strain when used from the concentration 80%, for 5 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:

- $1,5 \times 10^7$ CFU/mL $\leq N \leq 5,0 \times 10^7$ CFU/mL
- $30 \leq N_v \leq 160$ CFU/mL
- A, B and C $\geq 0,5 \times N_v0$
- 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



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

7. TRIAL - Candida albicans

Standard: EN 13624 Product : F1031V2 Batch N° : 611141 Study N° : 268D25-2018-03 Date of trials : 11/08/2018	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 2	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 30°C ± 1°C Diluent : sterile distilled water
---	---	--

STRAIN	Suspension of validation Nv		Suspension of validation NvB		Validation A		Validation B		Validation C		
<i>Candida albicans</i>	30	37	1.10 ⁻³	31	33	38	43	41	39	38	51
	\bar{x}	33,5	\bar{x}	32,0		\bar{x}	40,5	\bar{x}	40,0	\bar{x}	44,5
	30 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0		
	x oui <input type="checkbox"/> non		x oui <input type="checkbox"/> non		x oui <input type="checkbox"/> non		x oui <input type="checkbox"/> non		x oui <input type="checkbox"/> non		

STRAIN	Trial suspension			TRIAL			TRIAL			TRIAL		
				2 min			5 min			10 min		
<i>Candida albicans</i>	1.10 ⁻⁵	208	218	Vc	85	77	Vc	18	20	Vc	0	2
	1.10 ⁻⁶	24	22	Na	810,00		Na	190,00		Na	<140	
	N	2,15.10⁷		Log Na	2,91		Log Na	2,28		Log Na	<2,15	
	Log N ₀	6,33		Log R = log N ₀ -log Na	3,42		Log R = log N ₀ -log Na	4,05		Log R = log N ₀ -log Na	>4,18	



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

8. REPETITION – *Candida albicans*

Standard: EN 13624 Product : F1031V2 Batch N° : 611141 Study N° : 268D25-2018-03 Date of trials : 11/15/2018	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 2	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 30°C ± 1°C Diluent : sterile distilled water
---	---	--

STRAIN	Suspension of validation Nv		Suspension of validation NvB		Validation A		Validation B		Validation C		
	<i>Candida albicans</i>	38	43	1.10 ⁻³	40	40	44	44	40	48	39
\bar{x}		40,5	\bar{x}	40,0		\bar{x}	44,0	\bar{x}	44,0	\bar{x}	40,5
30 ≤ Nv0 ≤ 160		30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0			
× oui <input type="checkbox"/> non		× oui <input type="checkbox"/> non		× oui <input type="checkbox"/> non		× oui <input type="checkbox"/> non		× oui <input type="checkbox"/> non			

STRAIN	Trial suspension			TRIAL			TRIAL			TRIAL		
				2 min			5 min			10 min		
<i>Candida albicans</i>	1.10 ⁻⁵	231	227	Vc	83	84	Vc	15	19	Vc	0	0
	1.10 ⁻⁶	30	25	Na	835,00		Na	170,00		Na	<140	
	N	2,33.10⁷		Log Na	2,92		Log Na	2,23		Log Na	<2,15	
	Log N ₀	6,37		Log R = log N ₀ -log Na	3,45		Log R = log N ₀ -log Na	4,14		Log R = log N ₀ -log Na	>4,22	

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

9. TECHNICAL APPENDIX

MEDIA:

MEA (Malt Extract Agar), Dominique Dutscher, ref. 777304, batch #402241

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

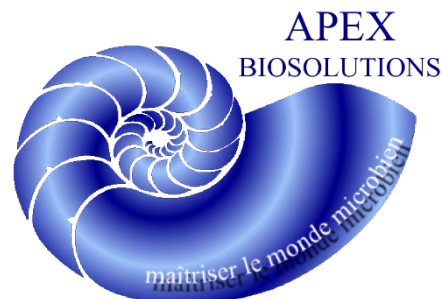
Writer	Supervisor
Ms Emilie CANTREL, laboratory technician	Ms Stephanie MOROT-BIZOT, director
	

TEST REPORT

**DETERMINATION OF YEASTICIDAL ACTIVITY OF THE F1031V2
PRODUCT ACCORDING TO THE EN 1650 STANDARD**

Delivered to **Ms CHAKCHOUK**

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



Date of request: 10/18/2018

Study : n°268D25-2018-05

FUNGICIDAL TESTS:

According to the European standard NF EN 1650 (July 2013) – antiseptics and chemical disinfectants– quantitative suspension tests for evaluation of fungicidal activity of antiseptics and chemical disinfectants used in food industrial, domestic and institutional areas (phase 2, step 1).

Trials on 1 reference strain: *Candida albicans*.

This test report included 7 pages.

Study completion date: 12/05/2018



Stephanie MOROT-BIZOT
PhD in microbiology
Study director



4, rue des Grandes Pièces, zone Eurespace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51
▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪
info@apexlabo.com

SUMMARY

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Rédacteur	Superviseur
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

Product	Batch #
F1031V2	611141

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, clearless



Diluent recommended by the manufacturer: nona, ready-to-use product

Date of receipt: 10/24/2018

Date of the study: from 11/18/2018 to 11/28/2018

3. EXPERIMENTAL CONDITIONS

- Final concentrations: 80%
- Method: EN 1650
- Exposure time: 2 min – 5 min - 10 min
- Trial temperature: 20°C
- Organic soil load: dirty conditions (BSA 3 g/L)
- Strains: *Candida albicans* CIP 48.72 lot 265.09 - Institut Pasteur
- Media and growth conditions: MEA (Malt Extract Agar), at 30°C ± 1°C.
- Product stability: limpid solution with organic soil load
- Stop solution: tween 80 (30g/L) and egg yolk (5%) in distilled water.

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

4. RESULTS

The F1031V2 product is active against the reference strains used, since the reduction obtained is greater than 4 log:

- *Candida albicans*, R = 4,18

5. CONCLUSION

According to the EN 1650 (July 2013), the F1031V2 product:

- **Demonstrated a yeasticidal activity against the reference strain *Candida albicans* when used from 100%, in dirty conditions at 20°C, for 5 min of exposure time.**

6. SHEETS OF RESULTS



Attached below.

