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Laboratoire MIDAC
Your expert in microbiology

Rapport d'essais **n° 13 253-1.**

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Accréditation n°1-0019
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Essai de suspension pour l'évaluation de l'activité bactéricide des désinfectants chimiques utilisés pour les instruments en médecine selon NF EN 13727 : 2004

Produit : 1226 IND01

Donneur d'ordre **CHRISTEYNS France**
Division santé Laboratoire Phagogène
9 rue Marcel Sembat
44100 NANTES

Loos, le 07 juin 2013

Le directeur :

aphaël D É

L'accréditation du COFRAC atteste de la compétence des laboratoires pour les seuls essais couverts par l'accréditation.

La reproduction de ce rapport d'essai n'est autorisée que sous sa forme intégrale.

Ce rapport d'essais ne concerne que les échantillons soumis à essais.

1 PRINCIPE :

L'activité bactéricide a été déterminée selon la norme européenne **NF EN 13727: "Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide des désinfectants chimiques pour les instruments utilisés en médecine - Méthode d'essai et exigences (Phase 2, étape 1) – juillet 2004."**

Souche(s) de référence testée(s) : **voir tableau(x) page(s) suivante(s).**

2 IDENTIFICATION DE(S) ECHANTILLON(S) :

Nom(s) : **1226 IND01.**

Société : **CHRISTEYNS France.**

Date de réception au laboratoire : 22/04/13.

Conditions de stockage au laboratoire : Température ambiante, à l'abri de la lumière.

3 METHODES D'ESSAI ET VALIDATION:

3.1 Dilution neutralisation :

Diluant : **neutralisant CEN tamponné**

3.2 Filtration sur membrane.

Nature des membranes : **Millipore type HAWG 0,45 *m.**
Liquide de rinçage : **Eau distillée stérile.**
Volume de chaque rinçage : **100 ml.**
Nombre de rinçages : **4.**
Neutralisant ajouté au milieu de dénombrement : **TSB Capitol IV à raison de 10% (v/v) du milieu gélosé.**

4 CONDITIONS EXPERIMENTALES:

Période d'analyse : **Du 14/05/13 au 03/06/13.**

Diluant du produit utilisé au cours des essais: **Eau distillée (produit prêt à l'emploi).**

Concentration(s) du produit soumis à essai : **voir tableau(x) page(s) suivante(s).**

Aspect des dilutions de produit : **dilutions homogènes.**

Température de contact : **20°C +/- 1°C.**

Temps de contact additionnel : **5 minutes+/- 10 secondes.**

Substance interférente : **Albumine bovine 3,0 g/l + erythrocytes de mouton 3,0 ml/l (conditions de saleté). Préparée selon les indications de la norme NF EN 13 727.**

Stabilité du mélange de la substance interférente et des produits soumis aux essais: **pas de précipité observé**

Température d'incubation: **37°C +/- 1°C.**

5 RESULTATS EXPERIMENTAUX:

Vérification de la méthodologie et de la validation de la méthode de neutralisation du produit soumis à l'essai dans les conditions expérimentales décrites ci-avant :

Essai 1 : neutralisation par dilution

Souche(s) :	Nombre de cellules viables (UFC/ml) :				
	Suspension bactérienne Nv_0	Témoin des conditions expérimentales : A	Témoin de non toxicité du neutralisant : B	Validation de la méthode de dilution-neutralisation **Concentration du produit : 80%** C	Conclusion :
Pseudomonas aeruginosa CIP 103467	Vc: 42 ; 36 Nv_0: 39,0	Vc: 42 ; 40 A: 41,0	Vc: 38 ; 42 B: 40,0	Vc: 0 ; 0 C: 0,0	Non validée
Staphylococcus aureus CIP 4. 83	Vc: 44 ; 42 Nv_0: 39,0	Vc: 46 ; 50 A: 41,0	Vc: 50 ; 48 B: 49,0	Vc: 0 ; 0 C: 0,0	Non validée
Enterococcus hirae CIP 58 55	Vc: 48 ; 42 Nv_0: 45,0	Vc: 40 ; 38 A: 39,0	Vc: 46 ; 40 B: 43,0	Vc: 0 ; 0 C: 0,0	Non validée

Vc : nombre d' ufc comptées.
N : Nombre d'ufc/ml de la suspension bactérienne d'essai (5.4.1.4.).
Nv_0 : N/10 soit Nombre d'ufc/ml de la suspension bactérienne de validation (5.4.1.5.).
Na : Nombre d'ufc/ml dans le mélange d'essai (5.5.2.2).
A :Nombre d'ufc/ml dans l'essai de validation des conditions expérimentales (5.5.2.3 ou 5.5.3.3).
B : Nombre d'ufc/ml dans l'essai de validation de la non-toxicité du neutralisant (5.5.2.4) ou du témoin de filtration sur membrane (5.5.3.4).
C : Nombre d'ufc/ml dans l'essai de validation de l'inactivation par dilution neutralisation (5.5.2.5) ou par filtration sur membrane (5.5.3.5).
Critères de validation :
A, B, C supérieurs ou égaux à 0,5 x Nv_0
Nv_0 compris entre 30 et 160 UFC/ml
N compris entre $1,5.10^8$ et $5,0.10^8$
N_0 compris entre $1,5.10^7$ et $5,0.10^7$
log N_0 compris entre 7,17 et 7,70
Quotient moyenne pondérée compris entre 5 et 15

Conclusion : **La méthode de neutralisation par dilution n'est pas validée.**

Essai 2 : neutralisation par filtration sur membrane :
(Essais réalisés en parallèle à l'essai proprement dit)

Souche(s) :	Nombre de cellules viables (UFC/ml) :				
	Suspension bactérienne **Nv_0**	Témoin des conditions expérimentales : **A**	Témoin de filtration : **B**	Validation de la méthode de Filtration sur membrane **Concentration du produit : 80%** **C**	Conclusion :
Pseudomonas aeruginosa CIP 103467	Vc: 53 ; 58 Nv_0: 55,5	Vc: 56 ; 54 A: 55,0	Vc: 54 ; 56 B: 55,0	Vc: 52 ; 54 C: 53,0	Validée
Staphylococcus aureus CIP 4. 83	Vc: 42 ; 46 Nv_0: 44,0	Vc: 38 ; 44 A: 41,0	Vc: 42 ; 46 B: 44,0	Vc: 44 ; 45 C: 44,5	Validée
Enterococcus hirae CIP 58 55	Vc: 60 ; 64 Nv_0: 62,0	Vc: 61 ; 57 A: 59,0	Vc: 59 ; 64 B: 61,5	Vc: 66 ; 55 C: 60,5	Validée

Vc : nombre d' ufc comptées.
N : Nombre d'ufc/ml de la suspension bactérienne d'essai (5.4.1.4.).
Nv_0 : N/10 soit Nombre d'ufc/ml de la suspension bactérienne de validation (5.4.1.5.).
Na : Nombre d'ufc/ml dans le mélange d'essai (5.5.2.2).
A :Nombre d'ufc/ml dans l'essai de validation des conditions expérimentales (5.5.2.3 ou 5.5.3.3).
B : Nombre d'ufc/ml dans l'essai de validation de la non-toxicité du neutralisant (5.5.2.4) ou du témoin de filtration sur membrane (5.5.3.4).
C : Nombre d'ufc/ml dans l'essai de validation de l'inactivation par dilution neutralisation (5.5.2.5) ou par filtration sur membrane (5.5.3.5).
Critères de validation :
A, B, C supérieurs ou égaux à 0,5 x Nv_0
Nv_0 compris entre 30 et 160 UFC/ml
N compris entre $1,5.10^8$ et $5,0.10^8$
N_0 compris entre $1,5.10^7$ et $5,0.10^7$
log N_0 compris entre 7,17 et 7,70
Quotient moyenne pondérée compris entre 5 et 15

Conclusion : **La méthode de neutralisation par filtration sur membrane est validée.**

Essais proprement dit (méthode par filtration sur membrane) :

Souche(s) :	Suspension d'essai : N et N_0	**Nombre de cellules viables (UFC/ml) pour le mélange d'essai pour les concentrations en % (v/v) de :** Na			
		0,08	0,8	8,0	80,0
Pseudomonas aeruginosa CIP 103467	10^{-6} : Vc1 : 218 Vc2 : 224 10^{-7} : Vc1 : 25 Vc2 : 27 N : 2,3 x 10^8 **N_0 : 2,3 x 10^7** log N_0 : 7,36	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**
Staphylococcus aureus CIP 4 83	10^{-6} : Vc1 : 174 Vc2 : 178 10^{-7} : Vc1 : 18 Vc2 : 20 N : 1,8 x 10^8 **N_0 : 1,8 x 10^7** log N_0 : 7,26	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**
Enterococcus hirae CIP 5855	10^{-6} : Vc1 : 207 Vc2 : 208 10^{-7} : Vc1 : 22 Vc2 : 23 N : 2,1 x 10^8 **N_0 : 2,1 x 10^7** log N_0 : 7,32	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 : > 330 Vc2 : > 330 Na : > 3,30 x 10^3 log Na :> 3,52	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**	Vc1 :**< 14** Vc2 : **< 14** Na : **< 1,40 x 10^2** log Na :**< 2,15**

Réduction du nombre de cellules viables aux concentrations d'essai :

	0,08	0,8	8,0	80,0
Pseudomonas aeruginosa CIP 103467	log R : < 3,82	log R : < 3,82	**log R : > 5,21**	**log R : > 5,21**
Staphylococcus aureus CIP 4 83	log R : < 3,74	log R : < 3,74	**log R : > 5,11**	**log R : > 5,11**
Enterococcus hirae CIP 5855	log R : < 3,80	log R : < 3,80	**log R : > 5,17**	**log R : > 5,17**

Critères de validation :
N compris entre 1,5 x 10^8 et 5,0 x 10^8
N_0 compris entre 1,5 x 10^7 et 5,0 x 10^7
log N_0 compris entre 7,17 et 7,70

6 CONCLUSION :

Conformément à la norme européenne **NF EN 13727** (juillet 2004),

- En **5 minutes de contact à 20°C, [conditions optionnelles],**
- Dans les **conditions de saleté (Albumine bovine 3,0 g/l + erythrocytes de mouton 3,0 ml/l),**
- Vis-à-vis des souches de
  ***Pseudomonas aeruginosa* CIP 103467,**
  ***Staphylococcus aureus* CIP 4. 83,**
  ***Enterococcus hirae* CIP 58 55,**

Le produit **1226 IND01** dilué à **8,0% (v/v) présente une activité bactéricide.**

Nordic Tersus
Laboratory

Test report No. sd2319

EVALUATION OF MYCOBACTERICIDAL ACTIVITY OF CHEMICAL DISINFECTANTS IN THE MEDICAL AREA INCLUDING INSTRUMENT DISINFECTANTS (EN 14348)

Name of the product: 1226
Batch number: 367929
Date of test report: 08.02.2019

Client, representative:
Christeyns France
31 Rue de la Maladrie 44124 Vertou
Jérôme Dubourgeois; +33 (0)2 40 57 56 23

