CHRISTEYNS

Laboratoire phagogène®
L'assurance désinfection

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ATTESTATION D'EQUIVALENCE

Je soussigné, Jérôme DUBOURGEOIS, agissant en qualité de Responsable R&D de la division santé de Christeyns France, atteste que le **PHAGORUB SOLUTION SPS**, mis à part le les ingrédients nécessaires à la gélification du produit, comporte les mêmes ingrédients que le **PHAGORUB GEL SPS**.

Les résultats essais permettant de valider l'absence de potentiel allergique ainsi que la compatibilité avec la peau du **PHAGORUB GEL SPS** peuvent également être attribués au **PHAGORUB SOLUTION SPS.**

Le **PHAGORUB SOLUTION SPS** peut donc être considéré que hypoallergénique (formulé pour minimiser les risques allergiques)

Fait à Nantes, le 11 mars 2014

Jérôme DUBOURGEOIS

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ATTESTATION D'EQUIVALENCE

Je soussigné, Jérôme DUBOURGEOIS, agissant en qualité de Responsable R&D de la division santé de Christeyns France, certifie que les produits :

PHAGORUB GEL SPS – AL3235

et

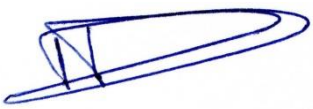
PHAGORUB SOLUTION SPS – DE528

ont un taux de matière active désinfectante identique.

Les essais d'efficacité et de tolérance réalisés sur le produit Phagorub gel SPS sont donc également valables pour la formule Phagorub solution SPS.

Fait à Nantes, le 11 mars 2014

Jérôme DUBOURGEOIS



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RAPPORT D'ESSAI N°3587-1

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Imprimé le : 01/04/15
Date de 1ère impression : 01/04/15

TEST D'EFFICACITE BACTERICIDE
SELON LA NORME NF EN 13727+A1 (Décembre 2013)
1592
Applications = Friction hygiénique des mains
Friction chirurgicale des mains
(Méthode par dilution/neutralisation)

DESTINATAIRE : CHRISTEYNS FRANCE

I- IDENTIFICATION DU DONNEUR D'ORDRE

Mr Jérôme DUBOURGEOIS
CHRISTEYNS FRANCE
31 rue de la Maladrie
44120 VERTOU
Tél. 02-40-80-27-27 - Fax. 02-40-03-09-73

II- IDENTIFICATION DE L'ECHANTILLON

- Nom du produit : **1592**
- Numéro de lot : PR288-F12
- Fabricant : CHRISTEYNS FRANCE
- Date de fabrication : 03/03/15
- Date de péremption : 03/03/18
- Date de réception au laboratoire : 23/03/15
- Aspect du produit : Liquide limpide incolore

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- 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

III- METHODE D'ESSAI

Norme NF EN 13727+A1 (Décembre 2013) : Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide en médecine. (Phase 2, Etape 1).

Application « Friction hygiénique des mains » : Réduction logarithmique au moins égale à 5 Log décimaux dans les conditions de l'essai.

Application « Friction chirurgicale des mains » : Réduction logarithmique au moins égale à 5 Log décimaux dans les conditions de l'essai.

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 26/03/15 au 30/03/15
- Analyse réalisée par : AF. GABILLET
- Diluant du produit utilisé au cours de l'essai : eau dure 30°f
- Concentrations de produit testé (V/V) : 20-40 et 80%
- Technique d'essai : dilution/neutralisation
- Aspect des dilutions : Opaques pour 20% et 40%, limpide pour 80%
- Stabilité du mélange substance interférente/dilutions du produit : absence de précipité au cours de l'essai
- Temps de contact : 30 secondes (+/-5 secondes)
- Température d'essai : 20°C (+/-1°C)

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- Substance interférente : 0,3 g/l d'albumine bovine (conditions de propreté)
- Température d'incubation : 37°C (+/-1°C)
- Identification des souches utilisées :
  Enterococcus hirae DSM 3320
  Staphylococcus aureus DSM 799
  Pseudomonas aeruginosa DSM 939
  Escherichia coli K12 CIP 54.117

V- RESULTATS D'ESSAI

Vc : nombre de colonies comptées sur les boîtes,
N: nombre d'UFC / ml dans la suspension microbienne d'essai,
N_0: nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de N,
Nv : nombre de cellules par ml de la suspension microbienne de validation,
Nv_0 : nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de Nv,
Nv_B : dans le cas du témoin de neutralisant B, il s'agit du nombre de cellules par ml après dilution au centième. Il représente un millième de Nv,
Na : nombre de survivants par ml dans le mélange d'essai à l'issue du temps de contact et avant neutralisation
A : nombre de survivants dans le témoin des conditions expérimentales,
B : nombre de survivants dans le témoin de neutralisant,
C : nombre de survivants dans le témoin de validation de la méthode,
R : réduction du nombre de cellules viables ($R=N_0/Na$) exprimé en logarithme

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Essai sur *Enterococcus hirae DSM 3320*

Souche testée	Suspension bactérienne d'essai	Essai de validation				
		Suspension bactérienne (NV)	Suspension bactérienne (NVB)	Conditions expérimentales (A)	Non toxicité du neutralisant (B)	Inactivation par dilution/neutralisation (C)
Enterococcus hirae DSM 3320 Lot 568	10^{-6} : Vc1 : 286 Vc2 : 302 10^{-7} : Vc1 :41 Vc2 : 42 N= 3,05.10^8 N_0= 3,05.10^7 Log N_0 = 7,48	Vc1 : 68 Vc2 : 79 (dilution 10^{-1}) Nv= 735 Nv_0= 74	Vc1 : 75 Vc2 : 88 (dilution 10^{-3}) NvB= 8,15.10^4	Vc1 : 53 Vc2 : 68 A= 61	Vc1 : 71 Vc2 : 69 B= 70	Vc1 : 49 Vc2 : 58 C= 54

L'essai est validé si :
N est compris entre 1,5.10^8 et 5.10^8 UFC/ml (8,17 ≤ lg N ≤ 8,70)
N_0 est compris entre 1,5.10^7 et 5.10^7 UFC/ml (7,17 ≤ lg N ≤ 7,70)
N_{v0} est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)
Nv_B est compris entre 3,0.10^4 et 1,6.10^5
A,B et C sont supérieurs ou égaux à 0,5xN_{v0}
B (dilution-neutralisation) est égal ou supérieur à 0,0005 x Nv_B
Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée	Concentrations testées % (V/V)									
		20%			40%			80%		
		10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
Enterococcus hirae DSM 3320 Lot 568	Vc1 Vc2	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	0 0	0 0	0 0
	Na	>3,3.10^5			>3,3.10^5			<140		
	lg Na	>5,52			>5,52			<2,15		
	Lg R	<1,96			<1,96			>5,33		

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Essai sur *Staphylococcus aureus DSM 799*

Souche testée	Suspension bactérienne d'essai	Essai de validation				
		Suspension bactérienne (NV)	Suspension bactérienne (NVB)	Conditions expérimentales (A)	Non toxicité du neutralisant (B)	Inactivation par dilution/neutralisation (C)
Staphylococcus aureus DSM 799 Lot 567	10^{-6} : Vc1 : 263 Vc2 : 234 10^{-7} : Vc1 : 27 Vc2 : 29 N= 2,51.10^8 N_0= 2,51.10^7 Log N_0 = 7,40	Vc1 : 52 Vc2 : 61 (dilution 10^{-1}) Nv= 565 Nv_0= 57	Vc1 : 48 Vc2 : 59 (dilution 10^{-3}) NvB= 5,35.10^4	Vc1 : 50 Vc2 : 54 A= 52	Vc1 : 53 Vc2 : 49 B= 51	Vc1 : 57 Vc2 : 61 C= 59
L'essai est validé si : *N est compris entre 1,5.10^8 et 5.10^8 UFC/ml (8,17 ≤ lg N ≤ 8,70)* *N_0 est compris entre 1,5.10^7 et 5.10^7 UFC/ml (7,17 ≤ lg N ≤ 7,70)* *N_{v0} est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)* *Nv_B est compris entre 3,0.10^4 et 1,6.10^5* *A,B et C sont supérieurs ou égaux à 0,5xN_{v0}* *B (dilution-neutralisation) est égal ou supérieur à 0,0005 x Nv_B* *Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15*						

Souche testée		Concentrations testées % (V/V)								
		20%			40%			80%		
		10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
Staphylococcus aureus DSM 799 Lot 567	Vc1 Vc2	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	0 0	0 0	0 0
	Na	>3,3.10^5			>3,3.10^5			<140		
	lg Na	>5,52			>5,52			<2,15		
	Lg R	<1,88			<1,88			>5,25		

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Escherichia coli K12 *CIP 54.117*

Souche testée	Suspension bactérienne d'essai	Essai de validation				
		Suspension bactérienne (NV)	Suspension bactérienne (NVB)	Conditions expérimentales (A)	Non toxicité du neutralisant (B)	Inactivation par dilution/neutralisation (C)
Escherichia coli CIP 54.117 Lot 323	10^{-6} : Vc1 : 224 Vc2 : 244 10^{-7} : Vc1 : 24 Vc2 : 20 N= 2,33.10^8 N_0= 2,33.10^7 Log N_0 = 7,37	Vc1 : 67 Vc2 : 42 (dilution 10^{-1}) Nv= 545 Nv_0= 55	Vc1 : 59 Vc2 : 54 (dilution 10^{-3}) NvB= 5,65.10^4	Vc1 : 41 Vc2 : 54 A= 48	Vc1 : 51 Vc2 : 47 B= 49	Vc1 : 56 Vc2 : 60 C= 58

L'essai est validé si :
N est compris entre 1,5.10^8 et 5.10^8 UFC/ml (8,17 ≤ lg N ≤ 8,70)
N_0 est compris entre 1,5.10^7 et 5.10^7 UFC/ml (7,17 ≤ lg N ≤ 7,70)
Nv_0 est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)
Nv_B est compris entre 3,0.10^4 et 1,6.10^5
A,B et C sont supérieurs ou égaux à 0,5xN_{v0}
B (dilution-neutralisation) est égal ou supérieur à 0,0005 x Nv_B
Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée		Concentrations testées % (V/V)								
		20%			40%			80%		
		10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
Escherichia coli CIP 54.117 Lot 323	Vc1	>330	>330	>330	>330	99	18	0	0	0
	Vc2	>330	>330	>330	>330	78	16	0	0	0
	Na	>3,3.10^5			9,59.10^3			<140		
	lg Na	>5,52			3,98			<2,15		
	Lg R	<1,85			3,39			>5,22		

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Pseudomonas aeruginosa DSM 939

Souche testée	Suspension bactérienne d'essai	Essai de validation				
		Suspension bactérienne (NV)	Suspension bactérienne (NVB)	Conditions expérimentales (A)	Non toxicité du neutralisant (B)	Inactivation par dilution/neutralisation (C)
Pseudomonas aeruginosa DSM 939 Lot 556	10^{-6} : Vc1 : 281 Vc2 : 263 10^{-7} : Vc1 : 27 Vc2 : 32 N= 2,74.10^8 N_0= 2,74.10^7 Log N_0 = 7,44	Vc1 : 89 Vc2 : 81 (dilution 10^{-1}) Nv= 850 Nv_0= 85	Vc1 : 100 Vc2 : 107 (dilution 10^{-3}) NvB= 1,04.10^5	Vc1 : 58 Vc2 : 73 A= 66	Vc1 : 76 Vc2 : 88 B= 82	Vc1 : 106 Vc2 : 118 C= 112

L'essai est validé si :
N est compris entre 1,5.10^8 et 5.10^8 UFC/ml (8,17 ≤ lg N ≤ 8,70)
N_0 est compris entre 1,5.10^7 et 5.10^7 UFC/ml (7,17 ≤ lg N ≤ 7,70)
N_{v0} est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)
Nv_B est compris entre 3,0.10^4 et 1,6.10^5
A,B et C sont supérieurs ou égaux à 0,5xN_{v0}
B (dilution-neutralisation) est égal ou supérieur à 0,0005 x Nv_B
Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée	Concentrations testées % (V/V)									
		20%			40%			80%		
Pseudomonas aeruginosa DSM 939 Lot 556		10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
	Vc1 Vc2	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	>330 >330	0 0	0 0	0 0
	Na	>3,3.10^5			>3,3.10^5			<140		
	lg Na	>5,52			>5,52			<2,15		
	Lg R	<1,92			<1,92			>5,29		

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VI- CONCLUSION

- Application « Friction hygiénique des mains » : Réduction logarithmique au moins égale à 5 Log décimaux dans les conditions de l'essai.

Conformément à la norme NF EN 13727 + A1 (Décembre 2013), le lot PR288-F12 du produit 1592 de la société CHRISTEYNS FRANCE, dans les conditions d'essai suivantes :
- en 30 secondes de temps de contact,
- à la température de 20°C,
- en présence d'albumine bovine à 0,3 g/l (conditions de propreté),

présente une activité bactéricide (réduction supérieure à 5 log décimaux dans le cas de l'application « Friction hygiénique des mains »), lorsqu'il est dilué à 80% (V/V), vis-à-vis des souches *Enterococcus hirae* DSM 3320, *Staphylococcus aureus* DSM 799, *Escherichia coli K12 CIP 54.117* et *Pseudomonas aeruginosa DSM 939.*

- Application « Friction chirurgicale des mains » : Réduction logarithmique au moins égale à 5 Log décimaux dans les conditions de l'essai.

Selon la méthodologie de la norme NF EN 13727 + A1 (Décembre 2013), le lot PR288-F12 du produit 1592 de la société CHRISTEYNS FRANCE, dans les conditions d'essai suivantes :
- en 30 secondes de temps de contact,
- à la température de 20°C,
- en présence d'albumine bovine à 0,3 g/l (conditions de propreté),

présente une activité bactéricide (réduction supérieure à 5 log décimaux dans le cas de l'application « Friction chirurgicale des mains »), lorsqu'il est dilué à 80% (V/V), vis-à-vis des souches *Enterococcus hirae* DSM 3320, *Staphylococcus aureus* DSM 799, *Escherichia coli* K12 CIP 54.117 et *Pseudomonas aeruginosa DSM 939.*

Les souches sont conservées et contrôlées selon la norme NF EN 12353.
Les souches d'essai ont été soumises à essai une seule fois.

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VII-SIGNATURES

Fait à DINARD,

Rédigé par	Validé par
AF . GABILLET Responsable d'essai	M.SESQUES Docteur en microbiologie Directeur technique

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DAkkS Deutsche Akkreditierungsstelle
D-PL-18411-01-01
D-PL-18411-01-02
HYGIENE NORD GMBH

HYGIENE NORD GMBH, c/o BIOTECHNIKUM, W.-RATHENAU-STR. 49 A, D-17489 GREIFSWALD

Christeyns France
31 rue de la Maladrie
44120 VERTOU
France

CUSTOMER NUMBER
815

DATE
March 27, 2015

REPORT A 15085
PHAGORUB SOLUTION SPS
HYGIENIC HANDRUB (EN 1500)

Purpose

The activity of the hygienic handrub product **Phagorub Solution SPS** (Christeyns France, Vertou, France) should be evaluated by a test simulating practical conditions according to EN 1500 (2013).