Methodology:

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

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 ($\log R = \log N_0 - \log N_a$)
- 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

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

7. TRIAL – VALIDATIONS AND RESULTS

STRAIN	Suspension of validation		Validation A		Validation B		Validation C	
<i>Candida albicans</i>	89	93	90	94	89	87	79	90
	\bar{X}	91,0	\bar{X}	92,0	\bar{X}	88,0	\bar{X}	84,5
	30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	× yes □ no		× yes □ no		× yes □ no		× yes □ no	



STRAIN	TRIAL SUSPENSION			TRIAL			TRIAL			TRIAL		
					2 min			5 min			10 min	
<i>Candida albicans</i>	1.10 ⁻⁵	280	274	Vc	55	61	Vc	18	19	Vc	0	0
	1.10 ⁻⁶	30	29	Na	580,00		Na	185,00		Na	<140	
	N	2,79.10 ⁷		log Na	2,76		log Na	2,27		log Na	<2,15	
	log N0	6,45		Log R = log No - log Na	3,69		Log R = log No - log Na	4,18		Log R = log No - log Na	>4,30	

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

8. REPETITION – VALIDATIONS AND RESULTS

STRAIN	Suspension of validation		Validation A		Validation B		Validation C	
<i>Candida albicans</i>	70	77	75	73	69	72	68	64
	\bar{x}	73,5	\bar{x}	74,0	\bar{x}	70,5	\bar{x}	66,0
	30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0	
	× yes □ no		× yes □ no		× yes □ no		× yes □ no	

STRAIN	TRIAL SUSPENSION			TRIAL		2 min	TRIAL			5 min	TRIAL			10 min
<i>Candida albicans</i>	1.10 ⁻⁵	295	301	Vc	49	43	Vc	21	20	Vc	2	4		
	1.10 ⁻⁶	30	33	Na	460,00		Na	205,00		Na	<140			
	N	3,00.10⁷		log Na	2,66		log Na	2,31		log Na	<2,15			
	log N0	6,48		Log R = log No - log Na		3,82	Log R = log No - log Na		4,17	Log R = log No - log Na		>4,33		

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

9. TECHNICAL APPENDIX

MEDIA:

MEA (Malt Extract Agar), *Dutscher*, réf 777304, lot n° 712042

ORGANIC SOIL LOAD :

Albumin Serum Bovine in powder, Fraction V, Dominique Dutscher, ref. 871001, batch D1304039

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
 - Egg yolk, 5% ----- 50 mL/L

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

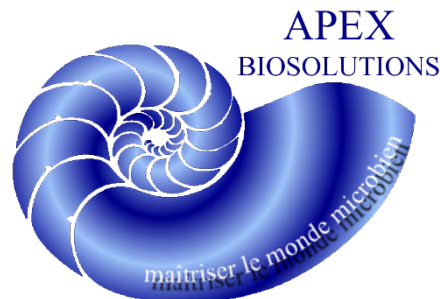
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

TEST REPORT

**DETERMINATION OF THE YESTICIDAL ACTIVITY OF THE
F1031V2 PRODUCT ACCORDING TO THE EN 13697 STANDARD**

Delivered to **Mme CHAKCHOUK**

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



Date of request: 10/18/2018

Study number: n°268D25-2018-07

BACTERICIDAL AND FUNGICIDAL TESTS:

Tests based on European standard NF EN 13697 (June 2015) – chemical antiseptics and disinfectants – quantitative non-porous surface tests for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in the field of food industries, in domestic areas and communities.

Trials on 1 reference strain: *Candida albicans*.

This test report includes 6 pages.

Study completion date: 12/07/2018

Stephanie MOROT - BIZOT

PhD in Microbiology
Study Director



4, rue des Grandes Pièces, zone Eurespace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51
▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪
info@apexlabo.com

SUMMARY

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

1 PERFORMING LABORATORY

APEX BIOSOLUTIONS
4, RUE DES GRANDES PIECES
25 770 SERRE LES SAPINS
FRANCE

2 PRODUCT IDENTITY

Product	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: tap water

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

Date of tests: from 11/18/2018 to 12/05/2018

3 EXPERIMENTAL CONDITIONS

Concentrations of the product: 100%

Appearance of the product and its dilutions: clear

Method used: dilution-neutralization

Contact time: 2 min - 5 min – 10 min

Temperature test: 20°C

Diluent of the product used in the tests: sterile distilled water

Diluent of microbial suspensions: solution tryptone-sterile salt



Strain : *Candida albicans* CIP 48.72 lot 265.09 - Institut Pasteur

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

Organic soil load: dirty conditions, BSA 3 g/L

Product stability: stable

Stop solution: Transferring the disc into 10 mL neutralizer.

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

4 VALIDATIONS AND RESULTS

- *Candida albicans*, R = 3,46

5 CONCLUSION

According to the EN 13697 standard (June 2015), the F1031V2 product:

- Demonstrated a yeasticidal activity on the reference strain *Candida albicans*, when used at the concentration of 100%, for 5 min of contact time, at 20 °C, in dirty conditions

6 RESULTS SHEETS

Attached below.



For all result sheets :

Methodology:

- $6.57 \leq N \leq 7.10$ for bacteria
- $Nc \geq 6.27$ for bacteria
- $NC > 0,5 * Nc$; $NT > 0,5 * Nc$
- $5,57 \leq \log N \leq 6,10$ for fungi
- $\log Nc \geq 5,27$ for fungi

Legend :

Nc = logarithm of the number of cfu per test area for the water control
 ND = logarithm of the number of cfu per test surface for testing with disinfectant
 N = logarithm of the number of cfu of the microbial test suspension
 NC = logarithm of the number of cfu per test area for the neutralization control
 NT = logarithm of the number of cfu per test area for the neutralizer control
 Nts = number of cfu remaining on the test surface.
 D = dilution factor for the dilution considered.
 R = logarithmic reduction obtained.
 VC = value counted per Petri dish



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

7 RESULTS SHEET – TRIALS

TRIAL																			
TRIAL SUSPENSION			VALIDATIONS						WATER CONTROL			CONTACT TIME							
			NT			NC			Nc			2 min		5 min		10 min			
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	VC1	VC2	VC1	VC2	
Candida albicans	1.10 ⁻⁵	300	288	1.10 ⁻³	215	180	1.10 ⁻³	202	197	1.10 ⁻³	185	188	1.10 ⁰	238	229	150	137	29	33
	1.10 ⁻⁶	35	30	1.10 ⁻⁴	23	21	1.10 ⁻⁴	22	20	1.10 ⁻⁴	20	21	1.10 ⁻¹	27	24	8	14	4	3
	Log N	5,87		Log NT	6,30		Log NC	6,30		Log Nc	6,27		Log Nd	3,37		3,16		2,49	
											Nts	9		Nts	22		2		0
										Drying time : 45 min			Log R	2,90		3,11		3,78	