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EN ISO/IEC 17025
L263

Nordic Tersus
Laboratory

Test report No. sd2319

EVALUATION OF MYCOBACTERICIDAL ACTIVITY (EN 14348)

Name of the product:	1226
Batch number:	367929
Order number:	18018
Manufacturer:	Christeyns France
Client, representative:	Christeyns France; 31 Rue de la Maladrie 44124 Vertou; Jérôme Dubourgeois; +33 (0)2 40 57 56 23
Date of delivery:	12.10.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark;
Active substance – conc.:	Peracetic acid: 340 ppm; Hydrogen peroxide: 3.26%
Appearance of the product:	Transparent liquid
Test concentration:	80%; 40%; 20%
Test conditions:	Dirty conditions
Contact time:	5 min; 15 min; 60 min (obligatory)
Interfering substance:	3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Test neutralizer:	Polysorbate 80 30 g/l; lecithin 3 g/l; sodium thiosulphate 12g/l
Rinsing liquid:	-
Test organisms:	*Mycobacterium terrae* ATCC 15755; *Mycobacterium avium* ATCC 15769
Testing method base:	**EVS-EN 14348:2005 – Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)**
Testing date:	26.11.2018 – 18.12.2018
Results:	look appendix 1-3

Allar Laaneleht
Chief specialist
Date of test report: 08.02.2019

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

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Appendix 3

Interpretation

The EN 14348 standard was used for testing a product **1226** – (Batch No. 367929) at 20 °C ± 1 °C, with the contact times of 5 min, 15 min and 60 min (obligatory) under dirty conditions. The dilution-neutralization method was used for testing products' effectiveness against the reference strains: *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769. Under dirty conditions the tested 80% of product was active against both of the testorganisms for all contact times; 40% within .15 min, 60 min and 20% within 60 min.

Conclusion

The surviving count of mycobacterial reference strains showed at least 4 lg reduction meaning that **under dirty conditions the 80% solution of product 1226 is mycobactericidal within 5 min, 40% solution within 15 min and 20% solution within 60 min.**

Allar Laaneleht

Chief specialist

08.02.2019

Chemila, spol. s r.o., Za Dráhou 4386/3, Hodonín 69501, Phone +420518340919, chemila@chemila.cz
Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2005.

Copy No.: 1
Issue No.: 1

Test report No. S268-1/2018

DETERMINATION OF FUNGICIDAL (EN 13624:2013) ACTIVITY OF THE PRODUCT **1226**

Sample ID: S268/2018
Sample name: **1226**
Client: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France
Producer: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France
Sampling point: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France

Incoming date:
10.10.2018

Delivery date:
14.2.2019

Hodonín, 14.2.2019

TESTING LABORATORY CERTIFIED BY CZECH ACCREDITATION INSTITUTE
-2-
Chemila, spol. s.r.o.
Za Dráhou 4386/3
695 01 Hodonín
CZ
č. 1273

.....
Ing. Jana Šlitrová, Head of Laboratory

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929

Subject of testing:
Determination of fungicidal activity of the product.

Identification of the sample:

Name of the product:	**1226**
Batch number:	267929
Date of manufacture:	12/01/2018
Expiry date:	12/01/2021
Manufacturer:	Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France
Incoming date:	10.10.2018
Storage conditions:	5 – 30 °C
Active compounds and concentrations:	CAS 7722-84-1 hydrogen peroxide 3.26 % CAS 79-21-0 peracetic acid 0.034 %
Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624:2013)
Period of analysis:	8.11. – 11.11.2018
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated) *
Contact time:	5 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis (niger)* ATCC 16404
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Presence of a high concentration (at least 75%) of *Aspergillus brasiliensis* spiny spores in the test suspension – yes.
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least a 4 lg reduction (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least a 4 lg reduction (10^4).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$
* The product can only be tested at a concentration of 97% (RTU product, used modified method) or less, as some dilution is always produced by adding the inoculum and interfering substance.

The standard:
EN 13624:2013 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929

The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **1226** on *Candida albicans* ATCC 10231

Tab No. 1.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	35	Φ_{Nvo}= 31	V_{c1}	35	Φ_A = 30.5	V_{c1}	29	Φ_B = 28.5	V_{c1}	14	Φ_C = 20
V_{c2}	27		V_{c2}	26		V_{c2}	28		V_{c2}	26	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	27	V_{c2}	36	Φ_{NVB}	31.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 1.2 Test suspension

Test suspension N $\Phi = 305 \times 10^6 = \lg 8.48$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0) $\lg N_0 = \lg N/100 = \lg 6.48$ $6.17 \leq \lg N_0 \leq 6.70$
	10^{-6}	296	313	
	10^{-7}	29	32	
				x yes no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **1226** on *Candida albicans* ATCC 10231

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.48$)**
100* / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.33**

2. Testing the efficacy of chemical disinfectant **1226** on *Aspergillus brasiliensis (niger)* ATCC 16404

Tab No. 2.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	39	Φ_{Nvo}= 45.5	V_{c1}	38	Φ_A = 35	V_{c1}	44	Φ_B = 35	V_{c1}	41	Φ_C = 37.5
V_{c2}	52		V_{c2}	32		V_{c2}	26		V_{c2}	34	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	47	V_{c2}	41	Φ_{NVB}	44	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 2.2 Test suspension

Test suspension N $\Phi = 19 \times 10^7 = \lg 8.28$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0) $\lg N_0 = \lg N/100 = \lg 6.28$ $6.17 \leq \lg N_0 \leq 6.70$
	10^{-6}	>165	>165	
	10^{-7}	22	16	
				x yes no

Tab No. 2.3 Testing the efficacy of chemical disinfectant **1226** on *Aspergillus brasiliensis (niger)* ATCC 16404

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.28$)**
100* / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.13**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

* The product can only be tested at a concentration of 97% (RTU product, used modified method) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929

3. Evaluation of fungicidal activity of the product **1226**

Tab No. 3.1 The efficacy of chemical disinfectant **1226** on test strains – fungicidal activity

Fungicidal activity of the product (EN 13624:2013)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 13624:2013	**lg R**
Candida albicans ATCC 10231	20	5	100	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	5	100	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a
* The product can only be tested at a concentration of 97% (RTU product, used modified method) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Prepared by: Ing. Barbora Stoklásková, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
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Testing date: 8.11. – 11.11.2018
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Batch No: 367929
Page: 5

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624:2013)
Period of analysis:	8.11. – 11.11.2018
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	distilled water
Appearance of the product:	colourless liquid
Test concentration:	20%, 40%, 100% (concentrated)*
Contact time:	5 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis (niger)* ATCC 16404
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Presence of a high concentration (at least 75%) of *Aspergillus brasiliensis* spiny spores in the test suspension – yes.
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least a 4 lg reduction (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least a 4 lg reduction (10^4).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$
* Product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.

The standard:
EN 13624:2013 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929

4. Testing the efficacy of chemical disinfectant **1226** on *Candida albicans* ATCC 10231

Tab No. 4.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%**		
V_{c1}	27	Φ_{Nvo}= 35	V_{c1}	37	Φ_A = 33.5	V_{c1}	35	Φ_B = 31.5	V_{c1}	25	Φ_C = 26.5
V_{c2}	43		V_{c2}	30		V_{c2}	28		V_{c2}	28	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	41	V_{c2}	31	Φ_{NVB}	36	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160			
							x	yes		no

Tab No. 4.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 34 \times 10^6 = \lg 7.53$	10^{-5}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 6.53$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	33	35	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 4.3 Testing the efficacy of chemical disinfectant **1226** on *Candida albicans* ATCC 10231

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N_0 = lg 6.53)**
20 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.38**
40 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.38**
100* / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.38**

5. Testing the efficacy of chemical disinfectant **1226** on *Aspergillus brasiliensis (niger)* ATCC 16404

Tab No. 5.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%**		
V_{c1}	42	Φ_{Nvo}= 46.5	V_{c1}	54	Φ_A = 44	V_{c1}	48	Φ_B = 35	V_{c1}	53	Φ_C = 41.5
V_{c2}	51		V_{c2}	34		V_{c2}	22		V_{c2}	30	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	53	V_{c2}	41	Φ_{NVB}	47	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160			
							x	yes		no

Tab No. 5.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 45 \times 10^6 = \lg 7.65$	10^{-5}	> 165	> 165	$\lg N_0 = \lg N/10 = \lg 6.65$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	37	53	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **1226** on *Aspergillus brasiliensis (niger)* ATCC 16404

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N_0 = lg 6.65)**
20 / 5 / dirty	10^{-3}	26	19	5.35	**1.30**
40 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.50**
100* / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 4.50**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), $R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

* Product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929

6. Evaluation of fungicidal activity of the product **1226**

Tab No. 6.1 The efficacy of chemical disinfectant **1226** on test strains – fungicidal activity

Fungicidal activity of the product (EN 13624:2013)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13624:2013	**lg R**
Candida albicans ATCC 10231	20	5	20	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	5	20	dirty	≥ 4	**< 4**
Candida albicans ATCC 10231	20	5	40	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	5	40	dirty	≥ 4	**> 4**
Candida albicans ATCC 10231	20	5	100*	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	5	100*	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a

* Product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Ing. Barbora Stoklásková, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: S268/2018
Rep No: 161
Sample name: **1226**
Sampled: by client
Sampling point: Christeyn France S.A., Vertou
Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 8.10.2018
Sample delivered: 10.10.2018
Testing date: 8.11. – 11.11.2018
Delivered amount: 800 ml
Batch No: 367929
Page: 8

Interpretation:
Results of tests are in Tabs.
According to EN 13624:2013 the tested product **1226,** batch No. 367929, in the concentrations 100%(97%)*, 100%(80%)*, 40% and 20%, diluted in distilled water, in the contact time 5 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number vegetative yeast cells of *Candida albicans* ATCC 10231 by at least a 4 lg reduction.

According to EN 13624:2013 the tested product **1226,** batch No. 367929, in the concentrations 100%(97%)*, 100%(80%)* and 40%, diluted in distilled water, in the contact time 5 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** of mould spores of *Aspergillus brasiliensis (niger)* ATCC 16404 by at least a 4 lg reduction.

* Product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the test organisms and interfering substance or product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.
** The test strain was used according to client's request.

Conclusion:
The product **1226** is capable of reducing the number vegetative yeast cells and the number of mould spores of the relevant organisms under defined conditions to the declared values and consequently, may be called fungicidal.