HYGIENE NORD GMBH
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BLZ: 13070024 KONTO: 224551200
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S.W.I.F.T./BIC: DEUTDEDBROS
GESCHÄFTSFÜHRER:
DIPL.BIOL. TORSTEN KOBURGER
SITZ DER GESELLSCHAFT:
GREIFSWALD
HANDELSREGISTER:
AG STRALSUND HRB 5168
UST.-ID (VAT):
DE211580653

Test Description

Manufacturer:	Christeyns France, Vertou, France
Product:	**Phagorub Solution SPS** Hygienic hand antiseptic
Sample number:	P 151167
Batch number:	PR195-F13
Manufacture date:	March 20, 2015
Best before:	not provided
Date of order:	March 23, 2015
Date of delivery:	March 23, 2015
Test date:	March 26, 2015 – March 27, 2015
Basis:	EN 1500 (2013): Chemical disinfectants and antiseptics – Hygienic handrub – Test method and requirements (phase 2/step 2)

Test organism:	*Escherichia coli* K 12 NCTC 10538
Test solutions:	100 %
Active ingredients in 100 g:	not provided
Odour:	alcoholic
Appearance:	colourless clear liquid
Appearance of dilution:	10 %: colourless clear liquid, white flocculation
pH – value (pH-meter)	100 %: 9.10 10 %: 7.68 WSH: 7.20
pH – value (pH-stripes)	100 %: 5 10 %: 7
Neutralizer:	4 % Tween 80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS (Neutralizer XXIV)
Test temperature:	20 ± 1 °C
Incubation temperature:	36 ± 1 °C

Method

Hygienic handrub - Evaluation of the activity of Phagorub Solution SPS in a test simulating practical conditions in accordance with EN 1500 (2013)

The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic handrub. A cross-over design was used for testing the activity of the test product in both parts of this study. The efficacy of the product formulation **Phagorub Solution SPS** was analysed using the test organism *E. coli* K12. A sufficient amount of the test product was applied to keep the subjects' hands well moistened with a volume of 3 ml for a contact time of **30 s**

Contamination fluid: $4.80*10^8$ cfu / ml

Prevalues. Hands were prepared by washing for 1 min with soft soap to remove natural transients. After thoroughly drying the hands with paper towels they were immersed in the contamination fluid up to the mid-metacarpals for 5 seconds. Avoiding the formation of droplets, hands were allowed to dry in the air for 3 minutes. Immediately after drying, the fingertips were rubbed for 1 min on the base of a Petri dish containing 10 ml of TSB without neutralizer to assess the release of test organisms before treatment of the hands (prevalues). For each of the required dilutions (10^{-3} and 10^{-4}) of these sampling fluids 0.1 ml were spread on the surface of TSA plates.

Postvalues. Immediately after sampling for the prevalues, the hygienic handrub procedure was performed with 2 x 3 ml of the reference product propan-2-ol (60 %) in 2 x 30 s by the first group, and keeping hands moist with **3 ml** of the test product formulation **Phagorub Solution SPS** for **30 s** by the second group, respectively, and switching groups afterwards. The reference, as well as the test procedure was completed by a 5 s rinse of the fingers under running tap water. Excess water was shaken of, after that fingertips were rubbed for 1 min on the base of a Petri dish containing 10 ml of TSB with neutralizer for 1 min. Appropriate volumes and dilutions of those sampling fluids (1 ml of the 10^0, and 0.1 ml of the 10^0 and 10^{-1} dilutions) were spread on the surface of TSA plates.

Plates were incubated aerobically at 36 ± 1 °C for 18 to 24 hours. The number of colony forming units per plate and dilution step was recorded. The viable counts per millilitre sampling fluid were calculated and transformed to decimal logarithms. From the difference of the mean log prevalues and the mean log postvalues of both hands a log reduction factor is established for each subject. The arithmetic means of all individual log reduction factors are calculated and compared for both the reference and the test procedure.

For the test validation, conformance of the results to the following criteria is required:

1. All results of at least 18 subjects shall be available.
2. The overall mean of the log prevalues for the reference and test procedure shall be at least 5.00.
3. In each procedure, not more than three individual log reduction factors fewer than 3.00 shall occur.
4. The absolute difference of mean differences between log reductions of RP and PP of group RP → PP and group PP → RP shall be less than 2.00.
5. The quotient of the cfu numbers of consecutive dilutions of the sampling fluids must not be < 5 or > 15, within the relevant range of 14 – 330 cfu.

- However, this criterion was only considered, if overall data indicated systematic errors or neutralization problems - as any other variations is taken care of by the weighted mean calculation already.

Results

Detailed results are presented in table 2. The results are valid, because the followings requirements according EN 1500 were fulfilled:

- all results of at least 18 volunteers are available
- the overall mean of the log prevalues for reference and test procedure is ≥ 5.00
- in each procedure (reference and test product) less than three individual log reduction factors are smaller than 3.00
- the absolute difference of mean differences between log reductions of RP and PP of group RP $\rightarrow$ PP and group PP $\rightarrow$ RP is be less than 2.00
- no systematic errors were detected by the calculation of the quotients of cfu numbers of consecutive dilutions of the sampling fluids.

For the test organism *E. coli*, the overall mean values of the reference product are:

prevalue	**5.80**
postvalue	**1.30**
reduction factor (RF)	**4.49**

The overall mean values of the test product formulation **Phagorub Solution SPS** at a contact time of **30 s** are:

prevalue	**5.88**
postvalue	**1.58**
reduction factor (RF)	**4.31**

The mean log reduction (RF) for the test product **(Phagorub Solution SPS)** is smaller than that of the reference product. According to the EN 1500 (2013), the test product was considered non inferior to the reference product in the Hodges-Lehman statistical analysis (see Table 4; p = 0.025, agreed inferiority margin = 0.6; calculated value = 0.42).

The test product formulation **Phagorub Solution SPS** does therefore **correspond** to the requirements of the EN 1500 (2013) for the **hygienic handrub** when hands are kept moist with 3 ml of the product for a contact time of **30 s**.

Greifswald, March 27, 2015

Dr rer. med. (Dipl. Biol.) T. Koburger
- General Manager -

Prof. Dr. med. A. Kramer
- MD for Hygiene and Environmental Medicine -

Table 1: Neutralization–control and validation (EN 1500:2013)

Date:	March 27, 2015	**Order number:**	A 15085
Product:	**Phagorub Solution SPS**	**Sample number:**	P 151167
Test organism:	*E. coli*	**Batch number:**	PR195-F13
Interfering substance:	none		
Incubation temperature:	36 ± 1 °C	**Neutralizer:**	II
Test suspension (N):	$4.80*10^8$ cfu / ml	**Incubation time:**	24 h – 48 h
Test suspension (Nv_B):	$1.25*10^5$ cfu / ml	**Test temperature**	20 ± 1 °C
Validation Suspension (N_V):	$1.58*10^3$ cfu / ml		

Neutralization – control and validation:

Validation suspension (Nv0)				
	cfu / Plate 1 & 2		V_c	$\bar{x}$ [cfu / ml]
V_{c1}	86	75	161	**158**
V_{c2}	72	83	155	
$30 \leq \bar{x}$ of $Nv_0 \leq 160$?				
[X] Yes [ ] No				

Neutralizer control (control B)				
	cfu / Plate 1 & 2		V_c	$\bar{x}$ [cfu / ml]
V_{c1}	69	76	145	**141.5**
V_{c2}	70	68	138	
$\bar{x}$ of B is $\geq 0.0005*$ $\bar{x}$ of Nv_B?				
[X] Yes [ ] No				

Method validation (Control C) 80 %				
	cfu / Plate 1 & 2		V_c	$\bar{x}$ [cfu / ml]
V_{c1}	83	88	171	**166.5**
V_{c2}	82	80	162	
$\bar{x}$ of C is $\geq 0.5*$ $\bar{x}$ of Nv_0?				
[X] Yes [ ] No				

* OK with regard to the increased bacterial count of the test suspension

Method validation (Control C) 10 %				
	cfu / Plate 1 & 2		V_c	$\bar{x}$ [cfu / ml]
V_{c1}	78	94	172	**167**
V_{c2}	73	89	162	
$\bar{x}$ of C is $\geq 0.5*$ $\bar{x}$ of Nv_0?				
[X] Yes [ ] No				

Control of group of subjects (EN 1500:2013)

Group 1 (RP → PP) subjects 1 - 10			Group 2 (PP → RP) subjects 11 - 20			Absolute difference of mean differences
mean $\log_{10}$ RF		difference	mean $\log_{10}$ RF		difference	
RP	PP		RP	PP		**abs (0.04 – 0.33) = 0.29**
4.52	4.48	0.04	4.47	4.14	0.33	
						result < 2.00 ?
						[X] Yes [ ] No

Table 2.1: Hygienic handrub – test results – reference product vs. test product

test date:	March 27, 2015	**order number:**	A 15085
reference:	60 % propan-2-ol	**batch number:**	**093192228**
test product:	**Phagorub Solution SPS**	**batch number:**	**PR195-F13**
neutralizer:	II	**sample number:**	P 151167

subject	hand	reference product					test product				
		prevalue [cfu]		postvalue [cfu]			prevalue [cfu]		postvalue [cfu]		
		0.1 ml 10^{-3}	0.1 ml 10^{-4}	1 ml 10^{0}	0.1 ml 10^{0}	0.1 ml 10^{-1}	0.1 ml 10^{-3}	0.1 ml 10^{-4}	1 ml 10^{0}	0.1 ml 10^{0}	0.1 ml 10^{-1}
1	right	152	26	12	4	0	247	24	22	5	0
	left	187	28	78	4	0	222	21	> 330	88	11
2	right	28	6	16	1	0	68	13	36	2	0
	left	55	9	10	0	0	102	7	32	5	0
3	right	84	22	130	17	0	93	8	202	16	1
	left	23	5	38	6	1	91	5	48	3	2
4	right	16	1	216	24	4	1	1	86	6	1
	left	20	1	20	1	0	9	1	0	0	0
5	right	76	14	2	0	0	76	15	4	0	0
	left	58	8	6	1	0	64	4	4	1	0
6	right	174	43	0	0	0	141	14	0	0	0
	left	146	15	0	0	0	138	13	0	0	0
7	right	115	12	0	0	0	138	12	0	0	0
	left	105	23	0	0	0	87	2	14	0	0
8	right	97	5	74	6	0	161	14	14	2	0
	left	58	5	0	0	0	136	18	0	0	0
9	right	54	8	224	23	2	82	4	110	8	0
	left	72	6	26	1	0	95	9	108	17	1
10	right	12	1	> 330	62	4	29	4	> 330	102	13
	left	9	0	> 330	59	8	24	2	> 330	181	19
11	right	171	32	2	0	0	> 330	54	> 330	49	11
	left	127	21	22	1	0	> 330	46	> 330	46	2
12	right	65	10	8	0	0	84	4	66	10	0
	left	70	4	16	1	0	117	17	36	5	0
13	right	132	27	0	0	0	96	2	20	2	0
	left	120	22	12	0	0	51	9	4	0	0
14	right	64	10	26	6	0	148	15	> 330	42	7
	left	69	11	62	5	1	110	8	42	5	0
15	right	35	2	26	2	0	76	15	4	0	0
	left	51	4	8	1	0	64	4	4	1	0
16	right	50	8	10	2	0	62	4	64	6	0
	left	44	8	42	3	0	57	4	16	2	0
17	right	105	25	> 330	38	2	124	19	> 330	> 330	32
	left	133	8	> 330	129	21	162	12	> 330	> 330	33
18	right	> 330	40	92	11	0	317	29	> 330	88	14
	left	141	19	24	2	0	226	18	> 330	41	5
19	right	59	8	6	0	0	27	5	12	1	0
	left	45	5	2	0	0	25	6	0	0	0
20	right	20	1	140	18	1	21	2	34	5	0
	left	3	0	108	10	1	6	0	38	6	0

Table 2.2: Hygienic hand rub – test results (logarithms and reduction factors) reference product vs. test product

test product: Phagorub Solution SPS
reference: 60 % propan-2-ol
sample number: P 151167
order number: A 15085

subject	hand	reference product					test product				
		prevalue log		postvalue log		reduc-tion-factors	prevalue log		postvalue log		reduc-tion-factors
		R/L	mean	R/L	mean		R/L	mean	R/L	mean	
1	R	6.21	6.25	1.08	1.49	**4.76**	6.39	6.37	1.34	2.14	**4.22**
	L	6.29		1.89			6.34		2.94		
2	R	5.45	5.59	1.20	1.10	**4.49**	5.83	5.92	1.56	1.53	**4.39**
	L	5.74		1.00			6.01		1.51		
3	R	5.98	5.67	2.13	1.85	**3.82**	5.97	5.96	2.30	1.99	**3.97**
	L	5.36		1.58			5.96		1.68		
4	R	5.20	5.25	2.34	1.82	**3.43**	4.00	4.48	1.93	0.97	**3.51**
	L	5.30		1.30			4.95		0.00		
5	R	5.91	5.84	0.30	0.54	**5.30**	5.92	5.86	0.60	0.60	**5.26**
	L	5.76		0.78			5.81		0.60		
6	R	6.30	6.23	0.00	0.00	**6.23**	6.15	6.14	0.00	0.00	**6.14**
	L	6.17		0.00			6.14		0.00		
7	R	6.06	6.06	0.00	0.00	**6.06**	6.14	6.04	0.00	0.57	**5.47**
	L	6.07		0.00			5.94		1.15		
8	R	5.99	5.88	1.87	0.93	**4.94**	6.20	6.17	1.15	0.57	**5.60**
	L	5.76		0.00			6.15		0.00		
9	R	5.73	5.79	2.35	1.88	**3.91**	5.91	5.95	2.04	2.05	**3.90**
	L	5.86		1.41			5.98		2.06		
10	R	5.08	5.02	2.79	2.78	**2.24**	5.46	5.42	3.01	3.13	**2.29**
	L	4.95		2.77			5.38		3.26		
11	R	6.27	6.20	0.30	0.82	**5.38**	6.73	6.70	2.69	2.68	**4.02**
	L	6.13		1.34			6.66		2.66		
12	R	5.81	5.83	0.90	1.05	**4.78**	5.92	6.00	1.82	1.69	**4.32**
	L	5.85		1.20			6.09		1.56		
13	R	6.16	6.14	0.00	0.54	**5.60**	5.98	5.84	1.30	0.95	**4.89**
	L	6.11		1.08			5.71		0.60		
14	R	5.81	5.82	1.41	1.60	**4.22**	6.17	6.11	2.62	2.12	**3.98**
	L	5.84		1.79			6.04		1.62		
15	R	5.54	5.63	1.41	1.16	**4.47**	5.92	5.86	0.60	0.60	**5.26**
	L	5.71		0.90			5.81		0.60		
16	R	5.70	5.67	1.00	1.31	**4.36**	5.79	5.77	1.81	1.51	**4.27**
	L	5.64		1.62			5.76		1.20		
17	R	6.07	6.10	2.58	2.86	**3.24**	6.11	6.16	3.51	3.51	**2.65**
	L	6.12		3.13			6.21		3.52		
18	R	6.60	6.38	1.96	1.67	**4.71**	6.50	6.42	2.97	2.79	**3.63**
	L	6.16		1.38			6.35		2.61		
19	R	5.77	5.71	0.78	0.54	**5.17**	5.43	5.41	1.08	0.54	**4.88**
	L	5.65		0.30			5.40		0.00		
20	R	5.30	4.89	2.16	2.10	**2.79**	5.32	5.05	1.53	1.56	**3.49**
	L	4.48		2.03			4.78		1.58		
arithm. mean		5.80		1.30		**4.49**	5.88		1.58		**4.31**
absolute SD		0.40		0.80		1.04	0.49		0.98		0.97
relative SD		6.88		61.09		23.14	8.40		62.00		22.56