8 RESULTS SHEET – REPETITIONS

REPETITION																			
TRIAL SUSPENSION			VALIDATIONS						WATER CONTROL			CONTACT TIME							
			NT			NC			Nc			2 min		5 min		10 min			
	VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2		VC1	VC2	VC1	VC2	VC1	VC2	
Candida albicans	1.10 ⁻⁵	244	241	1.10 ⁻³	170	164	1.10 ⁻³	160	159	1.10 ⁻³	137	139	1.10 ⁰	170	168	20	24	0	0
	1.10 ⁻⁶	24	24	1.10 ⁻⁴	19	16	1.10 ⁻⁴	18	16	1.10 ⁻⁴	15	14	1.10 ⁻¹	18	17	2	2	0	0
	Log N	5,78		Log NT	6,22		Log NC	6,21		Log Nc	6,14		Log Nd	3,23		2,34		<2,15	
											Nts	8		Nts	30		1		0
										Drying time : 52 min			Log R	2,91		3,80		>3,99	

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

9 TECHNICAL APPENDIX

Media:

MEA (Malt Extract Agar), Dominique DUTSCHER, ref. 777304, batch n° 402241

ORGANIC SOIL LOAD:

Bovine serum albumin powder, Dominique Dutscher, Ref. P6154, batch D1304039

Diluent

Trypton-Sel 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 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/L
- Egg yolk, 5%----- 50 g/L

Sterilised by autoclaving (without egg yolk)

STAINLESS STEEL CARRIERS – Discs 2 cm diameter in stainless steel 1.4301, grade 2B (MECAPOL).

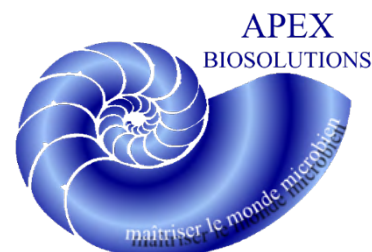
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

TEST REPORT

DETERMINATION OF FUNGICIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 13624 STANDARD

Delivered to Ms CHAKCHOUK

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



Date of request: 03/13/2019

Study number: n°072D08-2019-06

FUNGICIDAL TESTS :

According to the European standard NF EN 13624 (November 2013) – antiseptics and chemical disinfectants – quantitative suspension tests for evaluation of fungicidal activity of antiseptics and chemical disinfectants used in the medical area (phase 2, step 1).

Trials on 1 strain : *Aspergillus brasiliensis*.

This test report included 7 pages.

Study completion date: 04/10/2019

Stephanie MOROT-BIZOT
PhD in microbiology
Study director

A handwritten signature in black ink, appearing to read 'Stephanie Morot-Bizot', is located below the printed name and title.

SUMMARY

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

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



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

7. TRIAL – *Aspergillus brasiliensis*

Standard: EN 13624 Product : F1031V2 Batch N° : 611141B01 Study N° : 072D08-2019-06 Date of trials : 03/29/2019	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 2	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 30°C ± 1°C 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 ⁻³	43	51	42	44	50	50	48	39
	\bar{x}	64,5	\bar{x}	4,70.10⁴		\bar{x}	43,0	\bar{x}	50,0	\bar{x}	43,5
	30 ≤ Nv0 ≤ 160		3,10 ³ ≤ NvB ≤ 1,6.10 ⁵			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 ⁻⁵	>165	>165	Vc	121	133		Vc	18	19		Vc	2	2	
	1.10 ⁻⁶	41	42	Na	1270,00			Na	185,00			Na	<140		
	N	1,88.10⁷		Log Na	3,10			Log Na	2,27			Log Na	<2,15		
	Log N0	6,27		Log R = logN0-logNa			3,17	Log R = logN0-logNa			4,00	Log R = logN0-logNa			>4,12



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

8. REPETITION - *Aspergillus brasiliensis*

Standard: EN 13624 Product : F1031V2 Batch N° : 611141B01 Study N° : 072D08-2019-06 Date of trials : 04/03/2019	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 2	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 30°C ± 1°C 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 ⁻³	52	47	49	50	55	51	47	47
	\bar{x}	60,0	\bar{x}	4,95.10⁴		\bar{x}	49,5	\bar{x}	53,0	\bar{x}	47,0
	30 ≤ Nv0 ≤ 160		3,10 ³ ≤ NvB ≤ 1,6.10 ⁵			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 ⁻⁵	>165	>165	Vc	135	149	Vc	20	16	Vc	3	4
	1.10 ⁻⁶	53	50	Na	1420,00		Na	180,00		Na	<140	
	N	1,97.10⁷		Log Na	3,15		Log Na	2,26		Log Na	<2,15	
	Log N0	6,29		Log R = logN0-logNa		3,14	Log R = logN0-logNa		4,03	Log R = logN0-logNa		>4,14

Rédacteur	Superviseur
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, zone Eurespace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51
▪ SARI. au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪
info@apexlabo.com

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	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

4, rue des Grandes Pièces, zone Eureospace, 25 770 SERRE LES SAPINS ■ Tel: 03.81.25.09.04 ■ Fax: 03.81.25.53.51
■ SARL au capital de 10 000 € ■ RCS BESANÇON ■ N° SIRET 51786053200012 ■ N° TVA intra FR 23517860532 ■
info@apexlabo.com

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:

- $1,5 \times 10^8 \text{ CFU/mL} \leq N \leq 5 \times 10^8 \text{ CFU/mL}$
- $8,17 \leq \text{Log}N \leq 8,70$
- $1,4 \times 10^6 \text{ CFU/mL} \leq N_w \leq 1,3 \times 10^6 \text{ CFU/mL}$
- $R \geq 4$ 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 ⁻⁶	258	253	1.10 ⁻³	228	225	1.10 ⁰	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 ⁻⁷	27	26	1.10 ⁻⁴	23	27	1.10 ⁻¹	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 ⁻⁶	243	251	1.10 ⁻³	260	253	1.10 ⁰	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 ⁻⁷	28	27	1.10 ⁻⁴	29	28	1.10 ⁻¹	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	VC2	VC1	VC2	VC1	VC2	VC1	VC2
<i>Aspergillus brasiliensis</i>	66	68	60	57	62	66	57	52	1.10 ⁻⁶	160	154	1.10 ⁻³	158	160	1.10 ⁰	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 ⁻⁷	19	16	1.10 ⁻⁴	18	17	1.10 ⁻¹	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	VC2	VC1	VC2	VC1	VC2	VC1	VC2
<i>Aspergillus brasiliensis</i>	68	73	80	73	64	68	61	57	1.10 ⁻⁶	>165	>165	1.10 ⁻³	>165	>165	1.10 ⁰	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 ⁻⁷	18	20	1.10 ⁻⁴	21	19	1.10 ⁻¹	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

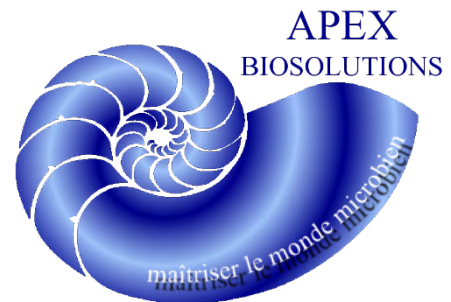
GLASS CARRIERS – blades of frosted glass 15 x 60 mm, 1 mm thick – Thermo scientific/ Menzel-Gläser
– ref. 100 OTM, batch #01 1794389.