12.2.2019, Hodonín

TESTING LABORATORY CERTIFICATE CZECH ACCREDITATION INSTITUTE
-2-
Chemila, spol. s.r.o.
Za Dráhou 4386/3
695 01 Hodonín
CZ
č. 1273

.....
Ing. Eva Kremlová, Leader of Study

Laboratoire MIDAC
Your expert in microbiology

Rapport d'essais **n° RE 17072-1**

cofrac ESSAIS
Accréditation n°1-0019
Portée disponible sur www.cofrac.fr

Evaluation des activités bactéricide et levuricide sur des surfaces non poreuses selon la norme NF EN 16615 : 2015

Produit : **1226–** lot **PR436-1226.170317**

Donneur d'ordre :

Christeyns France
31, rue de la Maladrie
44120 Vertou
France

Loos, le 30/05/2017

Mélinda MAUX, Ph.D
Responsable du laboratoire

Le rapport d'essai comporte : 5 pages

L'accréditation du COFRAC atteste de la compétence des laboratoires pour les seuls essais couverts par l'accréditation.
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Ce rapport d'essai ne concerne que l'échantillon soumis à essais.

1 PRINCIPE

Les activités bactéricides et levuricide ont été déterminées selon la norme **NF EN 16615** : "Antiseptiques et désinfectants chimiques – Méthode d'essai quantitative pour l'évaluation de l'activité bactéricide et levuricide sur de surfaces non poreuses, avec action mécanique à l'aide de lingettes dans le domaine médical (Essai à 4 zones) – Méthode d'essai et prescriptions (Phase 2, étape 2)." Mai 2015.

2 IDENTIFICATION DE(S) ECHANTILLON(S)

Nom(s) : **1226**– lot **PR436-1226.170317**

Société : **Christeyns France**

Date de réception au laboratoire : 21/03/2017

Conditions de stockage au laboratoire : Température ambiante, à l'abri de la lumière
Aspect du produit à réception : liquide, incolore

3 METHODE D'ESSAI ET VALIDATION

- Méthode de neutralisation : **Dilution neutralisation** (dénombrement par inclusion)

- Diluant neutralisant : **thiosulphate Sodium14 g/l**

4 CONDITIONS EXPERIMENTALES

Période d'analyse : Du **23/03/2017** au **27/04/2017**

Souche(s) de référence testée(s) : **voir tableau(x) page(s) suivante(s)**
Conservation et entretien au laboratoire conformément à la norme EN 12353

Concentration(s) du produit soumis à essai : **voir tableau(x) page(s) suivante(s)**
Diluant du produit utilisé au cours des essais: **Eau distillée**
Aspect des dilutions de produit : **incolore, homogène.**

Substance interférente : **Albumine bovine 3g/L + érythrocytes de mouton 3 mL/L** (conditions de saleté)

Température de contact : **20°C ± 2.5 °C**

Temps de contact : **5 minutes ± 15 secondes**

Température d'incubation : **37°C ± 1°C**

Identification surface d'essai : **PVC avec revêtement polyuréthane en surface** (dimension 20 cm x 50 cm)

Identification de la masse unitaire : **Bloc de granit** (dimension 12 cm x 8,6 cm x 8,6 cm)

Identification de la lingette utilisée : **Lingette de référence** (dimension 17,5 cm x 28cm : 55% pâte de bois, 45% polyéthylènetéréphtalate (PET)

Conditions de séchage : **<60 minutes** à la température de l'essai

5 RESULTATS EXPERIMENTAUX

Vérification de la méthodologie et de la validation de la méthode dilution-neutralisation

(Essais réalisés en parallèle à l'essai proprement dit)

Souche(s) d'essai	Nombre de cellules viables (UFC/ml)		
	Suspension de validation Nv_0	Témoin du neutralisant **B**	Validation de la méthode **C** product concentration: 100%
Pseudomonas aeruginosa DSM 939	VC1 : 73 VC2 : 94 Nv_0 : 83.5	VC1 : 98 VC2 : 91 **B** : 94.5	VC1 : 79 VC2 : 81 **C** : 80
Enterococcus hirae DSM 3320	VC1 : 145 VC2 : 126 Nv_0 : 135.5	VC1 : 133 VC2 : 137 **B** : 135	VC1 : 119 VC2 : 119 **C** : 119
Staphylococcus aureus DSM 799	VC1 : 36 VC2 : 42 Nv_0 : 39	VC1 : 34 VC2 : 34 **B** : 34	VC1 : 46 VC2 : 41 **C** : 43.5
Candida albicans DSM 1386	VC1 : 130 VC2 : 135 Nv_0 : 132.5	VC1 : 126 VC2 : 136 **B** : 131	VC1 : 140 VC2 : 141 **C** : 140.5
10^{-x} : nombre d'UFC comptées à la dilution x *Critères de validation :* *Nv0 compris entre 30 UFC/mL et 160 UFC/mL* *$B \geq 0,5\ Nv_0$* *$C \geq 0,5\ Nv_0$*			

Contrôles pour l'essai proprement-dit *(méthode par dilution-neutralisation)*

Souche(s) d'essai	Nombre de cellules viables pour l'essai **T** sur les zones tests 2 à 4 (UFC/25 cm²) pour le mélange d'essai aux concentrations v/v)		Nombre de cellules viables pour le Témoin eau **NW** sur les zones tests 2 à 4 (UFC/25 cm²) pour le mélange d'essai aux concentrations (v/v)
	1%	100%	1%
Pseudomonas aeruginosa DSM 939	VC_{T2} : 16 ; 16 VC_{T3} : 36 ; 32 VC_{T4} : 118 ; 97 $V_{T2\ à\ 4}$: 262.5	VC_{T2} : 0 ; 0 VC_{T3} : 0 ; 0 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: <14	VC_{T2} : >330 ; >330 VC_{T3} : >330 ; >330 VC_{T4} : 198 ; 217 $V_{NwT2\ à\ 4}$: >1650
Enterococcus hirae DSM 3320	VC_{T2} : 166 ; 126 VC_{T3} : 14 ; 16 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: 268.3	VC_{T2} : 0 ; 0 VC_{T3} : 0 ; 0 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: <14	VC_{T2} : >330 ; >330 VC_{T3} : >330 ; >330 VC_{T4} : >330 ; >330 $V_{NwT2\ à\ 4}$: >1650
Staphylococcus aureus DSM 799	VC_{T2} : 14 ; 18 VC_{T3} : 7 ; 9 VC_{T4} : 43 ; 36 $V_{T2\ à\ 4}$: 106	VC_{T2} : 0 ; 0 VC_{T3} : 0 ; 0 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: <14	VC_{T2} : 22 ; 15 VC_{T3} : 34 ; 34 VC_{T4} : 4 ; 0 $V_{NwT2\ à\ 4}$: 90.8
Candida albicans DSM 1386	VC_{T2} : 51 ; 54 VC_{T3} : 26 ; 30 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: 134.17	VC_{T2} : 0 ; 0 VC_{T3} : 0 ; 0 VC_{T4} : 0 ; 0 $V_{T2\ à\ 4}$: <14	VC_{T2} : >330 ; >330 VC_{T3} : 51 ; 74 VC_{T4} : 60 ; 60 $V_{NwT2\ à\ 4}$: >1650
Critères de validation : *$V_{T2\ à\ 4} \leq 50$ UFC / 25 cm²* *$V_{nw\ T2\ à\ 4} > 10$ UFC / 25 cm²*			

Résultats de l'essai proprement-dit *vis-à-vis des bactéries*

Souche(s) d'essai	Suspension d'essai N et **No**	Témoin de séchage D_{co}	Témoin temps de contact D_{ct}	Nombre de cellules viables pour la zone d'essai 1 **Na** (UFC/ml) pour le mélange d'essai aux concentrations (v/v)	
				1%	100%
Pseudomonas aeruginosa DSM 939	10^{-7}: >330 ; >330 10^{-8}: 38 ; 41 **Log N** : 9.60 **Log No** : 8.30	10^{-4}: 212 ; 225 10^{-5}: 24 ; 21 **Log Dco** : 7.04	10^{-4}: 175 ; 169 10^{-5}: 17 ; 17 **Log Dct** : 6.93	10^{0}: >330 ; >330 10^{-1}: 46 ; 41 **Na** : 2.18×10^{3} **Log Na** : 3.38	**10^{0}: 0 ; 0** **10^{-1}: 0 ; 0** **Na : <70** **Log Na : <1.85**
Enterococcus hirae DSM 3320	10^{-7}: 163 ; 163 10^{-8}: 17 ; 18 **Log N** : 9.22 **Log No** : 7.91	10^{-4}: 155 ; 137 10^{-5}: 27 ; 20 **Log Dco** : 6.89	10^{-4}: 161 ; 171 10^{-5}: 10 ; 14 **Log Dct** : 6.92	10^{0}: >330 ; >330 10^{-1}: >330 ; >330 **Na** : $>1.6 \times 10^{3}$ **Log Na** : >3.22	**10^{0}: 0 ; 0** **10^{-1}: 0 ; 0** **Na : <70** **Log Na : <1.85**
Staphylococcus aureus DSM 799	10^{-7}: 152 ; 165 10^{-8}: 16 ; 18 **Log N** : 9.20 **Log No** : 7.90	10^{-4}: >330 ; >330 10^{-5}: 261 ; 255 **Log Dco** : 8.11	10^{-4}: >330 ; >330 10^{-5}: 192 ; 152 **Log Dct** : 7.93	10^{0}: >330 ; >330 10^{-1}: >330 ; >330 **Na** : $>1.6 \times 10^{3}$ **Log Na** : >3.22	**10^{0}: 0 ; 0** **10^{-1}: 0 ; 0** **Na : <70** **Log Na : <1.85**

10^{x}: nombre d'UFC comptées à la dilution x

Critères de validation:
Log N compris entre 9.17 et 9.70
Log Dco et Log Dct compris entre 6.88 et 8.40

Réduction (R) du nombre de cellules viables aux concentrations d'essai testées (v/v) :

Souche(s) d'essai	1%	100%
Pseudomonas aeruginosa DSM 939	Log R : 3.55	**Log R : >5.08**
Enterococcus hirae DSM 3320	Log R : <1.85	**Log R : >5.07**
Staphylococcus aureus DSM 799	Log R : <4.71	**Log R : >6.08**

Réduction R : log R = log Dct - log Na

Critères d'interprétation :
concentration active si R ≥ 5
concentration non active si R < 5

Résultats de l'essai proprement-dit *vis-à-vis de la levure*

Souche(s) d'essai	Suspension d'essai N et **No**	Témoin de séchage D_{co}	Témoin temps de contact D_{ct}	Nombre de cellules viables pour la zone d'essai 1 **Na** (UFC/ml) pour le mélange d'essai aux concentrations (v/v)	
				1%	100%
Candida albicans DSM 1386	10^{-6}: 161 ; 169 10^{-7}: 24 ; 18 **Log N** : 8.23 **Log No** 6.93	10^{-3}: 256 ; 265 10^{-4}: 41 ; 42 **log Dco** 6.14	10^{-3}: 166 ; 178 10^{-4}: 18 ; 22 **log Dct** : 5.94	10^{0}: >330 ; >330 10^{-1}: 96 ; 107 **Na** : 3.71 **Log Na** : 2.23	**10^{0}: 0 ; 0** **10^{-1}: 0 ; 0** **Na : <70** **Log Na : <1.85**

10^{-x}: nombre d'UFC comptées à la dilution x

Critères de validation:
Log N compris entre 8.17 et 8.70
Log Dco et Log Dct compris entre 5.88 et 7.40

Réduction (R) du nombre de cellules viables aux concentrations d'essai testées (v/v) :

Souche(s) d'essai	1%	100%
Candida albicans DSM 1386	Log R : 2.23	**Log R : >4.09**

Réduction R : log R = log Dct - log Na

Critères d'interprétation :
concentration active si R ≥ 4
concentration non active si R <4

6 CONCLUSION

Selon la norme **NF EN 16615** (mai 2015), **le produit 1226**– lot **PR436-1226.170317**, présente une activité bactéricide et levuricide sur les surfaces, testé à la concentration de 100 % (v/v), après **5 minutes** de contact à **20° C**, en conditions de **saleté** (3,0 g/l d'albumine bovine + érythrocytes de mouton).