SD standard deviation R right L left

Table 3: Hygienic handrub – used volumes for keeping hands moist during contact time reference product vs. test product

reference product: Propan-2-ol 60 vol.%
test product: Phagorub Solution SPS

order number: A 15085
sample number: P 151167

subject	reference product [ml]	test product [ml]
1	6.0	3.0
2	6.0	3.0
3	6.0	3.0
4	6.0	3.0
5	6.0	3.0
6	6.0	3.0
7	6.0	3.0
8	6.0	3.0
9	6.0	3.0
10	6.0	3.0
11	6.0	3.0
12	6.0	3.0
13	6.0	3.0
14	6.0	3.0
15	6.0	3.0
16	6.0	3.0
17	6.0	3.0
18	6.0	3.0
19	6.0	3.0
20	6.0	3.0

Table 4: Sorting of individual differences and computation for Hodges-Lehmann 97.5 % upper confidence limits (EN 1500:2013)

Median of differences RP – PP: 0.10

Subject		Mean pairwise differences $(d_i+d_{ii})/2$ (no duplicates. only values ≥median)										
		11	18	13	17	7	1	12	19	14	2	6
	RP-PP	**1.36**	**1.08**	**0.71**	**0.59**	**0.59**	**0.54**	**0.46**	**0.29**	**0.24**	**0.10**	**0.09**
11	1.36	1.36										
18	1.08	1.22	1.08									
13	0.71	1.04	0.90	0.71								
17	0.59	0.98	0.84	0.65	0.59							
7	0.59	0.98	0.84	0.65	0.59	0.59						
1	0.54	0.95	0.81	0.63	0.57	0.57	0.54					
12	0.46	0.91	0.77	0.59	0.53	0.53	0.50	0.46				
19	0.29	0.83	0.69	0.50	0.44	0.44	**0.42**[53]	0.38	0.29			
14	0.24	0.80	0.66	0.48	0.42	0.42	0.39	0.35	0.27	0.24		
2	**0.10**	0.73	0.59	0.41	0.35	0.35	0.32	0.28	0.20	0.17	0.10	
6	**0.09**	0.73	0.59	0.40	0.34	0.34	0.32	0.28	0.19	0.17	0.10	
16	0.09	0.73	0.59	0.40	0.34	0.34	0.32	0.28	0.19	0.17	0.10	
5	0.04	0.70	0.56	0.38	0.32	0.32	0.29	0.25	0.17	0.14		
9	0.01	0.69	0.55	0.36	0.30	0.30	0.28	0.24	0.15	0.13		
10	-0.05	0.66	0.52	0.33	0.27	0.27	0.25	0.21	0.12	0.10		
4	-0.08	0.64	0.50	0.32	0.26	0.26	0.23	0.19	0.11			
3	-0.15	0.61	0.47	0.28	0.22	0.22	0.20	0.16				
8	-0.66	0.35	0.21									
20	-0.70	0.33	0.19									
15	-0.79	0.29	0.15									

The tabulated value for the smaller sum of ranks for n = 20 in the Wilcoxon Signed Ranks Test is 52 at a significance level of p = 0.025 in the directional test (1-tailed). Therefore, the upper confidence limit (97.5 %) is determined by the mean pairwise difference of the differences RP-PP with the rank 53 (c = 52+1 = **53**). With **0.42**, this value is smaller than the agreed inferiority margin of 0.6. Therefore, the hypothesis of inferiority of the test product (PP) can be rejected and it can be concluded that the test preparation PP is not inferior to RP.

Legend:

MW	=	mean
x	=	mean
RF	=	Reduction factor
> 330	=	not countable
n.d.	=	not determined
WSH	=	hard water (water of standardized hardness)
DGHM	=	Deutsche Gesellschaft für Hygiene und Mikrobiologie
cfu	=	colony forming unit

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ÖNORM EN ISO 17025

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Bischofshofen, 2016-07-17

Prüfbericht / *test report* B 20234

Labor-Nr. / *Identification of the test laboratory:*	B 20234
Prüfprodukt / *Test product*:	1592
Chargen-Bez. / *Batch number*:	LOT: PR195-F5
Hersteller / *Manufacturer*:	Christeyns
Auftragsdatum / *Date of order*:	2016-04-25
Materialeingang / *Date of delivery*:	2016-05-02
Lagerbedingungen / *storage conditions*:	gemäß Herstellerangaben / *those of the manufacturer*
Vom Hersteller empfohlenes Verdünnungsmittel / *product diluent recommended by the manufacturer for use*:	konzentrierte Anwendung / *concentrated application*
Aussehen / *Appearance*:	klare, farblose, viskose Flüssigkeit / *clear colourless viscous liquid*
Geruch / *Odour*:	alkoholisch / *alcoholic*
Methodik / *Method*:	**EN 12791 (2016) Chirurgische Händedesinfektion – Prüfverfahren und Anforderungen (Phase 2. Stufe 2) /** ***EN 12791 (2016) Surgical hand disinfection – Test method and requirements (phase2. step2)*** **SOP 02-054**

pH-Werte / *pH-values*:	100%: 8.20
Neutralisationsmittel / *Neutralizer*:	3,0 % Tween 80 + 3,0 % Saponin + 0,1 % Histidin + 0,1 % Cystein / *3.0 % polysorbate 80 + 3.0 % saponine + 0.1 % histidine + 0.1 % cysteine*
Prüfzeitraum / *Period of analysis*:	2016-06-01 to 2016-07-06
Wirkstoff(e) laut Herstellerangabe / *Active ingredient(s)*:	in 100ml: Not declared

Prüfung der Eignung für die Chirurgische Händedesinfektion nach EN 12791
Testing of Surgical hand rub product - EN 12791

Prüfdatum / *Date of test*: 2016-06-01; 2016-06-08; 2016-06-15; 2016-06-22; 2016-06-29; 2016-07-06

Referenzverfahren / Reference procedure:

Portionsweise 3 ml 60% (v/v) Propan-1-ol während 3 Minuten auf den Händen verreiben
Aliquots of 3 ml 60% (v/v) Propan-1-ol rubbed on the hand during 3 minutes.

Prüfverfahren / *Test procedure*:

3 ml von 1592 während 45 Sekunden auf den Händen verreiben, weitere 3 ml während 45 Sekunden auf den Händen verreiben (Gesamt 6ml, 90 Sekunden).
Rub 3ml of 1592 onto the hands for 45 seconds, rub further 3ml onto the hands for 45 seconds (total 6ml, 90 seconds).

Ergebnisse der Validierung der Neutralisation / *Results of validation of neutralisation*

Testdatum / *Date of test*: 2016-06-01 B 20234

Konzentration / *Concentration*: 80% (v/v) Endkonzentration / *end concentration*

Belastung / *load*: 0,3 g/l Rinderalbumin (niedrige Belastung) / *0.3 g/l bovine albumin (clean conditions)*

Aussehen der Produktverdünnung / *Appearance of product dilutions*: klar / clear

Einwirkungszeit / *Contact time*: 90 Sekunden / *seconds*

Testkeim / *Test strain*: S. aureus

Validierungs-suspension / *validation suspension* (Nv & Nv_0)	Kontrolle / *control* (Nv_B) x1000	Kontrolle / *control* (B)	Kontrolle / *control* (C)	Kontrolle / *control* (A)
Vc: 49 56	Vc: 57 53	Vc: 39 40	Vc: 28 25	Vc: 57 56
Nv_0: 52.5	NvB: 55	B: 39.5	C: 26.5	C: 56.5
Nv: 525				
Ergebnis gültig / *result valid*: ja / yes	**ja / yes**	**ja / yes**	**ja / yes**	**ja / yes**

Testkeim / *Test strain*: E. hirae

Validierungs-suspension / *validation suspension* (Nv & Nv_0)	Kontrolle / *control* (Nv_B) x1000	Kontrolle / *control* (B)	Kontrolle / *control* (C)	Kontrolle / *control* (A)
Vc: 60 53	Vc: 47 52	Vc: 34 42	Vc: 48 52	Vc: 65 55
Nv_0: 56.5	NvB: 49.5	B: 38	C: 49	C: 60
Nv: 565				
Ergebnis gültig / *result valid*: ja / yes	**ja / yes**	**ja / yes**	**ja / yes**	**ja / yes**

Testkeim / *Test strain*: E. coli

Validierungs-suspension / *validation suspension* (Nv & Nv_0)	Kontrolle / *control* (Nv_B) x1000	Kontrolle / *control* (B)	Kontrolle / *control* (C)	Kontrolle / *control* (A)
Vc: 53 58	Vc: 38 37	Vc: 33 38	Vc: 58 59	Vc: 66 62
Nv_0: 55.5	NvB: 37.5	B: 35.5	C: 58.5	C: 64
Nv: 555				
Ergebnis gültig / *result valid*: ja / yes	**ja / yes**	**ja / yes**	**ja / yes**	**ja / yes**

Testkeim / *Test strain*: P. aeruginosa

Validierungs-suspension / *validation suspension* (Nv & Nv_0)	Kontrolle / *control* (Nv_B) x1000	Kontrolle / *control* (B)	Kontrolle / *control* (C)	Kontrolle / *control* (A)
Vc: 52 44	Vc: 53 51	Vc: 46 42	Vc: 52 45	Vc: 51 45
Nv_0: 48	NvB: 52	B: 44	C: 48.5	C: 48
Nv: 480				
Ergebnis gültig / *result valid*: ja / yes	**ja / yes**	**ja / yes**	**ja / yes**	**ja / yes**

Testkeim / *Test strain*: C. albicans

Validierungs-suspension / *validation suspension* (Nv & Nv_0)	Kontrolle / *control* (Nv_B) x1000	Kontrolle / *control* (B)	Kontrolle / *control* (C)	Kontrolle / *control* (A)
Vc: 44 38	Vc: 56 53	Vc: 36 42	Vc: 42 34	Vc: 35 43
Nv_0: 41	NvB: 54.5	B: 39	C: 38	C: 39
Nv: 410				
Ergebnis gültig / *result valid*: ja / yes	**ja / yes**	**ja / yes**	**ja / yes**	**ja / yes**

Verifizierung / *Verification*

Nv ist zwischen / *is between* 3×10^2 and 1.6×10^3

Nv_0 ist zwischen / *is between* 30 and 160 (3×10^1 and 1.6×10^2)

A, B,C ist gleich oder größer als / *is equal to or greater than* 0.5 mal / *times* N_{v0}

Nv_B ist zwischen / *is between* 3×10^4 and 1.6×10^5

Legende / *Legend*

Vc = Lebendkeimzahl / *viable count*

Nv = Anzahl der KBE/ml in der Validierungsuspension / *number of cfu/ml in the validation suspension*

N_{v0}, = Anzahl der KBE/ml in den Prüfgemischen B und C zu Beginn der Einwirkzeit / *number of cfu/ml in the mixtures B and C at the beginning of the contact time*

N_{vB} = Im Falle der Neutralisationskontrolle B (Verdünnungs-Neutralisation) die Anzahl der KBE/ml nach 100- facher Verdünnung. Nv_0 ist 1/10 im Bezug auf die Validierungssuspension Nv, im Falle von Nv_B 1/1000. / *In the case of neutralizer control B (dilution neutralisation method) it is the number of cells per ml after 100 fold dilution. Nv_0 is one-tenth of the mean of the Vc values of validationsuspension Nv taken into account, in case of Nv_B it is one thousandth.*

A = Anzahl der überlebenden Zellen in der Kontrolle der Prüfbedingungen am Ende der Einwirkzeit. Sie entspricht dem Mittelwert der berücksichtigten Vc- Werte des Gemisches. / *Number of survivors of the experimental conditions control at the end of the contact time. It corresponds of the mean of the Vc-values of the mixture taken into account.*

B = Anzahl der überlebenden Zellen in der Kontrolle der Neutralisation in der definierten Zeit von 5 Minuten (im Falle von Produkten mit einer Einwirkzeit von ≤ 10 min nur 10 Sekunden) oder der Kontrolle der Filtration / *number of survivors in the neutralizer control at the defined times 5 minutes (in the case of products with a contact time of ≤ 10 min only 10 seconds) or the filtration control*

C = Anzahl der überlebenden Zellen in der Methodenvalidierung in der definierten Zeit von 30 Minuten / *number of survivors in the method validation at the defined times 30 minutes*

Prüfung der Eignung für die Chirurgische Händedesinfektion nach EN 12791
Reference surgical hand disinfection procedure – Experimental results - EN 12791

Versuchsdaten / *dates of experiments*: 2016-06-01; 2016-06-08; 2016-06-15; 2016-06-22; 2016-06-29; 2016-07-06
Anwendung / *application*: 3 min. Einreiben / 3 *min rub*
Nx: 4/4/3/4/3/3/4/3/3/4/3/3/3/3/3/3/5/3/3/3/4/3/3/3