TEST REPORT

DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 14476:2015 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°268D25-2018-09

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

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2	SAMPLE IDENTIFICATION	3
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Rédacteur	Superviseur
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 TCID₅₀

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% CO₂ 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 TCID₅₀ (calculated by Spearman-Kärber method) :

- For norovirus, titer = 7,125 log DICT₅₀

4 VALIDATION**a) Cytotoxicity**

For norovirus, the cell toxicity was observed until to the dilution 10⁻¹.

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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 ₅₀)		
	Untreated cell cultures	Treated cell cultures	Viral titer (log TCID ₅₀)
PRODUCT DILUTION			
F1031V2 10 ⁻²	7,125	6,750	0,375

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 ₅₀)	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 ₅₀)	Viral titer reduction (log TCID ₅₀)
Viral suspension control	7,125	
inactivation 5 min		0,375
inactivation 15 min	6,750	1,625
inactivation 30 min	5,500	2,125

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 TCID₅₀.

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

TRIAL 2 - The viral suspension was titrated at 7,125 log TCID₅₀.

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

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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 TCID₅₀ 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.

Rédacteur	Superviseur
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 TCID₅₀ = 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



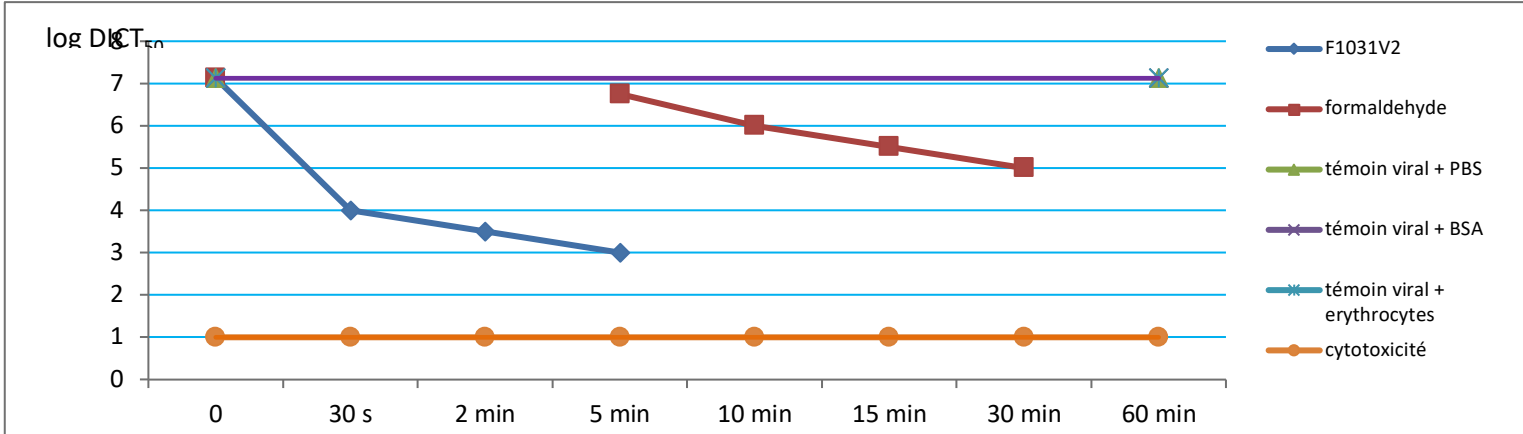
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Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Chart 1 – Trials on norovirus:

TRIAL 1



TRIAL 2

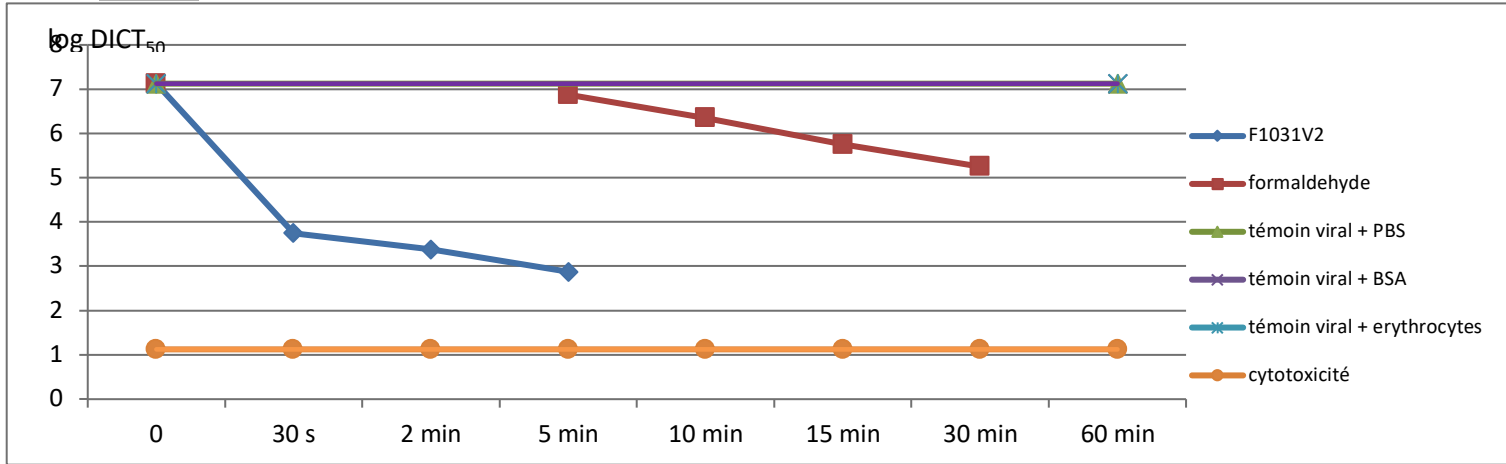


Table A2- Sensitivity of the cells to the norovirus:

PRODUCT	DILUTION	ORGANIC SOIL LOAD		Dilutions							
				-2	-3	-4	-5	-6	-7	-8	-9
F1031V2	10 ⁻²	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
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Table A3 — Results on norovirus

PRODUCT	Concentration	Organic soil load	Cytotoxicity level	Lg DICT ₅₀							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 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 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 ⁻²	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	

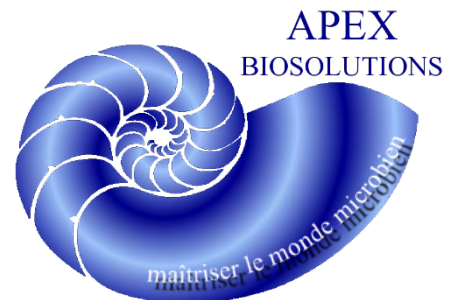
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

TEST REPORT

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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

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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', is located below the printed name and title.