DR. JOCHEN STEINMANN
Resp. techn. et scient.
chez MikroLab GmbH

Norderoog 2
D-28259 Bremen

Tél.:+49 (421) 27819102
Fax:+49 (421) 2760283
http://www.mikrolab-gmbh.de
E-mail: MikroLab.GmbH@t-online.de

11.06.2013
Dr St/NM

MikroLab GmbH, Norderoog 2, D-28259 Bremen

Christeyns France SA
9, rue Marcel Sembat
44100 NANTES
FRANCE

Efficacité contre polio virus du produit 1226 IND01 lors d'un essai de suspension quantitatif à 20 °C en conditions de saleté

RAPPORT D'EXPERTISE

Le désinfectant pour les surfaces 1226 IND01 de Christeyns France SA a été testé pour son efficacité à inactiver poliovirus type 1, souche LSc-2ab suivant la norme prEN 14476:2011.

Dans la norme prEN 14476:2011, on conclut à l'efficacité anti-virus d'un désinfectant si, après un temps d'action défini, la réduction du titre viral initial est supérieure ou égale à ≥ 4 unités log_{10} (inactivation ≥ 99,99 %).

Le désinfectant pour les surfaces 1226 IND01 a été utilisé non dilué à 20 °C, en 5, 10 et 15 minutes de temps de contact. Après 15 minutes une réduction du titre de virus ≥ quatre unités log_{10} a été constatée. L'efficacité est donc obtenue avec 1226 IND01:

non dilué **15 minutes**

Dr. Jochen Steinmann
MIKROLAB GmbH
Norderoog 2
D-28259 Bremen

MIKROLAB GMBH
Laboratory for applied microbiology

Anerkannt durch/Recognized by
Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
www.zlg.de
ZLG-AP-306.10.31

TEST REPORT

11.06.2013

Test report P13ML1536-2Po

Evaluation of the effectiveness of

1226 IND01

Test virus: poliovirus type 1 strain LSc-2ab

Method: prEN 14476:2011 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:

Christeyns France SA
9, rue Marcel Sembat
44100 NANTES

Norderoog 2, D-28259 Bremen
Tel.: +49 (0) 421-27819102, Fax: +49 (0) 421-2760283
info@mikrolab-gmbh.de, http://www.mikrolab-gmbh.de

1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the surface disinfectant 1226 IND01 against poliovirus type 1 using a quantitative suspension assay according to prEN 14476:2011 (1) under dirty conditions.

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	Christeyns France SA
Name of product	1226 IND01
Batch number	16.04.13
Application	surface disinfection
Production date	16.04.2013
Expiry date	-
Active compound (s) (100 g)	peracetic acid (CAS no.: 79-21-0)
Appearance, odour	clear, colourless liquid product specific
pH-values (in WSH)	undiluted: 2.21 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	25.04.2013

4. Materials

4.1 Culture medium and reagents

- Dulbecco`s Modified Eagle`s Medium (DMEM, Lonza Group Ltd., catalogue no. BE12-707F)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)

- sheep erythrocytes (Fiebig-Nährstofftechnik; article no. 31100100).

4.2 Virus and cells

The poliovirus type 1 strain LSc-2ab (Chiron-Behring) was obtained from PD Dr. Olaf Thraenhart, Eurovir, D-14943 Luckenwalde.

BGM cells (*buffalo green monkey* = permanent monkey kidney cell line; supplied by Prof. Dr. Lindl, Institut für angewandte Zellkultur, D-81669 München, Germany) were cultivated in a 175 cm^2 flask with Dulbecco`s Modified Eagle`s Medium (DMEM) and 10 % fetal calf serum (FCS).

The cells (passage 34) were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

4.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable volume automatic pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany).

5. Experimental conditions

Test temperature	20 °C ± 1 °C
Concentration of test product	undiluted (97.0 % and 80.0 %) and as 10.0 % (non-active range) solution
Contact times	5, 10 and 15 minutes
Interfering substance(s)	dirty conditions: 3.0 g/l bovine serum albumin (BSA) + 3.0 g/l erythrocytes
Diluent	water (10.0 % solution)
Stability of product in the mix with virus and interfering substance (97.0 % and 80.0 % solutions)	no flocculation
Procedure to stop action of product	immediate dilution
Test virus	poliovirus type 1 strain LSc-2ab (Chiron-Behring)
Period of analysis	25.04.2013 – 11.06.2013
End of testing	11.06.2013

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension according to prEN 5.4.1, *BGM cells* were infected with a multiplicity of infection of 0.1 at 37 °C. After cells showed a cytopathic effect, they were subjected to a threefold freeze/thaw procedure followed by a low speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation of the supernatant, test virus suspension was stored at -80 °C.

6.2 Disinfectant

The test product was tested undiluted under dirty conditions (1 part test virus suspension + 1 part interfering substance + 8 parts disinfectant). Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted. Additionally, the 97.0 % solution under dirty conditions was evaluated (0.1 parts virus suspension + 0.2 interfering substance (5x) + 9.7 parts disinfectant).

Furthermore the product was examined as 10.0 % (demonstration of non-active range) solution. This solution was prepared with water immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined as endpoint titration according to prEN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of freshly trypsinised *BGM cells* (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after seven days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

6.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the prEN 14476:2011, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

6.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to prEN 5.5. The test product 1226 IND01 was examined undiluted (97.0 % and 80.0 %) and as 10.0 % solution at 20 °C. 5, 10 and 15 minutes were chosen as exposure times.

Due to a more convenient handling, the volumes in this assay were 0.1 ml test virus suspension, 0.1 ml interfering substance and 0.8 ml test product (80.0 % assay) or 0.01 ml test virus suspension and 0.02 ml interfering substance and 0.97 ml test product (97.0 % assay). Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations of the virus control were performed after the longest exposure time (prEN 5.5.7).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

6.6 Determination of cytotoxicity

Determination of cytotoxicity was performed according to prEN 5.5.4.1 with 100 µl water, 100 µl interfering substance and 800 µl test product (80.0 % assay) and with 10 µl water, 20 µl interfering substance (5x) and 970 µl test product (97.0 % assay).

6.7 Cell sensitivity to virus

For the control of cell sensitivity to virus 0.3 parts by volume hard water were mixed with 9.7 parts by volume of the lowest apparently non-cytotoxic dilution of the product. This mixture or PBS as control was added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (prEN 6.6.4.2b). Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

6.8 Control of efficacy for suppression of disinfectant activity

Furthermore, a control of efficiency for suppression of disinfectant activity was included (prEN 5.5.4.1).

6.9 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to prEN 5.5.6.1 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined following prEN 6.6.7.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in prEN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction.

b) The test product was cytotoxic in the 1:100 dilutions (97.0 % and 80.0 %) thus allowing the demonstration of a 4 $\log_{10}$ reduction.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see prEN 5.7b) was 1.88 ± 0.66 (between 0.5 - 2.5) after 30 min and 3.13 ± 0.57 (between 2.0 - 4.5) after 60 min for poliovirus type 1.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BGM cells* showed no significant difference (< 1 $\log_{10}$; prEN 5.7) of virus titre: 8.13 ± 0.45 (PBS) versus 8.00 ± 0.44 (1:1,000 dilutions of disinfectant) $\log_{10}TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant activity (undiluted, 97.0 % solution) showed no decrease in virus titre.

Since all criteria according prEN 5.7 were fulfilled, examination with poliovirus type 1 according to prEN 14476:2011 is valid.

8. Results

Results of examination are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 (a + b) gives a summary of results.

The test product (97.0 %) was able to inactivate poliovirus type 1 after 15 minutes exposure time in this quantitative suspension test. The following reduction factors were measured at this time point: 4.25 ± 0.33 and ≥ 4.13 ± 0.43 (mean value ≥ 4.19 ± 0.27) (Tables 1 and 2). This corresponded to an inactivation of ≥ 99.99 %.

Tested in an 80.0 % assay, 1226 IND01 was not active within 15 minutes exposure time (Table 3).

The 10.0 % solution of 1226 IND01 was in addition not active against the poliovirus type 1 after 15 minutes of exposure time as well (Table 4).

9. Summary

The surface disinfectant 1226 IND01 demonstrated activity under dirty conditions against poliovirus type 1 undiluted (97.0 %) after a contact time of 15 minutes. Due to the lack of virological guidelines simulating practical conditions in Europe (phase 2, step 2 tests) the data of this quantitative suspension test lead to the recommendation to use 1226 IND01 for inactivation of poliovirus type 1 as follows:

undiluted 15 minutes

Bremen, 11.06.2013

- Dr. Jochen Steinmann -

10. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBl. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBl. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

11. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between MikroLab GmbH and the sponsor will be stored in the archives at MikroLab GmbH.