Proband / subject		Anzahl KBE je Platte aus Verdünnung $10^{(x)}$ - Referenz / *Number of cfu per plate from dilution $10^{(x)}$ - Reference*								
Nr./ no.	Hand / hand	Vorwert / *pre-count*			Nachwert / *post-count*					
					Sofortwirkung / *immediate*			3-Stundenwirkung / *3-hours*		
		-1	-2	-3	0	-1	-2	0	-1	-2
1	links / left	>330	>330	137	>330	162	17			
	rechts / right	>330	>330	296				>330	>330	53
2	links / left	>330	>330	228	51	5	0			
	rechts / right	>330	>330	85				49	4	0
3	links / left	>330	>330	33	>330	63	6			
	rechts / right	>330	197	20				>330	>330	84
4	links / left	>330	>330	272				>330	>330	66
	rechts / right	>330	>330	59	>330	73	7			
5	links / left	>330	>330	128	>330	83	8			
	rechts / right	>330	>330	70				>330	213	21
6	links / left	>330	>330	265				>330	>330	104
	rechts / right	>330	>330	229	>330	49	4			
7	links / left	>330	>330	219	>330	>330	120			
	rechts / right	>330	>330	149				>330	>330	89
8	links / left	>330	>330	259				>330	>330	70
	rechts / right	>330	>330	237	>330	>330	142			
9	links / left	>330	170	17	>330	81	8			
	rechts / right	>330	>330	84				>330	>330	58
10	links / left	>330	>330	77				>330	>330	49
	rechts / right	>330	>330	61	>330	116	11			
11	links / left	>330	>330	166				>330	>330	69
	rechts / right	>330	>330	159	144	15	1			
12	links / left	>330	>330	292	>330	112	12			
	rechts / right	>330	>330	289				92	9	0
13	links / left	>330	>330	59				>330	44	4
	rechts / right	>330	>330	95	185	18	1			
14	links / left	>330	>330	68	273	28	2			
	rechts / right	>330	>330	55				23	2	0
15	links / left	>330	161	16				>330	>330	68
	rechts / right	>330	230	23	75	8	1			
16	links / left	320	243	24	4	0	0			
	rechts / right	>330	223	23				88	9	1
17	links / left	>330	>330	53				89	9	1
	rechts / right	>330	>330	37	121	12	1			
18	links / left	>330	>330	84	>330	>330	106			
	rechts / right	>330	>330	119				>330	>330	266
19	links / left	>330	>330	280	>330	>330	58			

	rechts / right	>330	>330	269				>330	258	25
20	links / left	>330	>330	276				>330	81	8
	rechts / right	>330	>330	284	>330	>330	96			
21	links / left	>330	203	20	>330	166	17			
	rechts / right	>330	130	13				>330	>330	33
22	links / left	>330	>330	64				>330	>330	149
	rechts / right	>330	>330	48	>330	>330	208			
23	links / left	178	18	1	13	1	0			
	rechts / right	>330	>330	143				>330	91	9
24	links / left	>330	>330	97				271	27	2
	rechts / right	>330	>330	136	271	27	3			

Nx = Anzahl der 3ml- Portionen pro Proband / *3 ml portions used during procedure per subject*
__ = zur weiteren Berechnung verwendete Werte / *count used for further computation*

Chirurgische Händedesinfektion mit 1592 – Versuchsergebnisse
Surgical hand disinfection procedure with 1592 – Experimental results

Produkt / *product*: 1592

Versuchsdaten / *dates of experiments*: 2016-06-01; 2016-06-08; 2016-06-15; 2016-06-22; 2016-06-29; 2016-07-06

Anwendung / *application*: 3 ml von 1592 während 45 Sekunden auf den Händen verreiben, weitere 3 ml während 45 Sekunden auf den Händen verreiben (Gesamt 6ml, 90 Sekunden).

Rub 3ml of 1592 onto the hands for 45 seconds, rub further 3ml onto the hands for 45 seconds (total 6ml, 90 seconds).

Proband / subject		Anzahl KBE je Platte aus Verdünnung $10^{(x)}$ - Prüfprodukt / *Number of cfu per plate from dilution $10^{(x)}$ - Test product*								
Nr./ no.	Hand / hand	Vorwert / *pre-count*			Nachwert / *post-count*					
					Sofortwirkung / *immediate*			3-Stundenwirkung / *3-hours*		
		-1	-2	-3	0	-1	-2	0	-1	-2
1	links / left	>330	>330	87				>330	>330	48
	rechts / right	>330	>330	107	>330	>330	34			
2	links / left	>330	>330	67	23	2	0			
	rechts / right	>330	287	29				36	3	0
3	links / left	>330	>330	138				>330	108	10
	rechts / right	>330	>330	96	>330	>330	50			
4	links / left	>330	>330	283	>330	70	7			
	rechts / right	>330	>330	272				>330	34	3
5	links / left	>330	155	15				153	15	1
	rechts / right	>330	294	31	>330	34	3			
6	links / left	>330	>330	124	>330	34	3			
	rechts / right	>330	>330	77				>330	32	3
7	links / left	>330	>330	282				n z	75	7
	rechts / right	>330	>330	297	>330	>330	79			
8	links / left	>330	>330	251	>330	>330	81			
	rechts / right	>330	>330	257				>330	>330	60
9	links / left	>330	136	14				>330	71	7
	rechts / right	>330	128	13	64	6	0			
10	links / left	>330	>330	74	>330	35	3			
	rechts / right	>330	>330	63				>330	>330	32
11	links / left	>330	>330	168	70	7	0			
	rechts / right	>330	>330	80				>330	38	3
12	links / left	>330	>330	247				67	6	0
	rechts / right	>330	>330	272	37	3	0			
13	links / left	>330	>330	59	99	10	1			
	rechts / right	>330	>330	65				89	8	0
14	links / left	>330	>330	103				25	2	0
	rechts / right	>330	>330	138	224	22	2			
15	links / left	>330	100	9	47	4	0			
	rechts / right	>330	142	14				>330	81	8
16	links / left	>330	>330	151				68	6	0
	rechts / right	>330	>330	100	3	0	0			
17	links / left	>330	116	11	52	5	0			
	rechts / right	>330	>330	96				47	4	0

18	links / left	>330	169	17				>330	>330	37
	rechts / right	>330	133	14	24	2	0			
19	links / left	>330	>330	262				>330	85	8
	rechts / right	>330	>330	231	>330	67	6			
20	links / left	>330	>330	225	>330	120	12			
	rechts / right	>330	>330	224				>330	46	4
21[1)]	links / left	>330	>330	39				>330	154	15
	rechts / right	>330	80	58	11	1	0			
22[1)]	links / left	>330	>330	177	>330	33	3			
	rechts / right	>330	>330	164				>330	105	10
23[1)]	links / left	>330	82	8				>330	58	6
	rechts / right	>330	53	5	10	1	0			
24[1)]	links / left	>330	>330	102	16	1	0			
	rechts / right	>330	>330	124				81	8	0

__ = zur weiteren Berechnung verwendete Werte / *count used for further computation*

Liste der berechneten log-Werte und log-Reduktionsfaktoren Referenzverfahren (RP)
List of computed lg values and lg reduction factors Reference procedure (RP)

Produkt / product: Propan-1-ol mit einer Volumenkonz. von 60% / propan-1-ol 60% volume concentration

Proband / *subject* No.	Sofortwirkung / *immediate effect*			3-Stunden-Wirkung / *3-hours effect*		
	lg x	lg y	lg z	lg x	lg y	lg z
1	5.14	3.21	1.93	5.47	3.72	1.75
2	5.36	1.71	3.65	4.93	1.69	3.24
3	4.52	2.80	1.72	4.30	3.92	0.38
4	4.77	2.86	1.91	5.43	3.82	1.61
5	5.11	2.92	2.19	4.85	3.33	1.52
6	5.36	2.69	2.67	5.42	4.02	1.40
7	5.34	4.08	1.26	5.17	3.95	1.22
8	5.37	4.15	1.22	5.41	3.85	1.56
9	4.23	2.91	1.32	4.92	3.76	1.16
10	4.79	3.06	1.73	4.89	3.69	1.20
11	5.20	2.16	3.04	5.22	3.84	1.38
12	5.47	3.05	2.42	5.46	1.96	3.50
13	4.98	2.27	2.71	4.77	2.64	2.13
14	4.83	2.44	2.39	4.74	1.36	3.38
15	4.36	1.88	2.48	4.21	3.83	0.38
16	3.71	0.60	3.11	4.35	1.94	2.41
17	4.57	2.08	2.49	4.72	1.95	2.77
18	4.92	4.03	0.89	5.08	4.42	0.66
19	5.45	3.76	1.69	5.43	3.41	2.02
20	5.45	3.98	1.47	5.44	2.91	2.53
21	4.31	3.22	1.09	4.11	3.52	0.59
22	4.68	4.32	0.36	4.81	4.17	0.64
23	3.25	1.11	2.14	5.16	2.96	2.20
24	5.13	2.43	2.70	4.99	2.43	2.56
$\bar{x}$	4.85	2.82	2.02	4.97	3.21	1.76
s	0.57	0.96	0.79	0.42	0.89	0.93
N	24	24	24	24	24	24

lg x — lg Vorwert / *lg prevalue*
lg y — lg Nachwert / *lg postvalue*
lg z — lg Reduktionsfaktor / *lg reduction factor*
$\bar{x}$ — Gesamtmittelwert von lg x. lg y und lg z / *overall mean of lg x. lg y and lg z*
s — Standardabweichung / *standard deviation*
N — Anzahl der Werte (Probanden) in jeder Spalte / *number of values (=subjects) in each column*

Liste der berechneten log-Werte und log-Reduktionsfaktoren 1592 (PP)

List of computed lg values and lg reduction factors 1592 (PP)

Produkt / product: 1592

Proband / *subject* No.	Sofortwirkung / *immediate effect*			3-Stunden-Wirkung / *3-hours effect*		
	lg x	lg y	lg z	lg x	lg y	lg z
1	5.03	3.53	1.50	4.94	3.68	1.26
2	4.83	1.36	3.47	4.46	1.56	2.90
3	4.98	3.70	1.28	5.14	3.03	2.11
4	5.45	2.85	2.60	5.43	2.53	2.90
5	4.47	2.53	1.94	4.19	2.18	2.01
6	5.09	2.53	2.56	4.89	2.51	2.38
7	5.47	3.90	1.57	5.45	2.88	2.57
8	5.40	3.91	1.49	5.41	3.78	1.63
9	4.11	1.81	2.30	4.13	2.85	1.28
10	4.87	2.54	2.33	4.80	3.51	1.29
11	5.23	1.85	3.38	4.90	2.58	2.32
12	5.43	1.57	3.86	5.39	1.83	3.56
13	4.77	2.00	2.77	4.81	1.95	2.86
14	5.14	2.35	2.79	5.01	1.40	3.61
15	4.00	1.67	2.33	4.15	2.91	1.24
16	5.00	0.48	4.52	5.18	1.83	3.35
17	4.06	1.72	2.34	4.98	1.67	3.31
18	4.13	1.38	2.75	4.23	3.57	0.66
19	5.36	2.83	2.53	5.42	2.93	2.49
20	5.35	3.08	2.27	5.35	2.66	2.69
21	4.10	1.04	3.06	4.59	3.19	1.40
22	5.25	2.52	2.73	5.21	3.02	2.19
23	3.72	1.00	2.72	3.91	2.76	1.15
24	5.01	1.20	3.81	5.09	1.91	3.18
$\bar{x}$	4.84	2.22	2.62	4.88	2.61	2.26
s	0.55	0.96	0.79	0.48	0.69	0.86
N	24	24	24	24	24	24

lg x — lg Vorwert / *lg prevalue*
lg y — lg Nachwert / *lg postvalue*
lg z — lg Reduktionsfaktor / *lg reduction factor*
$\bar{x}$ — Gesamtmittelwert von lg x. lg y und lg z / *overall mean of lg x. lg y and lg z*
s — Standardabweichung / *standard deviation*
N — Anzahl der Werte (Probanden) in jeder Spalte / *number of values (=subjects) in each column*

Test auf Effekte der Reihenfolge / *Test for sequence effects*

Sofortwirkung / *Immediate effect*

Prozedur / *Procedure*	Reihenfolge / *Sequence*						Absolute Differenz / *Absolute Difference*
	RP -> PP			PP -> RP			
	Mittelwert / Mean	s	NN	Mittelwert / Mean	s	NN	
„RP" (Propan-1-ol 60% v/v)	1.95	0.71	12	2.10	0.88	12	
„PP" (1592)	2.13	0.61	12	3.11	0.65	12	
Differenz der Mittelwerte / *Difference of Means*							
RP - PP	-0.18			-1.01			0.83

3-Stunden Wirkung / *3-hour effect*

Prozedur / *Procedure*	Reihenfolge / *Sequence*						Absolute Differenz / *Absolute Difference*
	RP -> PP			PP -> RP			
	Mittelwert / Mean	s	NN	Mittelwert / Mean	s	NN	
„RP" (Propan-1-ol 60% v/v)	1.73	0.80	12	1.79	1.08	12	
„PP"(1592)	2.16	0.68	12	2.37	1.03	12	
Differenz der Mittelwerte / *Difference of Means*							
RP - PP	-0.43			-0.58			0.15

lg R	Dezimale log Reduktion / *decimal lg reduction*
RP -> PP	Reihenfolge zuerst RP dann PP / *sequence first RP. second PP*
PP -> RP	Reihenfolge zuerst PP dann RP / *sequence first PP. second RP*
s	Standardabweichung / *standard deviation*
NN	Anzahl der Werte (Probanden) / *number of values (volunteers)*

Individuelle Differenzen der log Reduktionen zwischen RP und PP für die Sofort- und 3h Wirkung /

Individual differences of lg reductions between RP and PP for immediate and 3-hour effects

Proband / *Volunteer*	Chronologische Reihenfolge / *Chronological sequence*	log R Sofortwirkung / *lg R immediate effect*			log R 3h Wirkung / *lg R 3-hour effect*		
		RP	PP	Differenz / *Difference* RP-PP	RP	PP	Differenz / *Difference* RP-PP
1	RP => PP	1.93	1.50	0.43	1.75	1.26	0.49
2	RP => PP	3.65	3.47	0.18	3.24	2.90	0.34
3	RP => PP	1.72	1.28	0.44	0.38	2.11	-1.73
4	PP => RP	1.91	2.60	-0.69	1.61	2.90	-1.29
5	RP => PP	2.19	1.94	0.25	1.52	2.01	-0.49
6	RP => PP	2.67	2.56	0.11	1.40	2.38	-0.98
7	RP => PP	1.26	1.57	-0.31	1.22	2.57	-1.35
8	RP => PP	1.22	1.49	-0.27	1.56	1.63	-0.07
9	RP => PP	1.32	2.30	-0.98	1.16	1.28	-0.12
10	RP => PP	1.73	2.33	-0.60	1.20	1.29	-0.09
11	PP => RP	3.04	3.38	-0.34	1.38	2.32	-0.94
12	PP => RP	2.42	3.86	-1.44	3.50	3.56	-0.06
13	PP => RP	2.71	2.77	-0.06	2.13	2.86	-0.73
14	PP => RP	2.39	2.79	-0.40	3.38	3.61	-0.23
15	PP => RP	2.48	2.33	0.15	0.38	1.24	-0.86
16	PP => RP	3.11	4.52	-1.41	2.41	3.35	-0.94
17	RP => PP	2.49	2.34	0.15	2.77	3.31	-0.54
18	PP => RP	0.89	2.75	-1.86	0.66	0.66	0.00
19	RP => PP	1.69	2.53	-0.84	2.02	2.49	-0.47
20	RP => PP	1.47	2.27	-0.80	2.53	2.69	-0.16
21	PP => RP	1.09	3.06	-1.97	0.59	1.40	-0.81
22	PP => RP	0.36	2.73	-2.37	0.64	2.19	-1.55
23	PP => RP	2.14	2.72	-0.58	2.20	1.15	1.05
24	PP => RP	2.70	3.81	-1.11	2.56	3.18	-0.62

lg R	Dezimale log Reduktion / *decimal lg reduction*
RP -> PP	Reihenfolge zuerst RP dann PP / *sequence first RP. second PP*
PP -> RP	Reihenfolge zuerst PP dann RP / *sequence first PP. second RP*