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Rédacteur	Superviseur
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 TCID₅₀

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-2cells, at 37°C, under 5% CO₂ 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 TCID₅₀ (calculated by Spearman-Kärber method) :

- For adenovirus, titer = 6,875 log DICT₅₀

4 VALIDATION**a) Cytotoxicity**

For adenovirus, the cell toxicity was observed until to the dilution 10⁻¹.

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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 ₅₀)		
	Untreated cell cultures	Treated cell cultures	Viral titer (log TCID ₅₀)
PRODUCT DILUTION			
F1031V2 10 ⁻²	6,875	6,625	0,250

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 ₅₀)	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 ₅₀)	Viral titer reduction (log TCID ₅₀)
Viral suspension control	6,875	
inactivation 5 min		0,375
inactivation 15 min	6,500	1,375
inactivation 30 min	5,500	1,625

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 TCID₅₀.

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

TRIAL 2 - The viral suspension was titrated at 7,000 log TCID₅₀.

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

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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 TCID₅₀ 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.

Rédacteur	Superviseur
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 TCID₅₀ = 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



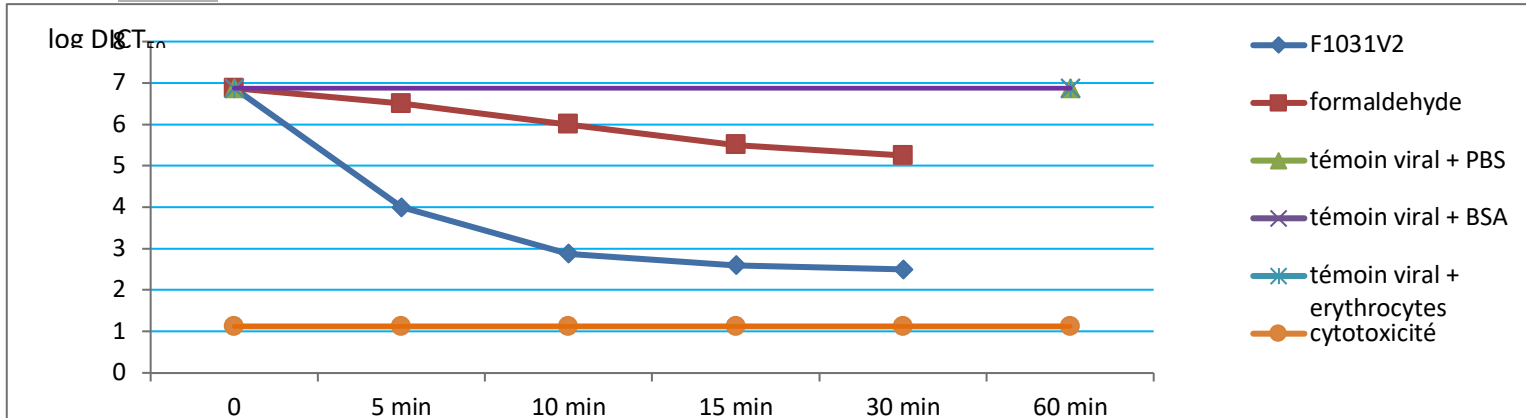
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Chart 1 – Trials on adenovirus:

TRIAL 1



TRIAL 2

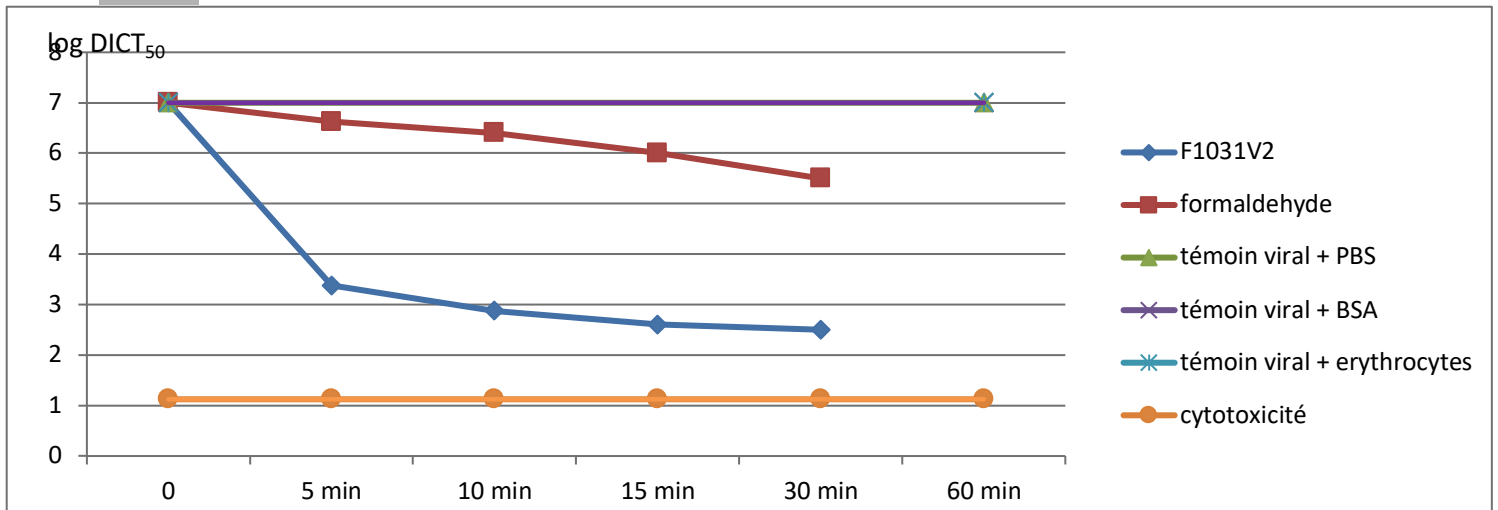


Table A2- Sensitivity of the cells to the adenovirus:

PRODUCT	DILUTION	ORGANIC SOIL LOAD		Dilutions								
				-2	-3	-4	-5	-6	-7	-8	-9	
F1031V2	10 ⁻²	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 ₅₀						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 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 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 ⁻²	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	4444	4444	4444	0000	0000	0000	0000	0000	0000
			10 min	4444	4444	4440	0000	0000	0000	0000	0000	0000	0000
			30 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
			VIRAL CONTROL	4444	4444	4444	4444	4444	1111	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	1111	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	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	0000	0000	
viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	0000	0000	
viral control of infectivity	N.A.	3 g/l BSA + 3 ml SE	0	4444	4444	4444	4444	4444	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	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	5 min	4444	4444	4444	0000	0000	0000	0000	0000	0000	0000
			10 min	4444	4444	4440	0000	0000	0000	0000	0000	0000	0000
			30 min	4444	4444	0000	0000	0000	0000	0000	0000	0000	0000
			VIRAL CONTROL	4444	4444	4444	4444	4444	1111	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	1111	1111	1000	0000	0000	
			15	4444	4444	4444	4444	1111	1111	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	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	0000	0000	
viral control of infectivity	N.A.	3 g/l BSA	0	4444	4444	4444	4444	4444	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	0000	0000	
viral control of infectivity	N.A.	3 g/l BSA + 3 ml SE	0	4444	4444	4444	4444	4444	1111	1111	0000	0000	
			60	4444	4444	4444	4444	4444	1111	1111	0000	0000	

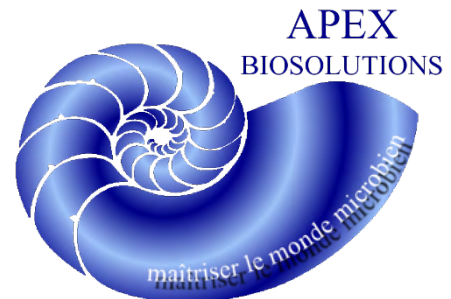
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

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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9 TECHNICAL APPENDIX 2 6

Rédacteur	Superviseur
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/m², 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% CO₂ 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 TCID₅₀ (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 ₀)
F1031V2	10^{-1}	6,60	6,42	0,18

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)
Viral suspension control	6,59	
inactivation 5 min	6,51	0,08
inactivation 15 min	5,56	1,03
inactivation 30 min	4,48	2,11



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	4,16
		30 min		1,96	4,63

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	4,20
		30 min		1,86	4,73

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

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

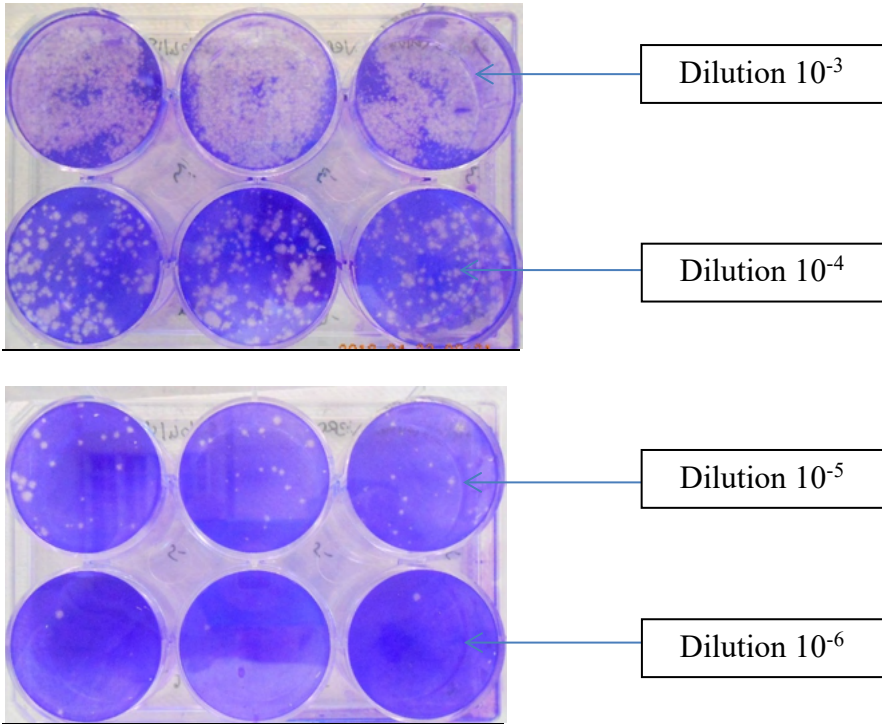
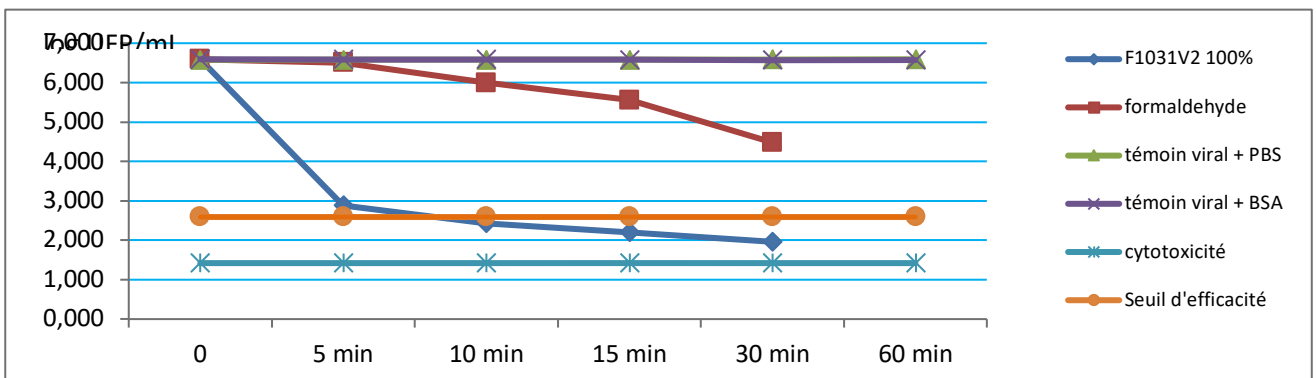
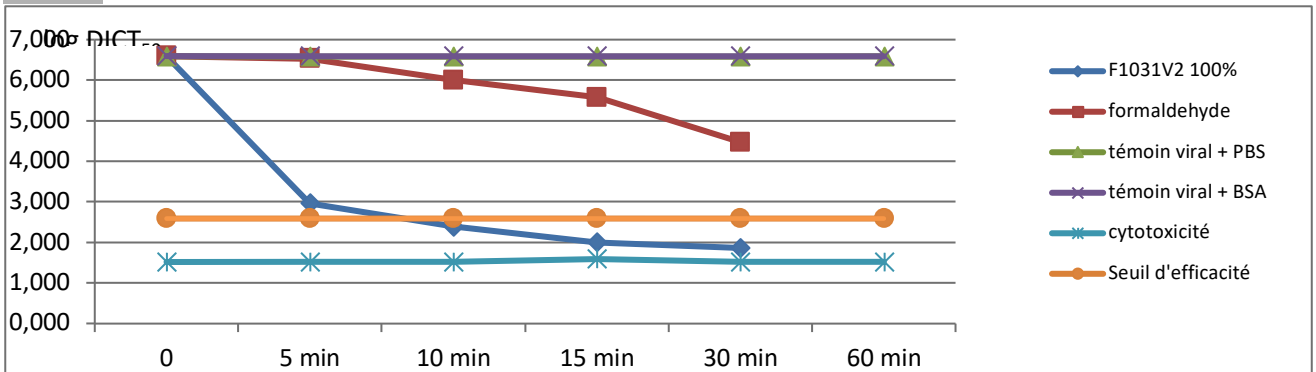