12. Literature

1. prEN 14476:2011: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae.
Brit J Psychol; 2, 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix:

Table 1: Raw data for 1226 IND01 (97.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3219) (1st assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	97.0%	dirty conditions	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0040 0440	0000 4000	0000 0000	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0%	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data for 1226 IND01 (97.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3238) (2nd assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	97.0%	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	tttt tttt	tttt tttt	4444 4444	4444 4444	0404 0400	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4440 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0%	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4044	0040 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data for 1226 IND01 (80.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3219)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0%	dirty conditions	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0004 0400	0000 0000	0000 0000	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	0044 4044	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0%	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 0444	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data for 1226 IND01 (10.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3238)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0%	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0044	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	10.0%	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 4044	0000 0400	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data for formaldehyde solution (0.7 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3238)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	n.a.	n.a.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4440 4444	0000 0044	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0000 4400	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	4404 0440	0000 0404	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	4444 4444	0404 4340	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 0444	0400 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6: Raw data for control of efficacy for suppression of disinfectant activity (97.0 %) (3238)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	clean conditions	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	dirty conditions	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0440 0044	0400 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7: Raw data (poliovirus type 1) for cell sensitivity (97.0 %) (3238)

Product	comparative virus titration with	Dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	dirty conditions	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 4044	0400 0000	n.d.
test product	dirty conditions	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	dirty conditions	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	dirty conditions	1:1,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0040 0440	0000 0400	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 8a: Summary of results with 1226 IND01 and poliovirus type 1

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				5	10	15	30	60	
test product	97.0%	dirty	3.50	7.00±0.44	n.d.	4.50±0.00	n.d.	n.d.	15 (RF = 4.25±0.33)
test product	97.0%	dirty	3.50	n.d.	5.88±0.37	≤4.38±0.25	n.d.	n.d.	15 (RF ≥ 4.13±0.43)
test product	80.0%	dirty	3.50	6.75±0.33	n.d.	6.13±0.37	n.d.	n.d.	> 15
test product	10.0%	dirty	2.50	n.d.	n.d.	8.00±0.38	n.d.	n.d.	> 15

n.a. = not applicable n.d. = not done

Table 8b: Summary of results with 1226 IND01 and poliovirus type 1

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7% (w/v)	PBS	3.50	n.d.	7.63±0.41	6.75±0.33	6.38±0.49	5.13±0.37	> 60.0
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	8.25±0.44	n.a.
virus contr. 1	97.0% assay	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.75±0.33	n.a.
virus contr. 2	97.0% assay	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.50±0.35	n.a.
virus contr. 1	80.0% assay	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.13±0.37	n.a.
virus contr. 2	80.0% assay	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.13±0.45	n.a.
suppression control	97.0 %	dirty	3.50	n.d.	n.d.	n.d.	7.13±0.45	n.d.	n.a.
sens.control PBS	n.a.	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.13±0.45	n.a.
sens. control test product	97.0% → 1:1,000	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	8.00±0.44	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

MIKROLAB GMBH
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ZLG-P-429.08.10

TEST REPORT

05.07.2013

Test report P13ML1536-2A

Evaluation of the effectiveness of

1226 IND01

Test virus: adenovirus type 5 strain Adenoid 75

Method: prEN 14476:2011 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Christeyns France SA
9, rue Marcel Sembat
44100 NANTES

Norderoog 2, D-28259 Bremen
Tel.: +49 (0) 421-27819102, Fax: +49 (0) 421-2760283
info@mikrolab-gmbh.de, http://www.mikrolab-gmbh.de

1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the surface disinfectant 1226 IND01 against adenovirus type 5 using a quantitative suspension assay according to the prEN 14476:2011 (1) under dirty conditions.

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	Christeyns France SA
Name of product	1226 IND01
Batch number	16.04.13
Application	surface disinfection
Production date	16.04.2013
Expiry date	-
Active compound (s) (100 g)	peracetic acid (CAS no.: 79-21-0)
Appearance, odour	clear, colourless liquid product specific
pH-values (in WSH)	undiluted: 2.21 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	25.04.2013

4. Materials

4.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Lonza Group Ltd., catalogue no. BE12-125F)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)

- sheep erythrocytes (Fiebig-Nährstofftechnik; article no. 31100100)

4.2 Virus and cells

The adenovirus type 5 strain adenoid 75 was obtained from PD Dr. A. Heim, Institute of Medical Virology, Hannover Medical School, Hannover, Germany. Before the inactivation assays, the virus had been passaged 3 times in *A549 cells* (human lung epithelial carcinoma cells).

The *A549 cells* also originated from the Institute of Medical Virology, Hannover Medical School.

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

4.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Water bath (JULABO, Julabo U 3)
- Adjustable volume automatic pipettes (Eppendorf AG)
- Polysterol 96-well microtiter plate (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany)

5. Experimental conditions

Test temperature(s)	20 °C ± 1 °C
Concentration(s) of test product	undiluted (80.0 %), 50.0 % and 10.0 % (non-active range) solutions
Contact time(s)	5 and 15 minutes
Interfering substance(s)	dirty conditions: 3.0 g/l BSA + 3.0 g/l erythrocytes
Diluent	water (50.0 % and 10.0 % solutions)
Stability of product in the mix with virus and interfering substance (80.0 %)	no flocculation
Procedure to stop action of product	immediate dilution
Test virus	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Period of analysis	25.04.2013 – 05.07.2013
End of testing	05.07.2013

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension according to prEN 5.4.1 *A549 cells* were cultivated in a 175 cm^2 flask with Eagle`s Minimum Essential Medium with Earle`s BSS and 10 % fetal calf serum (FCS). Adenovirus type 5 (stock virus suspension) was added to the monolayer for 1 h at 37 °C with gentle shaking every 15 min. After cells showed a cytopathic effect, they were treated with ultrasound (HD 2200, Bandelin electronic GmbH & Co. KG, D-12207 Berlin) followed by a low speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, test virus suspension was stored at –80 °C.

6.2 Disinfectant

The test product was evaluated undiluted. Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted. Furthermore, the product was additionally examined as 50.0 % and 10.0 % (demonstration of non-active range) solutions.
The 50.0 % and 10.0 % solutions were prepared with water immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined as endpoint titration according to prEN 5.5.2 transferring 0.1 ml of each dilution into eight wells of a microtitre plate, beginning with the highest dilution. This was followed by the addition of 0.1 ml of freshly trypsinized *A549 cells*. This cell suspension

was adjusted to reach 10-15 x 10^3 cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after ten days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

6.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the prEN 14476:2011, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period.

6.5 Inactivation assay

Investigations for determination of virucidal activity followed prEN 5.5. 1226 IND01 was examined undiluted (80.0 %) and as 50.0 % and 10.0 % solutions at 20 °C. 5 and 15 minutes were chosen as exposure times.

Due to a more convenient handling, the volumes in this assay were 0.1 ml test virus suspension, 0.1 ml interfering substance and 0.8 ml test product. Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titration of the virus control was performed at contact times 0 min and 60 min (prEN 5.5.7).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

6.6 Determination of cytotoxicity

Determination of cytotoxicity was performed according to prEN 5.5.4.1 with 100 µl water, 100 µl interfering substance and 800 µl test product.

6.7 Cell sensitivity to virus

For the control of cell sensitivity to virus the lowest apparently non-cytotoxic dilution of the test product or PBS as control were added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (prEN 5.5.4.2b).
Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

6.8 Control of efficacy for suppression of disinfectant activity

Furthermore, a control of efficiency for suppression of disinfectant activity was included (prEN 5.5.6).

6.9 Reference virus inactivation test

As reference for test validation 0.7 % formaldehyde according to prEN 5.5.6.1 was included. Contact times were 5, 15, 30 and 60 min. In addition, cytotoxicity of formaldehyde test solution was determined following prEN 5.5.6.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in prEN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of ≥ 4 $\log_{10}$ reduction.

b) The test product was cytotoxic in the 1:100 (80.0 % and 50.0 % solutions) dilutions thus allowing the detection of a four $\log_{10}$ reduction of the virus titre.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see prEN 5.7b) was ≥ 3.13 ± 0.25 (between 3.0 – 5.0) after 30 min and ≥ 3.13 ± 0.25 (between 3.5 – 5.5) after 60 min for adenovirus

type 5. The required value (3.50) after 60 min exposure time was not reached because the initial virus titre (7.63) was too low to reach 3.50 as RF with a cytotoxicity of 4.50. The virus titre had to reach 8.00 to demonstrate a $\log_{10}$ reduction of 3.50 (4.50 + 3.50 = 8.00). Therefore, despite this too low reduction of virus titre after 60 minutes exposure time the test is valid.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *A549 cells* showed an acceptable difference (<1 $\log_{10}$; prEN 5.7) of virus titre: 7.13 ± 0.37 (PBS) versus 6.88 ± 0.37 (1:1,000 dilutions of disinfectant, 80.0 % solution).

e) The control of efficacy for suppression of disinfectant activity demonstrated a reduction of viral infectivity after 30 minutes of exposure time due to the fact that even the 10.0 % solution produced a $\log_{10}$ reduction of 3.63 ± 0.60 after 15 minutes. For these examinations the activity of the disinfectant was stopped by immediate dilution.

Since all criteria according to prEN 5.7 were fulfilled, examination with adenovirus type 5 according to prEN 14476:2011 was valid.

8. Results

The surface disinfectant 1226 IND01 was examined undiluted (80.0 % solutions) and as 50.0 % and 10.0 % solutions at 20 °C under dirty conditions.

Results of examinations are shown in tables 1 to 7. Tables 1 to 6 demonstrate the raw data, whereas table 7 (a+b) gives a summary of results.

The undiluted solution of the test product (80.0 %) was able to inactivate adenovirus type 5 after 5 minutes in this quantitative suspension test. The following reduction factor was measured at this time point: ≥ 4.00 ± 0.34. This corresponded to an inactivation of ≥ 99.99 % (Table 1).

The 50.0 % solution was additionally active against adenovirus type 5. After 5 minutes exposure time, the following reduction factor was measured: ≥ 4.00 ± 0.34 (≥ 99.99 %) (Table 2).

The 10.0 % solution was not sufficient active against adenovirus type 5. After 15 minutes exposure time, the following reduction factor was achieved: 3.63 ± 0.60 (Table 3).

9. Summary

In summary, a sufficient reduction of virus titre could be achieved by 1226 IND01 undiluted after an exposure time of 5 minutes. Due to the lack of virological guidelines simulating practical conditions in Europe (phase 2, step 2 tests) the data of this quantitative suspension test lead to the recommendation to use the surface disinfectant 1226 IND01 for inactivation of adenovirus type 5 under dirty conditions as follows:

undiluted 5 minutes

Bremen, 05.07.2013

Dr. Jochen Steinmann

10. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

11. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between MikroLab GmbH and the sponsor will be stored in the archives at MikroLab GmbH.