Akzeptanzkriterien für die Testergebnisse nach EN12791 5.7.1. a) bis d) / *Acceptance criteria for test results according to EN12791 5.7.1. a) to d)*

a)	Vollständige Versuchsdaten sind vorhanden / *complete set of results available:* von 24 Personen */ from 24 volunteers*	Somit mehr als das Minimum von 23 / *hence. more than the minimum of 23*
b)	Die mittleren lg der Vorwerte des RP (Sofortwirkung / 3-h Wirkung) / *Mean of lg prevalues for RP (immediate / 3-h effect)* = 4.85 / 4.84 und für PP (Sofortwirkung / 3-h Wirkung) / *and for PP (immediate / 3-h effect)* = 4.85 / 4.84	Somit alle min. 3.5 / *hence all min. 3.5*
c)	Die absolute Differenz der mittleren Differenzen zwischen RP und PP / *Absolute difference of mean differences between RP and PP* d1) zwischen den Gruppen RP => PP und PP => RP (Sofortwirkung) / between the groups RP => PP and PP => RP *(immediate effect)*: 0.83 d2) zwischen den Gruppen RP => PP und PP => RP (3-h Wirkung) / between the groups RP => PP and PP => RP *(3-h effect)*: 0.15	Somit < 2 / *hence < 2* Somit < 2 / *hence < 2*
d)	Alle Resultate in den Tabellen Seite 6-9 welche zur Berechnung des gewichteten Mittelwertes verwendet wurden. haben einen Quotienten im Bereich 5 bis 15 / *Results in tables page 6-9 which were used for weighted mean counts; all quotions of weighted mean counts between 5 and 15.*	

Alle Akzeptanz- Kriterien erfüllt / *All acceptance criteria are fulfilled*

Legende:

RF = Reduktionsfaktor / *reduction factor*

RP = Referenzprodukt / *reference product*

PP = Prüfprodukt / *test product*

lg = $\log_{10}$ / lg_{10}

Sortierung der einzelnen Differenzen und Berechnung der oberen 97.5% Vertrauensgrenzen nach Hodges-Lehmann /

Sorting of individual differences and computation of the Hodges-Lehmann 97.5% upper confidence limit

Sofortwirkung / *immediate effect*

Sortierte Differenzen von / *Sorted differences of* RP-PP	**Mittlere paarweise Differenzen / *Mean pairwise differences*** $(d_i + d_{ii})/2$										
	0.44	0.43	0.25	0.18	0.15	0.15	0.11	-0.06	-0.27	-0.31	-0.34
0.44	0.44 [1]										
0.43	0.43 [2]	0.43 [2]									
0.25	0.34 [4]	0.34 [4]	0.25 [14]								
0.18	0.31 [6]	0.30 [7]	0.21 [15]	0.18 [19]							
0.15	0.29 [8]	0.29 [8]	0.20 [16]	0.16 [22]	0.15 [24]						
0.15	0.29 [8]	0.29 [8]	0.20 [16]	0.16 [22]	0.15 [24]	0.15 [24]					
0.11	0.27 [12]	0.27 [12]	0.18 [19]	0.14 [27]	0.13 [28]	0.13 [28]	0.11 [30]				
-0.06	0.19 [18]	0.18 [19]	0.09 [31]	0.06 [34]	0.04 [38]	0.04 [38]	0.02 [41]	-0.06 [48]			
-0.27	0.08 [32]	0.08 [32]	-0.01 [44]	-0.04 [46]	-0.06 [48]	-0.06 [48]	-0.08 [55]	-0.16 [71]	-0.27		
-0.31	0.06 [34]	0.06 [34]	-0.03 [45]	-0.06 [48]	-0.08 [55]	-0.08 [55]	-0.10 [63]	-0.18 [74]	-0.29	-0.31	
-0.34	0.05 [37]	0.04 [38]	-0.04 [46]	-0.08 [55]	-0.09 [61]	-0.09 [61]	-0.11 [64]	-0.20 [77]	-0.30	-0.32	-0.34
-0.40	0.02 [41]	0.01 [43]	-0.07 [52]	-0.11 [64]	-0.12 [66]	-0.12 [66]	-0.14 [70]	-0.23	-0.33	-0.35	-0.37
-0.58	-0.07 [52]	-0.07 [52]	-0.16 [71]	-0.20 [77]	**-0.21** [82]	-0.21	-0.23	-0.32	-0.42	-0.44	-0.46
-0.60	-0.08 [55]	-0.08 [55]	-0.17 [73]	-0.21 [81]	-0.22	-0.22	-0.24	-0.33	-0.43	-0.45	-0.47
-0.69	-0.12 [66]	-0.13 [69]	-0.22	-0.25	-0.27	-0.27	-0.29	-0.37	-0.48		
-0.80	-0.18 [74]	-0.18 [74]	-0.27	-0.31	-0.32	-0.32	-0.34	-0.43			
-0.84	-0.20 [77]	-0.20 [77]	-0.29	-0.33	-0.34	-0.34	-0.36	-0.45			
-0.98	-0.27	-0.27	-0.36	-0.40	-0.41	-0.41	-0.43				
-1.11	-0.33	-0.34	-0.43	-0.46	-0.48	-0.48					
-1.41	-0.48	-0.49									
-1.44											
-1.86											
-1.97											
-2.37											

Vorzeichenrangtest für gepaarte Stichproben nach Wilcoxon / *Wilcoxon`s matched-pairs signed-ranks test*

Sofortwirkung / *immediate effect*

Anzahl der Paare / *Number of pairs*	Signifikanzniveau / *Significance level*		
	p = 0.025 einseitig / *one sided*	p = 0.01 einseitig / *one sided*	p = 0.01 zweiseitig / *two sided*
23	73	62	54
24	81	69	61
25	89	76	68

Der Medianwert liegt zwischen dem 12. und 13. Wert: [-0,40+(-0,58)]/2 = -0,49. Die kleineren Exponenten geben die Ränge an.

Die mittleren paarweisen Differenzen, die den Medianwert (hier -0.49) nicht überschreiten, werden berechnet. Aus den kritischen Werten für Vorzeichenrangtests für gepaarte Stichproben nach Wilcoxon ergibt sich bei einem Eingangswert von n=24 und einem einseitigen Signifikanzniveau von 0.025 ein kritischer Wert von 81. **Folglich ist c=81+1=82**. Die paarweisen Differenzen werden in absteigender Reihenfolge sortiert. **Der 82. Wert ist -0.21.** Folglich ist die nach Hodges-Lehmann einseitige obere 97.5%-Vertrauensgrenze für die Differenz der log R zwischen RP und PP -0.21, was geringer ist als die vereinbarte Grenze für Unterlegenheit von 0.75.

Deshalb wird die Hypothese der Unterlegenheit von PP verworfen und es kann die Schlussfolgerung gezogen werden, dass das Prüfprodukt PP dem Referenzprodukt RP nicht unterlegen ist.

The median is between the 10th and 11th value: [-0.40+(-0.58)]/2 = -0.49. The small exponents represent the ranks.

The mean pair wise differences, that do not exceed the median (here: -0.49) are computed. From the critical values for Wilcoxon`s matched-pairs signed-ranks test the entry for n=24 and a one-sided 0.025 level of significance the critical value of 81 is found. ***Hence c=81+1=82****. In the body of the table the pair wise differences are sorted in descending order.* ***There the 82rd value is -0.21****. Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg R between RP and PP is -0.21, which is less than the agreed inferiority margin of 0.75.*

Therefore the hypothesis of inferiority of the immediate effect of PP versus RP can be rejected and it can be concluded that the test preparation PP is non- inferior to RP.

Sortierung der einzelnen Differenzen und Berechnung der oberen 97.5% Vertrauensgrenzen nach Hodges-Lehmann /

Sorting of individual differences and computation of the Hodges-Lehmann 97.5% upper confidence limit

3h- Wirkung / *3h- effect*

Sortierte Differenzen von / *Sorted differences of* RP-PP	**Mittlere paarweise Differenzen / *Mean pairwise differences* ($d_i + d_{ii}$) /2**										
	1.05	0.49	0.34	0.00	-0.06	-0.07	-0.09	-0.12	-0.16	-0.23	-0.47
1.05	1.05 [1]										
0.49	0.77 [2]	0.49 [5]									
0.34	0.69 [3]	0.41 [11]	0.34 [13]								
0.00	0.52 [4]	0.24 [17]	0.17 [23]	0.00 [39]							
-0.06	0.49 [5]	0.21 [18]	0.14 [26]	-0.03 [42]	-0.06 [45]						
-0.07	0.49 [5]	0.21 [18]	0.13 [27]	-0.03 [42]	-0.06 [45]	-0.07 [50]					
-0.09	0.48 [8]	0.20 [21]	0.12 [29]	-0.04 [44]	-0.07 [50]	-0.08 [53]	-0.09 [55]				
-0.12	0.46 [9]	0.18 [22]	0.11 [31]	-0.06 [45]	-0.09 [55]	-0.09 [55]	-0.10 [58]	-0.12 [63]			
-0.16	0.44 [10]	0.16 [24]	0.09 [32]	-0.08 [53]	-0.11 [60]	-0.11 [60]	-0.12 [63]	-0.14 [67]	-0.16 [72]		
-0.23	0.41 [11]	0.13 [27]	0.05 [34]	-0.11 [60]	-0.14 [67]	-0.15 [70]	-0.16 [72]	-0.17 [75]	-0.19 [77]	-0.23	
-0.47	0.29 [14]	0.01 [38]	-0.06 [45]	**-0.23** [82]	-0.26	-0.27	-0.28	-0.29	-0.31	-0.35	-0.47
-0.49	0.28 [15]	0.00 [39]	-0.07 [50]	-0.24	-0.27	-0.28	-0.29	-0.30	-0.32	-0.36	-0.48
-0.54	0.25 [16]	-0.02 [41]	-0.10 [58]	-0.27	-0.30	-0.30	-0.31	-0.33	-0.35	-0.38	-0.50
-0.62	0.21 [18]	-0.06 [45]	-0.14 [67]	-0.31	-0.34	-0.34	-0.35	-0.37	-0.39	-0.42	
-0.73	0.16 [24]	-0.12 [63]	-0.19 [77]	-0.36	-0.39	-0.40	-0.41	-0.42	-0.44	-0.48	
-0.81	0.12 [29]	-0.16 [72]	-0.23 [81]	-0.40	-0.43	-0.44	-0.45	-0.46	-0.48		
-0.86	0.09 [32]	-0.18 [76]	-0.26	-0.43	-0.46	-0.46	-0.47	-0.49	-0.51		
-0.94	0.05 [34]	-0.22 [79]	-0.30	-0.47	-0.50	-0.50	-0.51				
-0.94	0.05 [34]	-0.22 [79]	-0.30	-0.47	-0.50	-0.50	-0.51				
-0.98	0.03 [37]	-0.24	-0.32	-0.49							
-1.29	-0.12 [63]	-0.40	-0.47								
-1.35	-0.15 [70]	-0.43	-0.50								
-1.55	-0.25										
-1.73	-0.34										

Vorzeichenrangtest für gepaarte Stichproben nach Wilcoxon / *Wilcoxon`s matched-pairs signed-ranks test*

3h- Wirkung / 3h- effect

Anzahl der Paare / *Number of pairs*	Signifikanzniveau / *Significance level*		
	p = 0.025 einseitig / *one sided*	p = 0.01 einseitig / *one sided*	p = 0.01 zweiseitig / *two sided*
23	73	62	54
24	81	69	61
25	89	76	68

Der Medianwert liegt zwischen dem 12. und 13. Wert: [-0,49+(-0,54)]/2 = -0,515. Die kleineren Exponenten geben die Ränge an.

Die mittleren paarweisen Differenzen, die den Medianwert (hier -0.515) nicht überschreiten, werden berechnet. Aus den kritischen Werten für Vorzeichenrangtests für gepaarte Stichproben nach Wilcoxon ergibt sich bei einem Eingangswert von n=24 und einem einseitigen Signifikanzniveau von 0.025 ein kritischer Wert von 81. **Folglich ist c=81+1=82**. Die paarweisen Differenzen werden in absteigender Reihenfolge sortiert. **Der 82. Wert ist -0.23.** Folglich ist die nach Hodges-Lehmann einseitige obere 97.5%-Vertrauensgrenze für die Differenz der log R zwischen RP und PP -0.23, was geringer ist als die vereinbarte Grenze für Unterlegenheit von 0.85.

Deshalb wird die Hypothese der Unterlegenheit von PP verworfen und es kann die Schlussfolgerung gezogen werden, dass das Prüfprodukt PP dem Referenzprodukt RP nicht unterlegen ist.

The median is between the 10th and 11th value: [-0.49+(-0.54)]/2 = -0.515. The small exponents represent the ranks.

The mean pair wise differences, that do not exceed the median (here: -0.515) are computed. From the critical values for Wilcoxon`s matched-pairs signed-ranks test the entry for n=24 and a one-sided 0.025 level of significance the critical value of 81 is found. ***Hence c=81+1=82****. In the body of the table the pair wise differences are sorted in descending order.* ***There the 82rd value is -0.23****. Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg R between RP and PP is -0.23, which is less than the agreed inferiority margin of 0.85.*

Therefore the hypothesis of inferiority of the immediate effect of PP versus RP can be rejected and it can be concluded that the test preparation PP is non- inferior to RP.