Chart 1 – Trials on poliovirus:

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 ⁻¹	3 g/l BSA+ 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 + 3 mL/L SE	1,42	6,59	2,88	2,43	N.T.	1,96	N.T.	10 min R = 4,16
F1031V2 TRIAL 2	100,00%	3 g/l BSA + 3 mL/L SE	1,52	6,59	2,96	2,39	N.T.	1,86	N.T.	10 min 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 + 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 + 3 mL/L SE	N.A.	6,59	N.T.	N.T.	N.T.	N.T.	6,59	
CELL SENSITIVITY TO THE VIRUS	10 ⁻²	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			
26	4	0							
30 min	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
0							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			
30 min	26	3	0						
	8	1	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

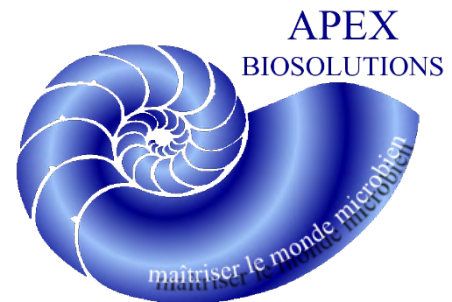
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

TEST REPORT

DETERMINATION OF THE VIRUCIDAL ACTIVITY OF THE F1031V2 PRODUCT ACCORDING TO THE EN 14476:2015 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°268D25-2018-10

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

1	PERFORMING LABORATORY	3
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Rédacteur	Superviseur
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 TCID₅₀

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% CO₂ 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 TCID₅₀ (calculated by Spearman-Kärber method) :

- For rotavirus, titer = 6,625 log DICT₅₀

4 VALIDATION**a) Cytotoxicity**

For rotavirus, the cell toxicity was observed until to the dilution 10⁻¹.

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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 ₅₀)		
PRODUCT DILUTION	Untreated cell cultures	Treated cell cultures	Viral titer (log TCID ₅₀)
F1031V2 10 ⁻²	6,625	6,500	0,125

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 ₅₀)	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 ₅₀)	Viral titer reduction (log TCID ₅₀)
Viral suspension control	6,625	
inactivation 5 min		0,375
inactivation 15 min	6,500	1,625
inactivation 30 min	5,375	1,750

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 TCID₅₀.

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

TRIAL 2 - The viral suspension was titrated at 6,625 log TCID₅₀.

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

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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 TCID₅₀ 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.

Rédacteur	Superviseur
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 TCID₅₀ = 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



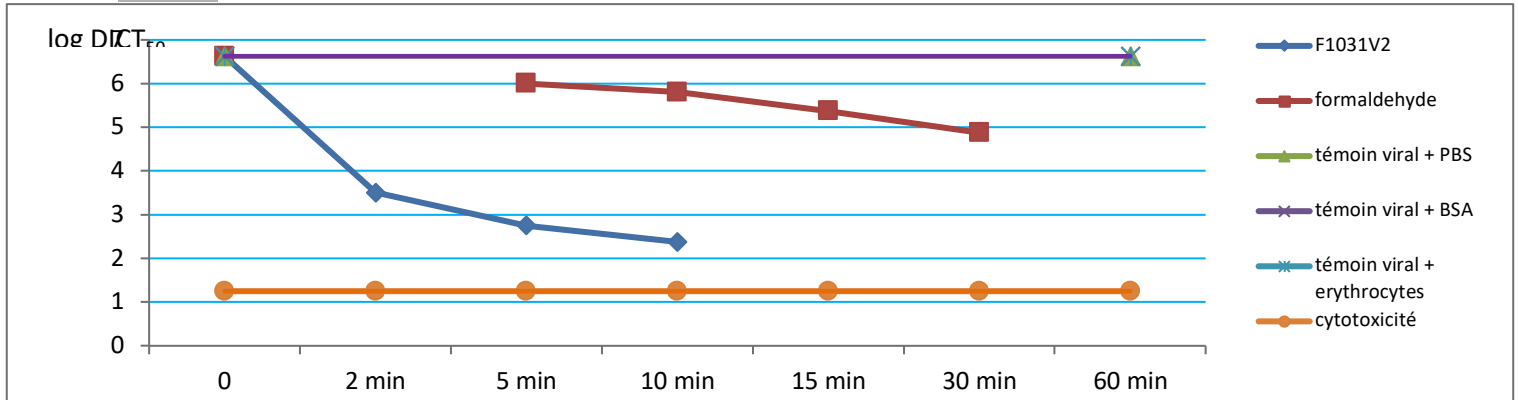
Rédacteur	Superviseur
Mme Emilie CANTREL, technicienne de laboratoire	Mme Stephanie MOROT-BIZOT, directrice
	

Chart 1 – Trials on rotavirus:

TRIAL 1



TRIAL 2

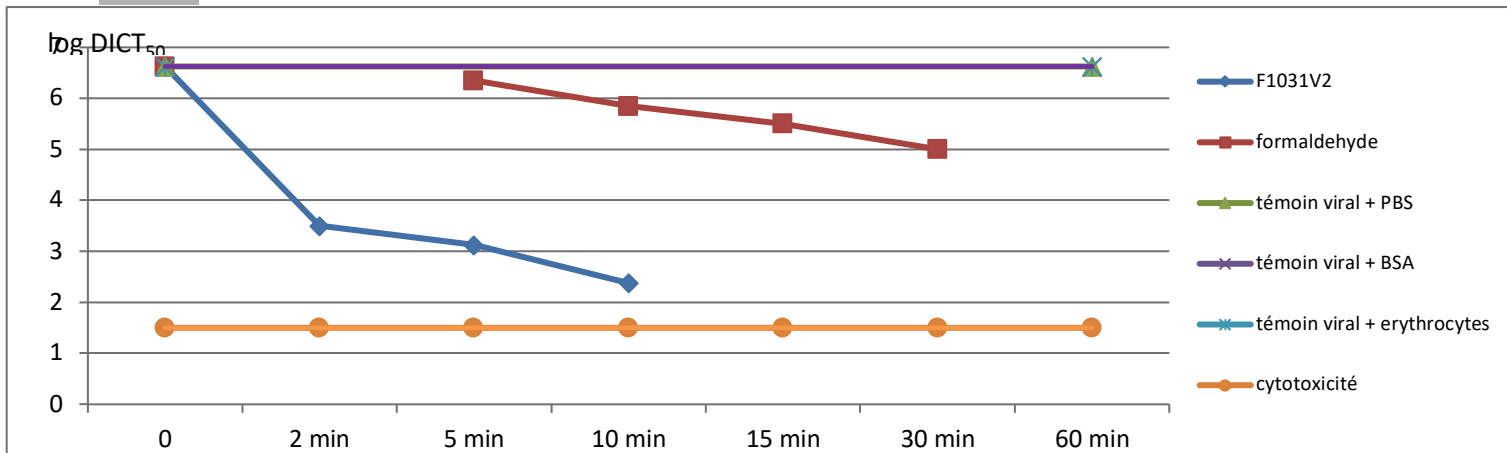


Table A2- Sensitivity of the cells to the rotavirus:

PRODUCT	DILUTION	ORGANIC SOIL LOAD		Dilutions							
				-2	-3	-4	-5	-6	-7	-8	-9
F1031V2	10 ⁻²	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 ₅₀							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 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 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 ⁻²	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	

Rédacteur	Superviseur
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

1	PERFORMING LABORATORY	3
2	PRODUCT IDENTITY	3
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9	TECHNICAL APPENDIX	7

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/m², 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.

Sponsor : FRANKLAB

4 VALIDATIONS AND ASSAYS

See results sheets.

The tuberculocidal activity is demonstrated if the reduction of the population is ≥ 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
- 3×10^2 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

5/7

7 *Mycobacterium terrae* - trial

Standard: EN 14348 Product : F1031V2 LINGETTES Batch N° : 701301 Study N° : 268D25-2018-24 Date of trials : 11/19/2018	Method: <input checked="" type="checkbox"/> Pour plating <input type="checkbox"/> Spread plating <input checked="" type="checkbox"/> Number of petri dishes: 2 petri dish / ml	Neutralizer : tween 80 + egg yolk 5% Temperature of trials : 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 37°C ± 1°C 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>	66	57	61	66	53	58	50	47	1.10 ⁻⁷	227	217	1.10 ⁰	>660	>660	301	298	46	35
	\bar{X}	61,5	\bar{X}	64,0	\bar{X}	55,5	\bar{X}	48,5	1.10 ⁻⁸	25	22	1.10 ⁻¹	266	248	34	30	8	5
	30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0					1.10 ⁻²	30	28	5	4	2	0
	x yes □ no		x oui □ non		x oui □ non		x oui □ non					1.10 ⁻³	4	3	0	1	0	0
										Log N	9,35	Na	2,60.10 ⁴	3,01.10 ³		4,05.10 ²		
										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									

4, rue des Grandes Pièces, zone Eureospace, 25 770 SERRE LES SAPINS ▪ Tel: 03.81.25.09.04 ▪ Fax: 03.81.25.53.51 ▪ SARL au capital de 10 000 € ▪ RCS BESANÇON ▪ N° SIRET 51786053200012 ▪ N° TVA intra FR 23517860532 ▪ info@apexlabo.com

Study No: 268D25-2018-24 F1031V2 lingettes

6/7

Sponsor : FRANKLAB

8 *Mycobacterium terrae* - repetition

Standard: EN 14348 Product : F1031V2 LINGETTES Batch N° : 701301 Study N° : 268D25-2018-24 Date of trials : 11/20/2018	Method: <input checked="" type="checkbox"/> Pour plating <input type="checkbox"/> Spread plating <input checked="" type="checkbox"/> Number of petri dishes: 2 petri dish / ml	Neutralizer : tween 80 + egg yolk 5% Temperature of trials : 20°C Organic soil load : 3 g/L BSA + 3 mL/L SE Incubation temperature : 37°C ± 1°C 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>	48	53	56	63	61	60	34	41	1.10 ⁻⁷	165	171	1.10 ⁰	662	662	223	191	49	50
	\bar{x}	50,5	\bar{x}	59,5	\bar{x}	60,5	\bar{x}	37,5	1.10 ⁻⁸	19	20	1.10 ⁻¹	401	389	26	31	6	6
	30 ≤ Nv0 ≤ 160		A ≥ 0,5 * Nv0		B ≥ 0,5 * Nv0		C ≥ 0,5 * Nv0					1.10 ⁻²	55	46	2	0	1	1
	x yes □ no		x yes □ no		x yes □ no		x yes □ no					1.10 ⁻³	6	10	0	0	0	0
										Log N	9,23	Na	4,05.10 ⁴	2,14.10 ³		4,95.10 ²		
										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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Rédacteur	Superviseur
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 :

N_a = 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 Product : F1031V2 Batch N° : 611141 Study N° : 268D25-2018-11 Date of trials : 11/19/2018	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 1	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA Incubation temperature : 37°C ± 1°C 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}	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 ⁻⁴	238	241	Vc	107	95	Vc	50	48	Vc	15	18		
	1.10 ⁻⁵	25	25	Na	1010,00		Na	490,00		Na	165,00			
	N	2,40.10 ⁶		Log Na	3,00		Log Na	2,69		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			



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

8 Clostridium difficile – REPETITION

Standard: EN 13704 Product : F1031V2 Batch N° : 611141 Study N° : 268D25-2018-11 Date of trials : 11/29/2018	Method: <input checked="" type="checkbox"/> pour plating <input type="checkbox"/> spread plating <input checked="" type="checkbox"/> Number of Petri dish/mL : 1	Neutralizer : polysorbate 80 (30g/L) + egg yolk 5% Temperature: 20°C Organic soil load : 3 g/L BSA Incubation temperature : 37°C ± 1°C 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}	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 ⁻⁴	238	241	Vc	107	95	Vc	50	48	Vc	15	18
	1.10 ⁻⁵	25	25	Na	1010,00		Na	490,00		Na	165,00	
	N	2,40.10 ⁶		Log Na	3,00		Log Na	2,69		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

Rédacteur	Superviseur
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

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

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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Siège social/Legal address : ZA de l'Observatoire - 3 avenue des Frênes - 78180 MONTIGNY LE BRETONNEUX – France.
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