12. Literature

1. prEN 14476:2011: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae.
Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix

Table 1: Raw data of 1226 IND01 (80.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20 °C (3257)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	dirty conditions	5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	80.0%	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4403 2444	0040 0040	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0202 3422	0000 0300	4000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data of 1226 IND01 (50.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20 °C (3257)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	50.0%	dirty conditions	5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	50.0%	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4403 2444	0040 0040	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0202 3422	0000 0300	4000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data of 1226 IND01 (10.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20 °C (3257)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	10.0%	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	4444 4444	0400 0403	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	10.0%	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4403 2444	0040 0040	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0202 3422	0000 0300	4000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data of formaldehyde solution (0.7 %) tested against adenovirus type 5 (quantal test; 8 wells) (3257)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	n.a.	n.a.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	5	tttt tttt	tttt tttt	tttt tttt	4444 4444	1333 1424	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	3000 2010	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2133 3443	0004 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Control of efficacy for suppression of disinfectant activity (80.0 %) (3257)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	clean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	dirty	tttt tttt	0000 0004	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6 : Raw data (adenovirus type 5) for cell sensitivity to virus (80.0 %) (3257)

Product	Interfering substance	Dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	-	without	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3322 4000	0000 0000	0000 0000	n.d.
test product	PBS	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	clean conditions	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	dirty conditions	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3000 1003	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7a : Summary of results with 1226 IND01 and adenovirus type 5

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				5	10	15	30	60	
test product	80.0%	dirty	3.50	≤3.50±0.00	n.d.	≤3.50±0.00	n.d.	n.d.	5 (RF = ≥4.00±0.34)
test product	50.0%	dirty	3.50	≤3.50±0.00	n.d.	≤3.50±0.00	n.d.	n.d.	5 (RF = ≥4.00±0.34)
test product	10.0%	dirty	2.50	n.d.	n.d.	3.88±0.37	n.d.	n.d.	> 15 (RF = 3.63±0.60)

n.a. = not applicable n.d. = not done

Table 7b: Summary of results with 1226 IND01 and adenovirus type 5

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0	5	15	30	60	
formaldehyde	0.7% (w/v)	PBS	4.50	n.d.	6.50±0.00	4.88±0.37	≤4.50±0.00	≤4.50±0.00	≥ 30.0
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
virus contr.	n.a.	dirty	n.a.	7.63±0.41	n.d.	n.d.	n.d.	7.50±0.48	n.a.
suppression control	n.a.	dirty	3.50	n.d.	n.d.	n.d.	≤2.63±0.25	n.d.	n.a.
sens.control PBS	n.a.	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	7.13±0.37	n.a.
sens. control test product	80.0% **à** 1:1,000	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.37	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

DR. JOCHEN STEINMANN
Resp. techn. et scient.
chez MikroLab GmbH

Norderoog 2
D-28259 Bremen

Tél.:+49 (421) 27819102
Fax:+49 (421) 2760283
http://www.mikrolab-gmbh.de
E-mail: info@mikrolab-gmbh.de

20.06.2013
Dr St/NM

MikroLab GmbH, Norderoog 2, D-28259 Bremen

Christeyns France SA
9, rue Marcel Sembat
44100 NANTES
FRANCE

Efficacité contre le norovirus murin du produit 1226 IND01 lors d'un essai de suspension quantitatif à 20 °C en conditions de saleté

RAPPORT D'EXPERTISE

Le désinfectant pour les surfaces 1226 IND01 de Christeyns France SA a été testé pour son efficacité à inactiver le norovirus murin suivant la norme prEN 14476:2011.

Dans la norme prEN 14476:2011, on conclut à l'efficacité anti-virus d'un désinfectant si, après un temps d'action défini, la réduction du titre viral initial est supérieure ou égale à ≥ 4 unités $\log_{10}$ (inactivation ≥ 99,99 %).

Le désinfectant pour les surfaces 1226 IND01 a été utilisé non dilué à 20 °C, en 5 et 15 minutes de temps de contact. Après 5 minutes une réduction du titre de virus ≥ quatre unités $\log_{10}$ a été constatée. L'efficacité est donc obtenue avec 1226 IND01:

non dilué **5 minutes**

Dr. Jochen Steinmann

MIKROLAB GMBH
Laboratory for applied microbiology

Anerkannt durch/Recognized by
Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
ZLG-AP-306.10.31

TEST REPORT

20.06.2013

Test report P13ML1536-2M

Evaluation of the effectiveness of

1226 IND01

Test virus: murine norovirus (as surrogate for human norovirus)

Method: prEN 14476:2011 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Christeyns France SA
9, rue Marcel Sembat
44100 NANTES

Norderoog 2, D-28259 Bremen
Tel.: +49 (0) 421-27819102, Fax: +49 (0) 421-2760283
info@mikrolab-gmbh.de, http://www.mikrolab-gmbh.de

1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the surface disinfectant 1226 IND01 against murine norovirus (MNV) as surrogate for human norovirus using a quantitative suspension assay according to prEN 14476:2011 (1) under dirty conditions.

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	Christeyns France SA
Name of product	1226 IND01
Batch number	16.04.13
Application	surface disinfection
Production date	16.04.2013
Expiry date	-
Active compound (s) (100 g)	peracetic acid (CAS no.: 79-21-0)
Appearance, odour	clear, colourless liquid product specific
pH-values (in WSH)	undiluted: 2.21 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	25.04.2013

4. Materials

4.1 Culture medium and reagents

- Dulbecco´s Modified Eagle´s Medium (DMEM, Lonza Group Ltd., catalogue no. BE12-614F)
- Fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 1.4 % formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)

- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).
- sheep erythrocytes (Fiebig-Nährstofftechnik; article no. 31100100).

4.2 Virus and cells

MNV was obtained from PD. Dr. E. Schreier, Head of FG15 Molecular Epidemiology of Viral Pathogens at the Robert Koch-Institute (RKI) in Berlin. Prior to inactivation, MNV was passaged three times in *RAW 264.7 cells* (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice, ATCC TIB-71).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

4.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Water bath (JULABO, Julabo U 3)
- Adjustable volume automatic pipettes (Eppendorf AG)
- Polysterol 96-well microtiter plate (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany).

5. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % solutions
Contact times	5 and 15 minutes
Interfering substance (s)	dirty conditions: 3.0 g/l bovine serum albumin (BSA) + 3.0 g/l erythrocytes
Diluent	water (50.0 % and 10.0 % solutions)
Procedure to stop action of disinfectant	immediate dilution
Stability of product in the mix with virus and interfering substance (80.0 % solution)	flocculation
Test virus	murine norovirus (Berlin 06 / 06 / DE Isolat S99)
Period of analysis	02.04.2013 – 20.06.2013
End of testing	20.06.2013

6. Methods

6.1 Preparation of test virus suspension

To prepare the test virus suspension, *RAW 264.7 cells* that had been cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and 10 % fetal calf serum with low endotoxin were inoculated with MNV (stock virus solution) in a 175 cm^2 cell culture flask. Once a cytopathic effect had been induced (approx. 1-3 days), freezing and thawing was carried out two times. The cell debris was removed by centrifugation at 770 x g and 4 °C for ten minutes and the supernatant was recovered as test viral suspension, aliquoted and stored at -80 °C.

6.2 Disinfectant

The test product was evaluated undiluted. Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted.

Furthermore the product was evaluated as 50.0 % and 10.0 % solutions (demonstration of non-active range). These solutions were prepared with water immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined according to prEN 5.5.2 by means of end point dilution method using the microtitre process. For this, 100 µl aliquots of the samples, which had been serially diluted with ice-cold DMEM, were transferred to eight wells of a 96-well microtitre plate. This was followed by the addition of 100 µl aliquots of *RAW 264.7 cells* (approx. 1-1.5 x 10^4 cells) freshly prepared by scraping. Incubation took place at 37 °C in a CO_2 incubator (5 % CO_2 content) for five days. Finally, cultures were observed for cytopathic effects with a reversed microscope and the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 + 0.5 - \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of positive determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

6.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to prEN 14476:2011, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period.

6.5 Inactivation assay

Investigations for determination of virucidal activity followed prEN 5.5. The test product 1226 IND01 was examined undiluted (80.0 %) and as 50.0 % and 10.0 % solutions at 20 °C. 5 and 15 minutes were chosen as contact times.

Due to a more convenient surfaceling, the volumes in this assay were 0.1 ml test virus suspension, 0.1 ml interfering substance and 0.8 ml test product.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by serial dilution.

Titration of the virus control was performed at maximum contact times (prEN 5.5.7).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

6.6 Determination of cytotoxicity

Determination of cytotoxicity was determined according to prEN 5.5.4.1 with 100 µl water, 100 µl interfering substance and 800 µl test product.
Values are given as $log_{10}CD_{50}$/ml (in analogy to $log_{10}TCID_{50}$/ml).

6.7 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. This mixture or PBS as control was added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (prEN 5.5.4.2b).
Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

6.8 Control of efficacy for suppression of disinfectant activity

In accordance with prEN 5.5.6, a control of efficiency for suppression of disinfectant activity was included.

6.9 Reference virus inactivation test

A 0.7 (w/v) % formaldehyde solution was included as reference for test validation according to prEN 5.5.6.1. Contact times were 5, 15, 30 and 60 minutes. In addition, cytotoxicity of formaldehyde test solution was determined following prEN 5.5.6.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in prEN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of 4 $\log_{10}$ reduction.

b) The test product was cytotoxic in the 1:100 dilutions (80.0 % solution).

c) The comparative titration on pre-treated (disinfectant in the lowest apparently non-cytotoxic dilution) and non-pre-treated (PBS) *RAW 264.7 cells* showed a difference < 1 $\log_{10}$ of virus titre: 7.38 ± 0.25 (PBS) versus 7.50 ± 0.00 (dirty conditions, 1:1,000 dilutions of disinfectant) $\log_{10}TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant activity demonstrated no reduction of viral infectivity after 30 minutes of exposure time (7.38 ± 0.41 versus 7.75 ± 0.33).

Since all criteria following prEN 5.7 were fulfilled, examinations with MNV according to prEN 14476:2011 were valid.

8. Results

Results of examination are shown in tables 1 to 7 Tables 1 to 6 demonstrate the raw data, whereas tables 7 (a + b) gives a summary of results.

The product tested undiluted (80.0 %) was able to inactivate MNV after 5 minutes in this quantitative suspension test under clean conditions (Table 1). The reduction factor was ≥ 4.13 ± 0.41.

Tested as 50.0 % solution 1226 IND01 was active against the MNV within 15 minutes of exposure time (table 2) (RF ≥ 4.00 ± 0.46).

Finally, 1226 IND01 was not active within 15 minutes of exposure time (table 3).

9. Summary

The surface disinfectant 1226 IND01 demonstrated an activity against MNV undiluted after a contact time of 5 minutes at 20 °C. Due to the lack of virological guidelines simulating practical conditions in Europe (phase 2, step 2 tests) the data of this quantitative suspension

test lead to the recommendation to use the surface disinfectant 1226 IND01 for inactivation of MNV (surrogate for human norovirus) as follows:

undiluted 5 minutes

Bremen, 20.06.2013

- Dr. J. Steinmann -

10. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBl. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBl. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assay.

11. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between MikroLab GmbH and the sponsor will be stored in the archives at MikroLab GmbH.