Statistischer paariger Vergleich der im Referenzverfahren und Prüfverfahren erhobenen Werte
Statistical comparison of values as obtained with R and P

(Vorzeichen-Rangtest für Paardifferenzen nach WILCOXON)
(WILCOXON matched-pairs signed-rank test)

3-Stunden-Wirkung - Langzeitwirkung / *3-hours effect - sustained effect*

Proband / *subject*	lg RF von / *lg RF derived from*		Differenz / *Difference*	Rang der Differenz / *Rank of Difference*	
Nr. / *no.*	R	P	R-P	ohne VZ / *without sign*	mit VZ / *with sign*
1	1.75	1.26	0.49	9.5	9.5
2	3.24	2.90	0.34	7	7
3	0.38	2.11	-1.73	23	-23
4	1.61	2.90	-1.29	20	-20
5	1.52	2.01	-0.49	9.5	-9.5
6	1.40	2.38	-0.98	18	-18
7	1.22	2.57	-1.35	21	-21
8	1.56	1.63	-0.07	2	-2
9	1.16	1.28	-0.12	4	-4
10	1.20	1.29	-0.09	3	-3
11	1.38	2.32	-0.94	16.5	-16.5
12	3.50	3.56	-0.06	1	-1
13	2.13	2.86	-0.73	13	-13
14	3.38	3.61	-0.23	6	-6
15	0.38	1.24	-0.86	15	-15
16	2.41	3.35	-0.94	16.5	-16.5
17	2.77	3.31	-0.54	11	-11
18	0.66	0.66	0.00		
19	2.02	2.49	-0.47	8	-8
20	2.53	2.69	-0.16	5	-5
21	0.59	1.40	-0.81	14	-14
22	0.64	2.19	-1.55	22	-22
23	2.20	1.15	1.05	19	19
24	2.56	3.18	-0.62	12	-12

RF = Reduktionsfaktor / reduction factor
VZ = Vorzeichen / sign

R = Referenzverfahren / reference
P = Prüfprodukt / test product
Rangsumme / rank sum (+): 35.5
Rangsumme / rank sum (-): 240.5

Vorzeichen-Rangtest für gepaarte Stichproben nach WILCOXON / *WILCOXON matched-pairs signed-rank test*

Kritische Werte für die untere der beiden Rangsummen mit (+) oder (-) Vorzeichen bei unterschiedlichen Signifikanzniveaus. /

Critical values for the lower of both sums of rank with (+) or (-) sign at different significance levels.

n Anzahl der Paare mit einer Differenz ≠ 0 / *number of pairs with difference = 0*	Signifikanzniveau / *level of significance*		
	0.025	0.01	
	einseitig / *one sided*	einseitig / *one sided*	zweiseitig / *two sided*
23	73	62	54
24	81	69	61
25	89	76	68
26	98	84	75

Bewertung von / *Evaluation of* 1592

Vergleich der durch das Prüfprodukt (PP) und das Referenzprodukt (RP) ermittelten mittleren Reduktionsfaktoren (log) / *Comparison of the mean lg reduction factors (RF) as obtained with product (PP) and reference (RP)*

Bewertete Wirkung / *assessed effects*	Mittelwert lg RF / *mean lg RF*		Signifikanz der Differenz / *Significance of difference*
	P	R	
Sofortwirkung / *immediate*	2.62	2.02	
3-Stunden-Wirkung - Langzeitwirkung / *3-hours effect - sustained effect*	2.26	1.76	s (P ≤ 0.01)
s = signifikant / *significant* ns = nicht signifikant / *not significant*			

Schlussfolgerung / *Conclusion:*

Das Prüfverfahren mit **1592** resultiert jeweils in einem mittleren Reduktionsfaktor sowohl in der Sofortwirkung, als auch in der 3-Stunden-Wirkung gegenüber dem Referenzverfahren nicht unterlegen.

Das Produkt wurde auch auf eine Langzeitwirkung hin untersucht. Die Prüfergebnisse, im Vergleich mit Propan-1-ol, haben gezeigt, dass das Produkt **1592** eine Langzeitwirkung aufweist.

Demzufolge ist in Übereinstimmung mit EN 12791 das Produkt **1592** geeignet als chirurgisches Händedesinfektionsmittel mit Langzeitwirkung für die folgende Anwendung:

3 ml von 1592 während 45 Sekunden auf den Händen verreiben, weitere 3 ml während 45 Sekunden auf den Händen verreiben (Gesamt 6ml, 90 Sekunden).

*The testing procedure with **1592** showed in each mean value, in both the immediate effect and the 3-hours effect non- inferior compared to the reference procedure.*

*The product was also tested for a 3-hours effect. The test results, in comparison with propan-1-ol, have shown that the product **1592** showed a 3-hours effect.*

*According to EN 12791 the product **1592** is suitable for surgical hand disinfection with a 3-hours effect by the following application:*

Rub 3ml of 1592 onto the hands for 45 seconds, rub further 3ml onto the hands for 45 seconds (total 6ml, 90 seconds).

Prof. Dr. med. H.-P. Werner
technischer Leiter / *technical manager*

BluTest
GLOBAL MICROBIOLOGY EXPERTISE

Test Report BS 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 method and requirements (phase 2, step 1)

Test Laboratory	BluTest Laboratories Ltd Robertson Incubator (Level 4) 56 Dumbarton Road Glasgow G11 6NU-UK
Identification of sample	
Name of the product	**PHAGORUB GEL SPS**
Client	LABORATOIRE PHAGOGENE, LOUZY, FRANCE
Project	BT-PHA-04
Date of Delivery	16 February 2011
Storage conditions	Ambient and darkness
Active substances	Not known
Test Method and its validation	
Method	Filtration-neutralization technique
Neutralizer	Lecithin 3g/l, Polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histidine 1g/l, phosphate buffer 0.0025mol/l, sterilized by autoclave
Experimental Conditions	
Period of analysis	5 April to 25 April 2011
Product diluent used	Sterile hard water
Product test concentrations	20.0 % V/V; 40.0% V/V; 80.0% V/V
Appearance product dilutions	Colourless, clear product solution
Contact time	t = 30s +/- 2 s
Test temperature	20°C ± 1°C
Interfering substance	0.3 g/l bovine albumin
Stability of mixture	Precipitate absent throughout the test
Temperature of incubation	37°C ± 1°C + 5.0% CO_2
Identification of strain	*Mycobacterium terrae* ATCC 15755

Conclusion

Following the EN 14348 (2005) protocol, **PHAGORUB GEL SPS,** possesses tuberculocidal activity (6.02 $\log_{10}$ reduction) at a concentration of 80% V/V in 30 seconds at 20°C under **CLEAN** conditions (0.3 g/l bovine albumin) for reference strain *Mycobacterium terrae* ATCC 15755

Signed

Dr Chris Woodall, Director
BluTest Laboratories Limited
Glasgow, UK
29 April 2011

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Tel: +44 (0)141 330 7340 Fax: +44 (0)141 330 7342 Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

RESULTS

TABLE 1

	Number of cells per ml in the mycobacterial suspensions	Number of cells per ml in the test mixtures at the beginning of the contact time (time 0)	Number of survivors per ml in the test mixtures at the end of the contact-time.
Test	$N = 1.77 \times 10^9$	$No = 1.77 \times 10^8$	Na (SEE TABLE 2)
Controls	$Nv = 5.20 \times 10^2$	$Nvo = 5.20 \times 10^1$	$A = 4.10 \times 10^1$ $B = 3.95 \times 10^1$ $C = 4.70 \times 10^1$

a) N is between $1,5 \times 10^9$ cfu/ml and $5,0 \times 10^9$ cfu/ml ($9,17 \leq \lg N \leq 9,70$) and N_0 is between $1,5 \times 10^8$ cfu/ml and $5,0 \times 10^8$ cfu/ml ($8,17 \leq \lg N_0 \leq 8,70$)
b) N_{v0} is between 30 and 160 cfu/ml ($3,0 \times 10^1$ and $1,6 \times 10^2$) and N_v is between 3.0×10^2 and $1,6 \times 10^3$ cfu/ml
c) A,B,C are equal to or greater than $0.5 \times N_{v0}$

TABLE 2

CONCENTRATION	Na	No/Na	Log Reduction (R)
80.0% W/V	1.68×10^2	1.05×10^6	6.02
40.0% W/V	$>3.3 \times 10^4$	$<5.36 \times 10^3$	<3.73
20.0% W/V	$>3.3 \times 10^4$	$<5.36 \times 10^3$	<3.73

Robertson Incubator, Level 4, Robertson Building, 56 Dumbarton Road, Glasgow, G11 6NU
Tel: +44 (0)141 330 7340 Fax: +44 (0)141 330 7342 Email: info@blutest.com Website: www.blutest.com
Company Registration Number: SC364409 VAT Registration Number: GB 979 1131 96

RAPPORT D'ESSAI INTERNE N° 2012/001

Norme : Antiseptiques et désinfectants chimiques.Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide de base des antiseptiques et des désinfectants chimiques. Méthode d'essai et prescriptions (phase 1) AFNOR EN 1275 avril 2006

Identification de l'échantillon de désinfectant

Nom du produit : PHAGORUB SOLUTION

Numéro de lot : lot 20011 07.2013

Fabricant : PHAGOGENE

Condition de stockage : à température ambiante au laboratoire

Diluant du produit dont l'utilisation est recommandée par le fabricant : néant, pur

Substance(s) active(s) et sa (leur) concentration : éthanol 72% m/m

Aspect du produit : liquide limpide fluide

Période d'essai : le vendredi 03/02/2012

Résultats : (voir page 3/3)

Conclusion :

Le produit PHAGORUB SOLUTION lot 20011 07.2013 utilisé pur (80%V/V au contact) est levuricide en 15 secondes de contact vis-à-vis de la souche de référence Candida albicans ATCC 10231 selon la méthodologie adaptée de la norme AFNOR NF EN 1275 2006.

SIGNATAIRES :

Alain LE BEC, Microbiologiste Laboratoire de Contrôle : 07 02 2012

Guillaume MACOUIN, Responsable Laboratoire de Contrôle : 07 02 2012

(N° procès verbal : 4 chiffres de l'année du début de l'essai / 3 chiffres chronologiques)

2012/001

RAPPORT D'ESSAI INTERNE N° 2012/001

Résultats d'essai :

rattaché à l'instruction I-109/F
version du fichier : a
date de dernière modification 25/08/2010
résultats de rapport d'essai interne n° 2012/001

résultats de la norme AFNOR NF EN 1275 avril 2006

Candida albicans ATCC 10231

concentration=>	80%V/V	x	x
Vc1	1	x	x
Vc2	1	x	x
Na	10	#DIV/0!	#DIV/0!
R	5,4	#DIV/0!	#DIV/0!

	Vc1	Vc2	moyenne
Nv			1420
Nvo	140	144	142
A	120	134	127,0
B	130	124	127,0
C	93	107	100,0
N	234	218	2,26E+07

Aspergillus niger ATCC 16404

concentration=>	X	X	X
Vc1	x	x	x
Vc2	x	x	x
Na	#DIV/0!	#DIV/0!	#DIV/0!
R	#DIV/0!	#DIV/0!	#DIV/0!

	Vc1	Vc2	moyenne
Nv			#DIV/0!
Nvo	X	X	#DIV/0!
A	X	X	#DIV/0!
B	X	X	#DIV/0!
C	X	X	#DIV/0!
N	X	X	#DIV/0!

pour chaque souche d'essai, vérifier que :

suspension d'essai	N	est compris entre $1,5^{e7}$ et $5x10^{e7}$ UCF/ml
UFC/ml dans le mélange d'essai à T0	No	est compris entre $1,5^{e6}$ et $5x10^{e6}$ UCF/ml (N/10)
suspension de validation	Nv	est compris entre 300 et 1600 UFC/ml
UFC/ml dans les mélanges A,B,C	Nvo	est compris entre 30 et 160 (Nv/10)
témoin des conditions expérimentales	A	supérieur ou égal à 0,5xNvo
le témoin de toxicite du neutralisant	B	supérieur ou égal à 0,5xNvo
l'essai de validation du neutralisant	C	supérieur ou égal à 0,5xNvo
le nombre d'UFC après contact	Na	doit être inférieur ou égal à 15
la réduction logarithmique	R	est supérieur ou égal à 4 (R=No/Na)
	Vc	est le nombre de colonnie par boite de Pétri
	Na	UFC/ml dans le mélange d'essai après temps de contact

condition expérimentales :

date des essais vendredi 03/02/2012
produit testé : PHAGORUB SOLUTION/Phagogène lot 2001 07.2013
temps de contact en min : 15 secondes
température de contact en °C : 21°C
lot *candida albicans* ATCC 10231: 443-170-1 2013-04 MICROBIOLOGICS
lot *aspergillus niger* ATCC 16404: non réalisé
lot gélose GEM: 130834 01/2014 AESCHEMUNEX
lot tryptone sel: 135004 15/12/2012
lot neutralisant: N6 RIVADIS
analyste : LE BEC alain

conclusion :

Le produit PHAGORUB SOLUTION lot 20011 07.2013 utilisé pur (80%V/V au contact) est levuricide en 15 secondes de contact vis-à-vis de la souche de référence Candida albicans ATCC 10231 selon la méthodologie adaptée de la norme AFNOR NF EN 1275 2006.

Rivadis Groupe

LABORATOIRES : ☐ PHAGOGENE ☒ RIVADIS	Réf. : F-278/A	Page : 2/3
Fiche : Rapport d'essai de normes européennes sur les antiseptiques et désinfectants chimiques	Date d'application : 11 février 2009	

RAPPORT D'ESSAI INTERNE N° 2010/007

Norme : EN 1650, **Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine de l'agro-alimentaire, dans l'industrie, dans les domaines domestiques et en collectivité. Oct. 2008**

Méthode d'essai et prescriptions (phase 2, étape 1)

Identification de l'échantillon de désinfectant

Nom du produit : solution hydroalcoolique AL 3235
Numéro de lot : P02891V01/01 du 25/03/2010
Fabricant : INTERSPRAY
Condition de stockage : à température ambiante
Diluant du produit dont l'utilisation est recommandée par le fabricant : utilisé pur
Substance(s) active(s) et sa (leur) concentration : alcool éthylique à 72% m/m
Aspect du produit : liquide limpide, incolore
Période d'essai : vendredi 04 juin 2010
Résultats : (voir page 3/3)

Conclusion : le produit " solution hydroalcoolique AL3235 " lot P02891V01/01 est levuricide en 15 secondes à 80%V/V à 20°C vis-à-vis de la souche de référence *candida albicans* ATCC 10231, selon la norme AFNOR EN 1650 oct. 2008 en condition de propreté.

SIGNATAIRES :

Alain LE BEC, Microbiologiste Laboratoire de Contrôle : 15 06 2010
Guillaume MACOUIN, Responsable Laboratoire de Contrôle : 15/06/10

(N° procès verbal : 4 chiffres de l'année du début de l'essai / 3 chiffres chronologiques)

RAPPORT D'ESSAI INTERNE N° 2010/007

Résultats d'essai :

rattaché à l'instruction 51/I-063/E
version du fichier : 1
date de la dernière modification: 25/11/2009
résultats de rapport d'essai interne n° **2010/007**

résultats de la norme AFNOR EN 1650 octobre 2008

candida albicans ATCC 10231

concentration=>	80%V/V		
Vc1	0		
Vc2	0		
Na	0	#DIV/0!	#DIV/0!
R	4,0	**#DIV/0!**	**#DIV/0!**

	Vc1	Vc2	moyenne
A	non réalisé	non réalisé	#DIV/0!
B	204	221	212,5
C	229	180	204,5
Nv	1970	2110	2040,0
N	197	211	1,97E+07

la validation de l'innocuité de l'eau dure (A) n'est pas réalisé car le produit est testé pur.

pour chaque souche d'essai, vérifier que :

l'inoculum	**N**	est compris entre 1,5e7 et 5e7 UCF/ml
l'inoculum de validation	**Nv**	est compris entre 600 et 1500 UFC/ml
le témoin de toxicite de l'eau dure	**A**	(est supérieur ou égal à 0,05*Nv)
le témoin de toxicite du neutralisant	**B**	(est supérieur ou égal à 0,05*Nv)
l'essai de validation du neutralisant	**C**	doit être supérieur ou égal à (0,5*B) :
la réduction logarithmique	**R**	doit être supérieur ou égal à 4
UFC/ml du mélange après contact	**Na**	doit etre inférieur à 150
le nombre de bactéries par boite	**Vc**	est compris entre 15 et 150

condition expérimentales :

date des essais : vendredi 4 juin 2010
produit testé : solution hydroalcoolique AL3235 lot P02891V01/01
temps de contact en min : 15 secondes
substances interférentes : condition de propreté
lot *candida albicans* ATCC 10231 : 443502 08/2010, MICROBIOLOGICS
lot *Aspergillus niger* ATCC 16404 : non réalisé
température au contact en °C : 20
lot albumine bovine : K39019318, Merck exp. 16/06/2010
lot TCS en pente : 932406 20/11/2010 AESCHEMUNEX
lot gélose GEM : 006843 exp. 07/2012 AESCHEMUNEX
lot de neutralisant : N6 du 27/04/2010
lot tryptone sel : 004809 exp.17/02/2011 AESCHEMUNEX

conclusion : le produit "solution hydroalcoolique AL3235" lot P02891V01/01 du 25/03/2010 est levuricide en 15 secondes à 80%V/V à 20°C vis-à-vis de la souche de référence *candida albicans* ATCC 10231, selon la norme AFNOR EN 1650 oct. 2008 en condition de propreté.