12. Literature

1. prEN 14476:2011: Chemical disinfectants and antiseptics – virucidal quantitative suspension test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae.
Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Besurfacelung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix:

Table 1:	Raw data for 1226 IND01 (80.0 %) tested against MNV under dirty conditions
Table 2:	Raw data for 1226 IND01 (50.0 %) tested against MNV under dirty conditions
Table 3:	Raw data for 1226 IND01 (10.0 %) tested against MNV under dirty conditions
Table 4:	Raw data for formaldehyde (0.7 %) tested against MNV
Table 5:	Control of efficacy for suppression of disinfectant activity (80.0 %)
Table 6:	Raw data (MNV) for cell sensitivity (80.0 %)
Table 7 (a + b):	Summary of results with 1226 IND01 and MNV

Table 1: Raw data for 1226 IND01 (80.0 %) tested against MNV (quantal test; 8 wells) (3249)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	80.0%	dirty	5	tttt tttt	tttt tttt	0000 4000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	80.0%	dirty	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Formaldehyde	0.7% (w/v)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Formaldehyde cytotoxicity	0.7% (w/v)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	dirty	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 3444	0000 3000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4434	0030 4000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2 : Raw data for 1226 IND01 (50.0 %) tested against MNV (quantal test; 8 wells) (3249)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	50.0%	dirty	5	tttt tttt	tttt tttt	4400 0440	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	tttt tttt	tttt tttt	0004 0040	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	50.0%	dirty	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Formaldehyde	0.7% (w/v)	PBS	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Formaldehyde cytotoxicity	0.7% (w/v)	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	dirty	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 3444	0000 3000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4434	0030 4000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4 : Raw data for formaldehyde (0.7 %) tested against MNV (quantal test; 8 wells) (3247)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	n.a.	n.a.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Formaldehyde	0.7% (w/v)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4444 0444	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	3343 3443	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	0444 4444	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	4444 4444	4044 4444	0404 0000	0000 0000	0000 0000	0000 0000	n.d.
Formaldehyde cytotoxicity	0.7% (w/v)	PBS	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 3444	0004 0004	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Control of efficacy for suppression of disinfectant activity (80.0 %) (3249)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
Test product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product	clean conditions	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product	dirty conditions	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4404 3044	4000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7a:Summary of results with 1226 IND01 and MNV

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				5	10	15	30	60	
test product	80.0%	dirty	3.50	≤3.63±0.25	n.d.	≤3.50±0.00	n.d.	n.d.	5 (4.13±0.41)
test product	50.0%	dirty	3.50	≤4.00±0.38	n.d.	≤3.75±0.33	n.d.	n.d.	15 (4.00±0.46)
test product	10.0%	dirty	2.50	n.d.	n.d.	5.25±0.33	n.d.	n.d.	> 15.0

n.a. = not applicable n.d. = not done

Table 7b: Summary of results with 1226 IND01 and MNV

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7% (w/v)	PBS	3.50	n.d.	7.38±0.25	6.50±0.00	6.38±0.41	5.63±0.41	> 60
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.75±0.33	n.a.
virus contr.	n.a.	dirty	n.a.	7.50±0.35	n.d.	n.d.	n.d.	7.75±0.33	n.a.
suppression control	n.a	dirty	3.50	n.d.	n.d.	n.d.	7.38±0.41	n.d.	n.a.
sens.control PBS	n.a.	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.25	n.a.
sens. control test product	80.0% (1:1,000)	dirty	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.00	n.a.

n.a. = not applicable n.d. = not done

Laboratoire MIDAC
Your expert in microbiology

Rapport d'essais **n° 13 251-1.**

Activité bactéricide selon NF EN 13 704 (avril 2002)

Produit : 1226 IND01 (le 16/04/13)

Donneur d'ordre : **CHRISTEYNS France**
Division santé Laboratoire Phagogène
9 rue Marcel Sembat
44100 NANTES

Loos, le 13 mai 2013.

Le directeur :

Raphaël DUGUÉ.

Laboratoire MIDAC - Parc Eurasanté - Le Galénis - 85 C, rue Nelson Mandela - 59120 LOOS - FRANCE
Tél. : +33 (0)3 20 90 52 55 - Fax : +33 (0)3 20 58 21 66 - contact@midac-lab.fr
SARL au capital de 80 000 € - R.C.S. Lille 329 303 242

I. PRINCIPE :

L'activité sporicide a été déterminée selon la norme européenne **NF EN 13 704**: **"Désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité sporicide des désinfectants chimiques utilisés dans le domaine de l'agro-alimentaire, dans l'industrie, dans les domaines domestiques et en collectivité. - Méthode d'essai et prescriptions - (Phase 2, étape 1) – Avril 2002."**

Souche(s) de référence testée(s) : **voir tableau(x) page(s) suivante(s).**

II. IDENTIFICATION DE(S) ECHANTILLON(S) :

Nom(s) : **1226 IND01 (le 16/04/13).**

Société : **CHRISTEYNS France.**

Aspect du produit **: Liquide incolore.**

Date de réception au laboratoire : 22/04/13.

Conditions de stockage au laboratoire : Température ambiante, à l'abri de la lumière.

III. METHODE D'ESSAI ET VALIDATION:

- Dilution neutralisation :

1. **Diluant :** neutralisant CEN tamponné

2. **Filtration sur membrane.**

   Nature des membranes : Millipore type HAWG 0,45 µm.
   Liquide de rinçage : Eau distillée stérile.
   Volume de chaque rinçage : 100 ml.
   Nombre de rinçages : 4.

   Neutralisant ajouté au milieu de dénombrement : TSB Capitol IV à raison de 10% (v/v) du milieu gélosé.

IV. CONDITIONS EXPERIMENTALES:

Période d'analyse : Du 23/04/13 au 10/05/13.

Diluant du produit utilisé au cours des essais: **Eau distillée (produit prêt à l'emploi).**

Concentration(s) du produit soumis à essai : **voir tableau(x) page(s) suivante(s).**

Aspect des dilutions de produit : **limpides.**

Température de contact : **20°C +/- 1°C.**

Temps de contact additionnel : **15 minutes+/- 10 secondes.**

Substance interférente : **Albumine bovine 0,3 g/l (conditions de propreté).**

Stabilité du mélange de la substance interférente et des produits soumis aux essais: **précipité non décelé.**

Température d'incubation: **30°C +/- 1°C.**

V. RESULTATS EXPERIMENTAUX:

I. Vérification de la méthodologie et de la validation de la méthode de neutralisation du produit soumis à l'essai dans les conditions expérimentales décrites ci-avant :

Essai 1 : neutralisation par dilution
(Essais réalisés en parallèle à l'essai proprement dit)

Souche(s) :	Nombre de cellules viables (u.f.c/ml) :					
	Suspension de validation (spores) : **Nv_0**	Témoin des conditions expérimentales : **A**	Témoin de non toxicité du neutralisant : **B**	Concentration du produit mise en œuvre pour la validation :	Validation de la méthode de dilution-neutralisation **C**	Conclusion :
Bacillus subtilis DSM 347	Vc : 200 ; 213 Nv : 2,1 x 10^3	Vc : 198 ; 203 Nv : 2,0 x 10^2	Vc : 188 ; 179 Nv : 1,8 x 10^2	80% (v/v)	Vc : 3 ; 4 Nv : < 15	Non validée

Nv : Nombre d'ufc/ml de la suspension de spores (B.2.).
A : Nombre d'ufc/ml dans l'essai de validation des conditions expérimentales (B. 4.1.2 a ou B.4.2.2 a).
B : Nombre d'ufc/ml dans l'essai de validation de la non-toxicité du neutralisant (B.4.1.2.b) ou du témoin de filtration sur membrane (B.4.2.2 b).
C : Nombre d'ufc/ml dans l'essai de validation de l'inactivation par dilution neutralisation (B.4.1.2 c) ou par filtration sur membrane (B.4.2.2 c).

Critères de validation :
Nv compris entre 6 x 10^2 et 3 x 10^3 UFC / ml.
A et B supérieurs ou égaux à 0,05 x Nv
C supérieur ou égal à 0,5 x B

Conclusion : **La méthode de neutralisation par dilution n'est pas validée.**

Essai 2 : neutralisation par filtration
(Essais réalisés en parallèle à l'essai proprement dit)

Souche(s) :	Nombre de cellules viables (u.f.c/ml) :					
	Suspension de validation (spores) : Nv_0	Témoin des conditions expérimentales : A	Témoin de non toxicité du neutralisant : B	Concentration du produit mise en œuvre pour la validation :	Validation de la méthode de filtration sur membranes: C	Conclusion :
Bacillus subtilis DSM 347	Vc : 200 ; 186 Nv : 1,9 x 10^3	Vc : 172 ; 188 Nv : 1,8 x 10^2	Vc : 182 ; 193 Nv : 1,9 x 10^2	80% (v/v)	Vc : 185 ; 172 Nv : 1,8 x 10^2	Validée

Nv : Nombre d'ufc/ml de la suspension de spores (B.2.).
A : Nombre d'ufc/ml dans l'essai de validation des conditions expérimentales (B. 4.1.2 a ou B.4.2.2 a).
B : Nombre d'ufc/ml dans l'essai de validation de la non-toxicité du neutralisant (B.4.1.2.b) ou du témoin de filtration sur membrane (B.4.2.2 b).
C : Nombre d'ufc/ml dans l'essai de validation de l'inactivation par dilution neutralisation (B.4.1.2 c) ou par filtration sur membrane (B.4.2.2 c).

Critères de validation :
Nv compris entre 6 x 10^2 et 3 x 10^3 UFC / ml.
A et B supérieurs ou égaux à 0,05 x Nv
C supérieur ou égal à 0,5 x B

Conclusion : **La neutralisation est validée avec la méthode de filtration testée.**

Essai proprement-dit (méthode par filtration sur membranes) :

Souche(s) :	Suspension d'essai de spores : N	Nombre de cellules viables (u.f.c/ml) pour le mélange d'essai pour les concentrations en % (v/v) de : Na		
		20%	40%	80%
Bacillus subtilis DSM 347	10^{-4} : 212 10^{-5} : 240 N= 2,25 x 10^{6}	Vc1 : > 300 Vc2 : > 300 Na : > 3,0 x 10^{3}	Vc1 : 112 Vc2 : 95 Na : 1,1 x 10^{3}	**Vc1 : < 15** **Vc2 : < 15** **Na : < 1,5 x 10^{2}**

Réduction du nombre de cellules viables aux concentrations d'essai en % (v/v) :

	20%	40%	80%
Bacillus subtilis DSM 347	log R : < 0,75 x 10^{2}	log R : 2,05 x 10^{2}	**log R : > 1,5 x 10^{3}**

Critères de validation :
N compris entre 1,5 x 10^{6} et 5,0 x 10^{6}

Vc : nombre d' ufc comptées.
N : Nombre d'ufc/ml de la suspension d'essai de spores (5.4.1.3.).
Nv : Nombre d'ufc/ml de la suspension de spores (B.2.).
Na : Nombre d'ufc/ml dans le mélange d'essai (5.5.2.2.3. ou 5.5.2.3.3).
R : Réduction du nombre de cellules viables (5.6.3.)

VI. CONCLUSION :

Conformément à la norme européenne **NF EN 13 704** (avril 2002),

- En **15 minutes de contact à 20°C [conditions optionnelles],**
- Dans les **conditions de propreté (0,3 g/l d'albumine bovine),**
- Vis-à-vis de la souche ***Bacillus subtilis*** **DSM 347,**

Le produit **1226 IND01 (le 16/04/13)** dilué à **80,0% (v/v) présente une activité sporicide.**

BILAN TEST PHAGOSPORE

Philippe SALIOU*, Anne LE GRAND*, Madeleine RAGUENES**
* Service Hygiène Hospitalière CHRU BREST
** DAHL CHRU BREST

Introduction :

Phagospore est un produit nettoyant-désinfectant pour sol et surfaces hautes formulé à base d'acide peracétique (300 ppm). En plus des activités bactéricide (EN 13727) et fongicide (EN 13697) classiques pour ce type de produit, Phagospore est virucide (EN 14476) et sporicide (*Clostridium difficile* selon EN 13704).

De novembre 2013 à avril 2014, Phagospore a été testé, dans trois services du CHRU de BREST (2 services de SSR et un service de médecine à orientation gériatrique).

Pendant la première phase de l'essai, Phagospore était utilisé 15 jours consécutifs pour nettoyer et désinfecter le sol (bidon de 5 litres) et les surfaces hautes (flacon de 750 mL) d'une chambre afin de s'assurer de sa compatibilité avec les matériaux.

Pendant la seconde phase, Phagospore était utilisé dans la chambre des malades ayant une gastroentérite aigue (GEA) à Norovirus ou une diarrhée à *Clostridium difficile* afin de vérifier, son acceptabilité par les professionnels.

Pendant l'essai, les utilisateurs remplissaient une fiche d'évaluation comprenant 6 parties : conditionnement, tolérance (odeur, cutanée, respiratoire), efficacité (vitesse de séchage, pouvoir nettoyant, facilité d'utilisation, aspect des surfaces après utilisation, traces), compatibilité avec les matériaux, comparaison avec l'eau de javel (odeur, aspect surfaces après utilisation, facilité d'utilisation, tolérance, compatibilité avec les matériaux) et commentaires libres.

Résultats :

49 fiches d'évaluations ont été remplies et analysées.

95,6% des utilisateurs sont satisfaits ou très satisfaits du flacon 750 ml et du pulvérisateur. Mais, 1/3 sont peu ou pas satisfaits du bidon de 5 litres jugé trop lourd et peu maniable.

L'ensemble des utilisateurs trouve l'odeur du Phagospore parfumé à la menthe satisfaisante, et plus agréable que celle de l'eau de javel. Mais ils étaient 74% à ne pas apprécier l'odeur initiale (parfum Niaouli), 57% trouvaient l'odeur d'acide acétique (vinaigre) trop marquée. Près de la moitié (47%) trouvait son odeur moins agréable que celle de l'eau de javel.

Près de 80% des utilisateurs jugent que la tolérance cutanée du Phagospore est bonne et 70% d'entre eux qu'elle est meilleure que celle de l'eau de javel. Ces deux produits doivent être utilisés avec des gants de protection.

Presque tous les utilisateurs apprécient le pouvoir détergent du Phagospore (98%) et sa rapidité de séchage (96%). La majorité (77%) juge l'aspect des surfaces satisfaisant ou très satisfaisant (absence de trace : 70%) et 70% jugent le résultat meilleur qu'avec l'eau de javel. Cependant, 6 utilisateurs notent que Phagospore mousse lors de son application sur le sol ou que celui-ci est collant après son utilisation. Enfin 82% trouvent Phagospore facile d'utilisation, et 85% plus facile d'utilisation que l'eau de javel.

Discussion :

L'entretien des chambres se fait avec des produits détergents désinfectants bactéricides et fongicides. Les surfaces de la chambre d'un malade ayant une GEA à Norovirus ou une diarrhée à *Clostridium difficile*, doivent être désinfectées à de l'eau de javel (1, 2). Cette désinfection nécessite de réaliser un bionettoyage en 5 étapes (nettoyage, rinçage, séchage, désinfection javel, séchage). Cette désinfection lourde à mettre en place et chronophage est souvent difficile à appliquer surtout en période d'épidémie, notamment à Norovirus, qui touche les malades et le personnel. Phagospore, produit nettoyant désinfectant permet de réduire ce protocole à deux étapes (application du produit et séchage). L'eau de Javel nécessite un temps de contact de 10 minutes. Phagospore est actif en 10 minutes sur *Clostridium difficile* selon la norme EN 13704 et en 5 minutes sur Norovirus selon la norme EN 14476.

L'essai montre que Phagospore parfum menthe a une odeur jugée agréable par les utilisateurs. Aucun effet secondaire cutané ou respiratoire notable n'a été signalé dans la mesure ou les précautions d'utilisation (port de gants) sont respectées.

Il n'a pas été constaté d'altération ou d'incompatibilité avec le matériel et les surfaces traitées durant la période de l'essai.

La facilité d'utilisation, le pouvoir nettoyant et la qualité du nettoyage sont jugés excellents par la majorité des utilisateurs.

Références :

1- Avis relatif à la maîtrise de la diffusion des infections à Clostridium difficile dans les établissements de santé français. Haut Conseil de la Santé Publique juin 2008, 11 pages.
2- Recommandations relatives aux conduites à tenir devant des gastro-entérites aiguës en établissement d'hébergement pour personnes âgées. Commission spécialisée Maladies transmissibles Haut Conseil de la Santé Publique janvier 2010, 77 pages.

RAPPORT D'ESSAI N°3410-1

55 Boulevard Jules Verger
35803 DINARD Cedex
Tél. 02 99 16 50 72
Fax. 02 99 16 52 75

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Test d'efficacité sporicide
contre les spores de *Clostridium difficile* CIP 104282
selon une méthodologie adaptée de la norme
NF EN 13697 (Novembre 2001)
1226 IND01

DESTINATAIRE : CHRISTEYNS FRANCE

I- IDENTIFICATION DU DONNEUR D'ORDRE

Monsieur Jérôme DUBOURGEOIS
CHRISTEYNS FRANCE
31 rue de la Maladrie
44 120 VERTOU
Tél.02-40-80-27-27 - Fax. 02-40-03-09-73

II- IDENTIFICATION DE L'ECHANTILLON

- Nom du produit : **1226 IND01**
- Numéro de lot : PR178-4
- Fabricant : CHRISTEYNS FRANCE
- Date de fabrication : 02/07/14
- Date de péremption : non communiquée
- Date de réception au laboratoire : 18/07/14
- Aspect du produit : Produit limpide transparent
- Conditions de stockage : à température ambiante et à l'abri de la lumière
- Diluant du produit recommandé par le fabricant : non concerné
- Matière(s) active(s) : Non communiquées

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Fax. 02 99 16 52 75

III- METHODE D'ESSAI

Méthodologie adaptée de la norme NF EN 13697 (Novembre 2001) : « Essai quantitatif de surface non-poreuse pour l'évaluation de l'activité bactéricide et/ou fongicide des désinfectants chimiques utilisés dans le domaine de l'agro-alimentaire, dans l'industrie, dans les domaines domestiques et en collectivité ». (Phase 2, étape 2)

Réduction recherchée demandée par le client : 4 Log.

Neutralisant : 3% Polysorbate 80 ; 3% Saponine ; 0,3% lécithine d'œuf ; 0,1% L-Histidine ; 0,5% Thiosulfate de sodium (stérilisé à 121°C pendant 20 minutes).

IV- CONDITIONS EXPERIMENTALES

- Période d'analyse : du 16/09/14 au 19/09/14
- Analyse réalisée par : AF. GABILLET
- Diluant du produit utilisé au cours de l'essai : eau distillée
- Concentrations de produit testé (V/V) : 10%-50% et 100%
- Aspect des dilutions : limpides
- Temps de contact : 15 minutes (+/- 10 secondes)
- Température d'essai : 20°C (+/-1°C)
- Substance interférente : 3 g/ d'albumine bovine (conditions de saleté)
- Température d'incubation : 37°C (+/-1°C) en anaérobiose
- Identification des spores utilisées :
    - Spores de *Clostridium difficile* CIP 104282 (séchage pendant 35 minutes à 37°C+/-1°C)

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35803 DINARD Cedex
Tél. 02 99 16 50 72
Fax. 02 99 16 52 75

V- RESULTATS D'ESSAI

Vc : nombre de colonies comptées sur les boîtes,
N: nombre de cellules dénombrées par 0,05 ml dans la suspension microbienne d'essai et de la suspension de validation,
Nd: logarithme du nombre d'UFC/surface d'essai pour l'essai avec le désinfectant,
Nc: logarithme du nombre d'UFC/surface d'essai pour le témoin d'eau,
NC: logarithme du nombre d'UFC/surface d'essai du témoin de neutralisation,
NT : logarithme du nombre d'UFC/surface d'essai de neutralisation,
ME : effet microbicide (ME= Nc-Nd).

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55 Boulevard Jules Verger
35803 DINARD Cedex
Tél. 02 99 16 50 72
Fax. 02 99 16 52 75

Essai sur *les spores de Clostridium difficile* CIP 104282

Souche testée	Suspension microbienne d'essai	Essai de validation		
		Témoin d'eau (Nc)	Témoin de neutralisation (NC)	Essai de neutralisation (NT)
Spores de Clostridium difficile CIP 104282	10^{-5} : Vc1 = 63 Vc2 = 31 10^{-6} : Vc1= 3 Vc2= 4 N= 6,37	10^{-2} : Vc1 =134 Vc2 =114 10^{-3} : Vc1=13 Vc2= 24 Nc =6,09 Nts>100	10^{-2} : Vc1 =93 Vc2 =91 10^{-3} : Vc1=25 Vc2=12 NC= 5,96	10^{-2} : Vc1=91 Vc2 =75 10^{-3} : Vc1= 21 Vc2= 14 NT= 5,92

L'essai est validé si :
N-Nc est inférieur ou égal à 2 Log
N-NC est inférieur ou égal à 2 Log
NC-NT est inférieur ou égal à +/- 0,3
Nts est inférieur à 100 UFC pour les concentrations actives. Pour les concentrations non actives, Nts peut ne pas être dénombrable.

Souche testée		Concentrations testées (V/V)				
		10%	50%	100%		
Spores de Clostridium difficile CIP 104282	10^0	>300 ;>300	0 ;0	0 ;0		
	10^{-1}	>300 ;>300	0 ;0	0 ;0		
	10^{-2}	67 ;77	0 ;0	0 ;0		
	Dénombrement surface (Nts)	>100	0	0		
	Nd	5,86	<1	<1		
	ME	0,23	>5,09	>5,09		

(la technique de dénombrement utilisée est la technique par étalement en surface de 0,1 ml)

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VI- CONCLUSION

Selon une méthodologie adaptée de la norme NF EN 13697 (Novembre 2001), le lot PR178-4 du produit 1226 IND01 de la société CHRISTEYNS FRANCE, présente une réduction supérieure à 5,09 Log sur les surfaces, lorsqu'il est testé à 50% et 100% (V/V), en présence d'albumine bovine à 3 g/l (conditions de saleté), en 15 minutes de temps de contact, à la température de 20°C vis-à-vis des spores de *Clostridium difficile* CIP 104282.

Les spores ont été soumises à essai une seule fois.

Commentaires :
Les spores ont été dénombrées sur de la gélose cœur/cervelle additionnée de 5% de sang de mouton et de 0,1% de taurocholate. L'incubation est de 3 jours à 37°C (+/-1°C) sous atmosphère anaérobie.

VII-SIGNATURES

Fait à DINARD, le

Rédigé par	Validé par
AF. GABILLET Responsable d'essai 19/9/14	M. SESQUES Docteur en microbiologie Directeur technique 22/09/2014

LMH
Laboratoire Microbiologie et Hygiène
55 Boulevard Jules Verger - BP 10180
35803 DINARD CEDEX
Tél. 02 99 16 50 72 - Fax 02 99 16 52 75