RAPPORT D'ESSAI N°3588-1

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Imprimé le : 01/04/15
Date de 1ère impression : 01/04/15

TEST D'EFFICACITE LEVURICIDE
SELON LA NORME NF EN 13624 (Novembre 2013)
1592
Applications = Friction hygiénique des mains
Friction chirurgicale des mains
(Méthode par dilution/neutralisation)

DESTINATAIRE : CHRISTEYNS FRANCE

I- IDENTIFICATION DU DONNEUR D'ORDRE

Mr Jérôme DUBOURGEOIS
CHRISTEYNS FRANCE
31 rue de la Maladrie
44120 VERTOU
Tél. 02-40-80-27-27 - Fax. 02-40-03-09-73

II- IDENTIFICATION DE L'ECHANTILLON

- Nom du produit : **1592**
- Numéro de lot : PR288-F12
- Fabricant : CHRISTEYNS FRANCE
- Date de fabrication : 03/03/15
- Date de péremption : 03/03/18
- Date de réception au laboratoire : 23/03/15
- Aspect du produit : Liquide limpide incolore
- Conditions de stockage : à température ambiante et à l'abri de la lumière

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- Diluant du produit recommandé par le fabricant : non concerné
- Matière(s) active(s) : Non communiquées

III- METHODE D'ESSAI

Norme NF EN 13624 (Novembre 2013) : Essai quantitatif de suspension pour l'évaluation de l'activité fongicide ou levuricide en médecine. (Phase 2, Etape 1).

Application « Friction hygiénique des mains » : Réduction logarithmique au moins égale à 4 Log décimaux dans les conditions de l'essai.

Application « Friction chirurgicale des mains » : Réduction logarithmique au moins égale à 4 Log décimaux dans les conditions de l'essai.

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 18/03/15 au 20/03/15
- Analyse réalisée par : M. TEULIER
- Diluant du produit utilisé au cours de l'essai : eau dure 30°f
- Concentrations de produit testé (V/V) : 20-40 et 80%
- Technique d'essai : dilution/neutralisation
- Aspect des dilutions : Opaques pour 20% et 40%, limpide pour 80%
- Stabilité du mélange substance interférente/dilutions du produit : absence de précipité au cours de l'essai
- Temps de contact : 30 secondes (+/-5 secondes)
- Température d'essai : 20°C (+/-1°C)
- Substance interférente : 0,3 g/l d'albumine bovine (conditions de propreté)

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- Température d'incubation : 30°C (+/-1°C)
- Identification de la souche utilisée :
  Candida albicans DSM 1386

V- RESULTATS D'ESSAI

Vc : nombre de colonies comptées sur les boîtes,
N: nombre d'UFC / ml dans la suspension microbienne d'essai,
N_0: nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de N,
Nv : nombre de cellules par ml de la suspension microbienne de validation,
Nv_0 : nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de Nv,
Nv_B : dans le cas du témoin de neutralisant B, il s'agit du nombre de cellules par ml après dilution au centième. Il représente un millième de Nv,
Na : nombre de survivants par ml dans le mélange d'essai à l'issue du temps de contact et avant neutralisation
A : nombre de survivants dans le témoin des conditions expérimentales,
B : nombre de survivants dans le témoin de neutralisant,
C : nombre de survivants dans le témoin de validation de la méthode,
R : réduction du nombre de cellules viables ($R=N_0/Na$) exprimé en logarithme

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Essai sur *Candida albicans DSM 1386*

Souche testée	Suspension microbienne d'essai	Essai de validation				
		Suspension microbienne (NV)	Suspension microbienne (NVB)	Conditions expérimentales (A)	Non toxicité du neutralisant (B)	Inactivation par dilution/neutralisation (C)
Candida albicans DSM 1386 Lot 534	10^{-5} : Vc1 : 151 Vc2 : 155 10^{-6} : Vc1 : 17 Vc2 : 14 N= 1,53.10^7 N_0= 1,53.10^6 Log N_0 =6,18	Vc1 : 43 Vc2 : 38 (dilution 10^{-1}) Nv= 405 Nv_0= 41	Vc1 : 48 Vc2 : 38 (dilution 10^{-3}) NvB= 4,30.10^4	Vc1 : 29 Vc2 : 40 A= 35	Vc1 : 36 Vc2 : 34 B= 35	Vc1 : 34 Vc2 : 31 C= 33

L'essai est validé si :
N est compris entre 1,5.10^7 et 5.10^7 UFC/ml (7,17 ≤ lg N ≤ 7,70)
N_0 est compris entre 1,5.10^6 et 5.10^6 UFC/ml (6,17 ≤ lg N ≤ 6,70)
Nv_0 est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)
Nv_B est compris entre 3,0.10^4 et 1,6.10^5
A,B et C sont supérieurs ou égaux à 0,5xN_{v0}
B (dilution-neutralisation) est égal ou supérieur à 0,0005 x Nv_B
Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée	Concentrations testées % (V/V)									
		20%			40%			80%		
		10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}	10^0	10^{-1}	10^{-2}
Candida albicans DSM 1386 Lot 534	Vc1	>330	>330	>330	>330	264	27	0	0	0
	Vc2	>330	>330	>330	>330	248	25	0	0	0
	Na	>3,3.10^5			2,56.10^4			<140		
	lg Na	>5,52			4,41			<2,15		
	Lg R	<0,66			1,77			>4,03		

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VI- CONCLUSION

- Application « Friction hygiénique des mains » : Réduction logarithmique au moins égale à 4 Log décimaux dans les conditions de l'essai.

Conformément à la norme NF EN 13624 (Novembre 2013), le lot PR288-F12 du produit 1592 de la société CHRISTEYNS FRANCE, dans les conditions d'essai suivantes :
- en 30 secondes de temps de contact,
- à la température de 20°C,
- en présence d'albumine bovine à 0,3 g/l (conditions de propreté),

présente une activité levuricide (réduction supérieure à 4 log décimaux dans le cas de l'application « Friction hygiénique des mains »), lorsqu'il est dilué à 80% (V/V), vis-à-vis de la souche *Candida albicans* DSM 1386.

- Application « Friction chirurgicale des mains » : Réduction logarithmique au moins égale à 4 Log décimaux dans les conditions de l'essai.

Selon la méthodologie de la norme NF EN 13624 (Novembre 2013), le lot PR288-F12 du produit 1592 de la société CHRISTEYNS FRANCE, dans les conditions d'essai suivantes :
- en 30 secondes de temps de contact,
- à la température de 20°C,
- en présence d'albumine bovine à 0,3 g/l (conditions de propreté),

présente une activité levuricide (réduction supérieure à 4 log décimaux dans le cas de l'application « Friction chirurgicale des mains »), lorsqu'il est dilué à 80% (V/V), vis-à-vis de la souche *Candida albicans* DSM 1386.

La souches est conservée et contrôlée selon la norme NF EN 12353.
La souche d'essai a été soumise à essai une seule fois.

RAPPORT D'ESSAI N°3588-1

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VII-SIGNATURES

Fait à DINARD, le 02/04/15

Rédigé par	Validé par
M. TEULIER Responsable d'essai	M.SESQUES Docteur en microbiologie Directeur technique

DR. BRILL + DR. STEINMANN
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-306.10.31

TEST REPORT

18.06.2015

Test report C15L0111Po

Evaluation of the effectiveness of

1592

Test virus: poliovirus type 1 strain LSc-2ab

Method: EN 14476:2013

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

Sponsor:
Christeyns France
Rue de la maladrie 31
FR – 44120 Vertou

Norderoog 2, D - 28259 Bremen
Tel.: +49 421-27819102, Fax: +49 421-2760283
info@brillhygiene.com, http://www.brillhygiene.com

1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, D - 28259 Bremen

2. Identification of sample

Manufacturer	Christeyns France
Name of product	1592
Product diluent recommended by the manufacturer	-
Batch number	PR195-F10
Application	-
Production date	02.03.2015
Expiry date	-
Active compound (s) (100 g)	72 % (w/w) ethanol
Appearance, odour	clear, colorless liquid alcoholic
pH-values (in WSH)	not applicable
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	09.03.2015

3. Materials

3.1 Culture medium and reagents

- Dulbecco`s Modified Eagle`s Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880021)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).

3.2 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 15) were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected**.**

3.3 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 and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (97.0 % and 80.0 %) and as 50.0 %, 25.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30 and 60 seconds and 30 minutes
Interfering substance	0.3 g/l bovine serum albumin (BSA, clean conditions EN 14476:2013)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water (50.0 %, 25.0 % and 10.0 % solutions)
Stability of product in the mix with virus and interfering substance (97.0 % and 80.0 % solutions)	no flocculation, no precipitation
Virus strain	poliovirus type 1 strain LSc-2ab (Chiron-Behring)
Date of testing	09.03.2015 – 18.06.2015
End of testing	18.06.2015

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 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 in order to sediment cell debris. After aliquotation of the supernatant, test virus suspension was stored at -80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated undiluted. Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted. Additionally, the test product was examined as 97.0 % solution (0.1 parts virus suspension + 0.2 parts interfering substance (5-fold) + 9.7 parts disinfectant).

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.

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 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.

5.4 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 EN 14476:2013, 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 %.

5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (97.0 % and 80.0 %) and as 50.0 %, 25.0 % and 10.0 % (demonstration of non-active range) solutions in water at 20 °C according to EN 14476:2013. 30 and 60 seconds and 30 minutes were chosen as contact times.

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 (EN 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.

5.6 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.7 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 (EN 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.

5.8 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

5.9 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined following EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction (maximal virus reduction ≥ 4.25 ± 0.23).

b) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see 6.6.7) was 1.63 ± 0.57 (between 0.5 - 2.5) after 30 min and 2.38 ± 0.37 (between 2.0 - 4.5) after 60 min for poliovirus type 1.

c) The test product (97.0 %) showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BGM cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 6.75 ± 0.33 (PBS) versus 6.75 ± 0.33 (1:100 dilutions of disinfectant, 97.0 %) $\log_{10}TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (97.0 %) showed no decrease (< 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (6.75 ± 0.35 versus 6.75 ± 0.33 $\log_{10}TCID_{50}$/ml).

f) One concentration demonstrated a 4 $\log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with poliovirus type 1 according to EN 14476:2013 is valid.

7. Results

Results of examination are shown in tables 1 to 10. Tables 1 to 9 demonstrate the raw data, whereas table 10 (a+b) gives a summary of results.

The undiluted test product (97.0 % assay) was able to inactivate poliovirus type 1 after 30 seconds in this quantitative suspension test (Tables 1 and 2). The reduction factors were ≥ 4.25 ± 0.23 and ≥ 4.25 ± 0.23 at this time point (mean value ≥ 4.25 ± 0.16). This corresponded to an inactivation of ≥ 99.99 %.

The test product in an 80.0 % assay was not able to inactivate poliovirus type 1 after 30 seconds in this quantitative suspension test (Table 3).

Tested as 50.0 % and 25.0 % solutions, the test product was not active within 30 seconds of exposure time (Tables 4 and 5).

Tested as 10.0 % solution, the test product was not active within 30 minutes of exposure time (Table 6).

8. Conclusion

The disinfectant 1592 tested undiluted demonstrated effectiveness against poliovirus type 1 after an exposure time of 30 seconds under clean conditions.
Therefore, the disinfectant 1592 can be declared as active against poliovirus type 1 as follows:

undiluted 30 seconds

Bremen, 18.06.2015

- Dr. Jochen Steinmann -
Scientific Director

9. 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.

10. Records to be maintained

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

11. Literature

1. EN 14476:2013: 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:

Legend to the Tables

Table 1: Raw data for 1592 (97.0 %) tested against poliovirus type 1 (1st assay)

Table 2: Raw data for 1592 (97.0 %) tested against poliovirus type 1 (2nd assay)

Table 3: Raw data for 1592 (80.0 %) tested against poliovirus type 1

Table 4: Raw data for 1592 (50.0 %) tested against poliovirus type 1

Table 5: Raw data for 1592 (25.0 %) tested against poliovirus type 1

Table 6: Raw data for 1592 (10.0 %) tested against poliovirus type 1

Table 7: Raw data for formaldehyde solution (0.7 %) tested against poliovirus type 1

Table 8: Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %)

Table 9: Raw data (poliovirus type 1) for cell sensitivity (97.0 %)

Table 10 (a+b): Summary of results with 1592 and poliovirus type 1

Legend to the Figures

Figure 1: Virus-inactivating properties of 1592 (97.0 %)

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)

Table 1: Raw data for 1592 (97.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3852) (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%	clean conditions	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1.5	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.
test product cytotoxicity	97.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean 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 4334	0000 0033	0000 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 2: Raw data for 1592 (97.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945) (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%	clean conditions	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.5	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.
test product cytotoxicity	97.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 0444	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0040 0400	0000 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 3: Raw data for 1592 (80.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0%	clean conditions	0.5	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4440	0040 0040	0000 0000	n.d.	n.d.
			1.0	tttt tttt	4444 4444	4444 4444	0003 0040	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.5	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.
test product cytotoxicity	80.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4404	4000 4000	0000 4000	4000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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 4: Raw data for 1592 (50.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0%	clean conditions	0.5	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 4040	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1.5	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.
test product cytotoxicity	50.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4404	4000 4000	0000 4000	4000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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: Raw data for 1592 (25.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	25.0%	clean conditions	0.5	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0000 4004	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1.5	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.
test product cytotoxicity	25.0%	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4404	4000 4000	0000 4000	4000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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 6: Raw data for 1592 (10.0 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0%	clean conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4004 0440	0000 0000	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	10.0%	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4404	4000 4000	0000 4000	4000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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 for formaldehyde solution (0.7 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (3945)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7% (m/V)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4440 4444	0400 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0000 0440	4400 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	4004 4044	0004 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	4444 4444	4444 4444	0000 0000	0000 0000	0000 0000	n.d.	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	3000 4040	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 8: Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %) (3945)

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	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	0040 0000	4000 0000	0000 0000	n.d.
test product	dirty conditions	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 9: Raw data (poliovirus type 1) for cell sensitivity (97.0 %) (3945)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4433 4444	0040 0040	0000 0000	0000 0000	n.d.
test product	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 0004	0000 0000	0000 0000	n.d.
test product	1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 10a: Summary of results with 1592 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	1	1.5	30	60	
test product	97.0%	clean conditions	2.50	≤ 2.50±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.25±0.23)
test product	97.0%	clean conditions	2.50	≤ 2.50±0.00	≤ 2.50±0.00	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.25±0.23)
test product	80.0 %	clean conditions	2.50	6.63±0.41	4.75±0.33	n.d.	n.d.	n.d.	> 0.5
test product	50.0%	clean conditions	2.50	7.75±0.33	n.d.	n.d.	n.d.	n.d.	> 0.5
test product	50.0%	clean conditions	1.50	7.63±0.41	n.d.	n.d.	n.d.	n.d.	> 0.5
test product	10.0%	clean conditions	1.50	n.d.	n.d.	n.d.	8.00±0.38	n.d.	> 30

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

Table 10b: Summary of results with 1592 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.	7.50±0.35	7.00±0.46	6.25±0.44	5.50±0.00	> 60
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.88±0.37	n.a.
virus control 1	97.0%	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.
virus control 2	97.0%	clean conditions	n.a.	7.00±0.38	n.d.	n.d.	n.d.	6.75±0.33	n.a.
virus control	80.0%	clean conditions	n.a.	7.88±0.54	n.d.	n.d.	n.d.	7.63±0.25	n.a.
suppression control	97.0%	clean conditions	2.50	n.d.	n.d.	n.d.	6.75±0.35	n.d.	n.a.
sens.control PBS	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.
sens. control test product	97.0% → 1:100	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.

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

Figure 1: Virus-inactivating properties of 1592 (97.0 %)

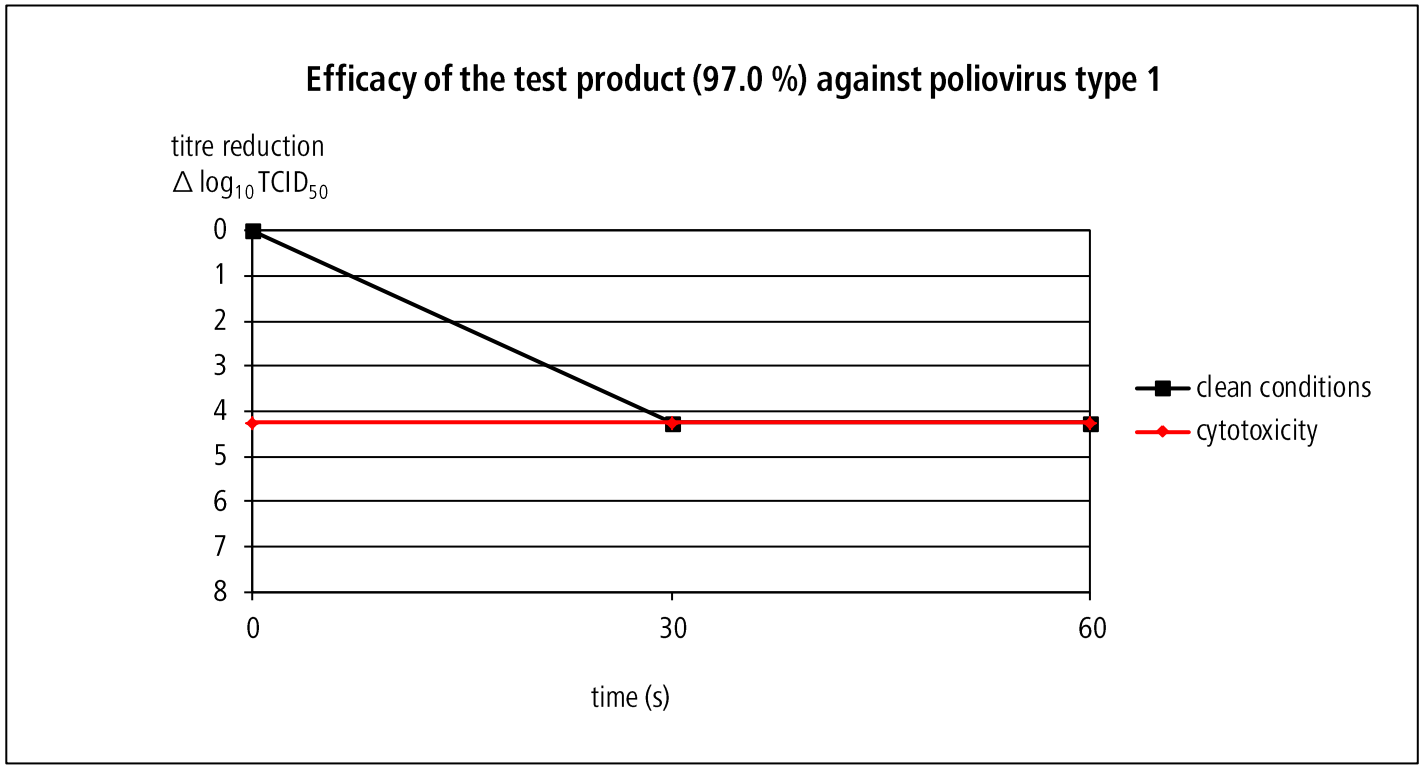
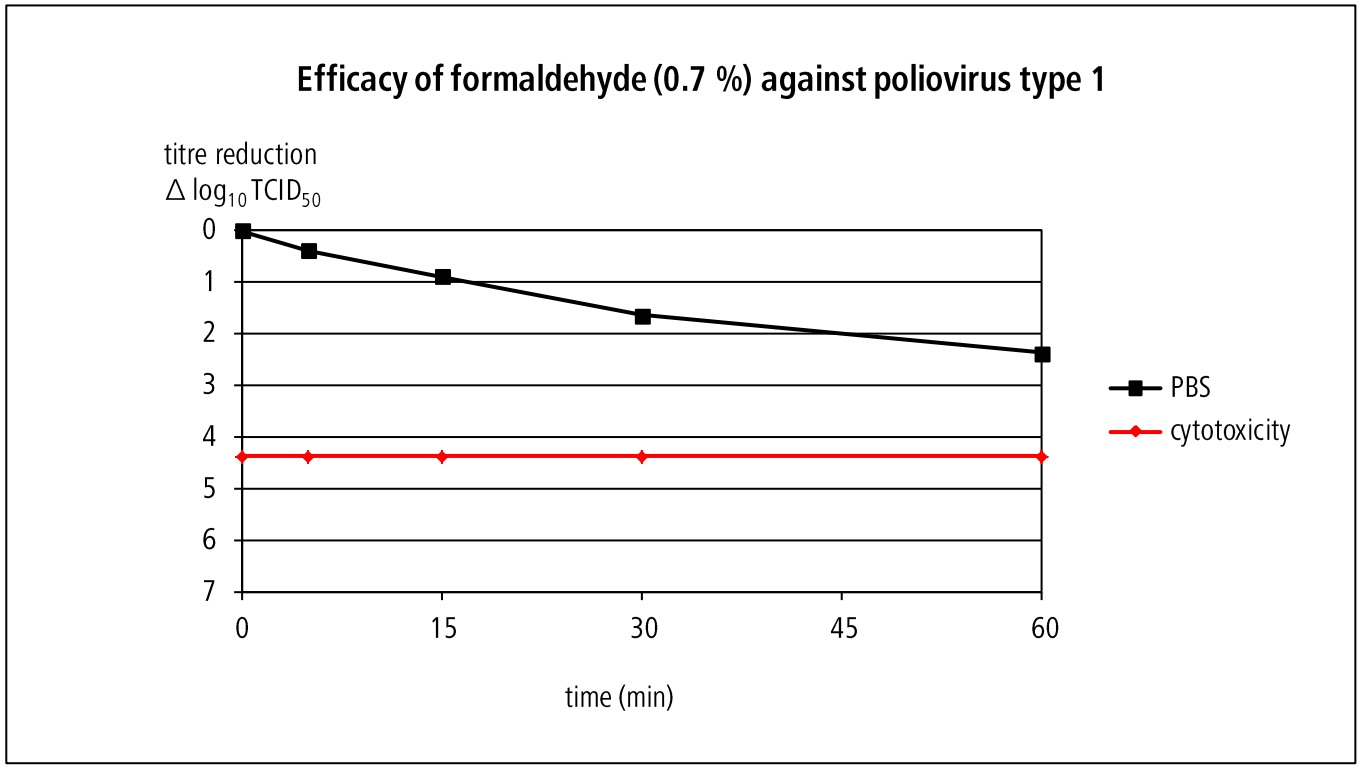


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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LABORATOIRE PHAGOGENE
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Efficacité du PHAGORUB sur l'adenovirus type 5 lors d'un essai de suspension quantitatif à 20°C

RAPPORT D'EXPERTISE

Le PHAGORUB, désinfectant hydro-alcoolique pour les mains du LABORATOIRE PHAGOGENE, a été testé sur son efficacité à inactiver l'adenovirus type 5, souche adenoïd 75, selon la norme EN 14476 : 2007-02.

Dans la norme EN 14476 : 2007-02, 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 mains PHAGORUB a été utilisé non dilué, à une température de 20°C, en 15, 30 et 60 secondes de temps de contact. Après 15 secondes, une réduction du titre viral supérieure à quatre unités $\log_{10}$ a été constatée. L'efficacité du produit est donc démontrée selon les conditions suivantes:

Non dilué **15 secondes**

Dr. J. Steinmann

MIKROLAB GMBH
Laboratory for applied microbiology

Accredited by
Zentralstelle der Länder
für Gesundheitsschutz
bei Arzneimitteln
und Medizinprodukten
ZLG-P-429.08.10

TEST REPORT

20/01/2011

Test report P10ML1138A

Evaluation of the effectiveness of

PHAGORUB

Test virus: adenovirus type 5

Method: NF T72-185; NF EN 14476+A1:2007-01-01

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the hand disinfectant PHAGORUB against adenovirus type 5 using a quantitative suspension assay following EN 14476 (1).

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	LABORATOIRE PHAGOGENE
Name of product	PHAGORUB
Batch number	JD0006.13
Application	hand disinfectant
Expiry date	-
Active compound (s) (100 g)	72 % ethanol
Appearance odour	clear, colourless liquid alcoholic
pH-value (in WSH)	undiluted: 7.75 (20°C)
Storage conditions	room temperature in the dark (area with limited access)
Date of arrival in the laboratory	03/11/2010

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)

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 plates (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flasks (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 (100.0 %), 50.0 % and 10.0 % solutions (non-active range)
Contact time(s)	0.25, 0.5 and 1.0 minutes
Interfering substance(s)	PBS
Diluent	water of standardised hardness (50.0 % and 10.0 % solutions)
Stability of product in the mix with virus and interfering substance (100.0 %)	minor flocculation
Procedure to stop action of product	immediate dilution
Test virus	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Period of analysis	03/11/2010 – 20/01/2011
End of testing	20/01/2011

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 6.3 *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. The active ingredients of this test product had been increased by the factor 1.25 by the producing company. Due to the addition of test virus suspension and interfering substance a 100.0 % solution resulted. The product was additionally tested as 50.0 % and 10.0 % solutions (demonstration of non active range).
These solutions were prepared with water of standardised hardness immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 6.5.1 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 EN 14476: 2007-02, 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 EN 6.6. PHAGORUB was examined undiluted (100.0 %) and as 50.0 % and 10.0 % solutions at 20°C. 0.25, 0.5 and 1.0 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 (EN 6.6.8).

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 EN 6.6.4.1 with 200 µl hard water and 800 µl test product.

6.7 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume hard 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 (EN 6.6.4.2b).
Finally, a comparative titration of the test virus suspension was performed on the pretreated (disinfectant) and non pretreated (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 (EN 6.6.6).

6.9 Reference virus inactivation test

As reference for test validation 0.7 % formaldehyde according to EN 6.6.7.1 was included. Contact times were 5, 15, 30 and 60 min. In addition, cytotoxicity of formaldehyde test solution was determined following EN 6.6.7.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in EN 8.3 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 1:10 dilutions allowing the detection of a four $\log_{10}$ reduction of the virus titre.

c) The comparative titration on pretreated (disinfectant) and non-pretreated (PBS) *A549 cells* showed an acceptable difference (<1 log_{10}; EN 8.3) of virus titre: 7.75 (PBS) versus 8.25 (1:100 dilutions of disinfectant, 100.0 % solution).

d) The control of efficacy for suppression of disinfectant activity demonstrated no reduction of viral infectivity after 30 minutes of exposure time.

Since all criteria according to EN 8.3 were fulfilled, examination with adenovirus type 5 according to EN 14476 was valid.

8. Results

The hand disinfectant PHAGORUB was examined undiluted (100.0 %) and as 50.0 % and 10.0 % solutions at 20°C. 0.25, 0.5 and 1.0 minutes were chosen as exposure times.

Results of examinations are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 gives a summary of results.

In the first assay the undiluted solution of the test product (100.0 %) was able to inactivate adenovirus type 5 after 15 seconds in this quantitative suspension test. The following reduction factor was measured at this time point: ≥ 5.88 (1st assay). In the second assay the activity was confirmed after 15 seconds exposure time: ≥ 5.38 (2nd assay). Both values corresponded to an inactivation of ≥ 99.999 % (mean ≥ 5.63).

The 50.0 % solution was not active against adenovirus type 5. After one minute exposure time, the following reduction factor was measured: 1.75 (50.0 %)

The 10.0 % solution was not active against adenovirus type 5 (Table 8).

9. Summary

In summary, a sufficient reduction of virus titre could be achieved by PHAGORUB of LABORATOIRE PHAGOGENE undiluted after an exposure time of 15 seconds. 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 hand disinfectant PHAGORUB for inactivation of adenovirus type 5 as follows:

undiluted **15 seconds**

Bremen, 20/01/2011

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. EN 14476:2007-02: 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 Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix

Table 1: Raw data of PHAGORUB (100.0 %) tested against adenovirus type 5 at 20°C (1st assay)

Table 2: Raw data of PHAGORUB (100.0 %) tested against adenovirus type 5 at 20°C (2nd assay)

Table 3: Raw data of PHAGORUB (50.0 %) tested against adenovirus type 5 at 20°C

Table 4: Raw data of PHAGORUB (10.0 %) tested against adenovirus type 5 at 20°C

Table 5: Raw data of formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20°C

Table 6: Raw data for the control of efficacy for suppression of disinfectant activity (100.0 %)

Table 7: Raw data (adenovirus type 5) for cell sensitivity to virus (100.0 %)

Table 8: Results with PHAGORUB and adenovirus type 5 (summary)

Table 1: Raw data of PHAGORUB (100.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2449)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	100.0%	PBS	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	100.0%	PBS	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.	PBS	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	0000 0020	0000 0003	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	3323 0003	0101 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 of PHAGORUB (100.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2449)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	100.0%	PBS	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	100.0%	PBS	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.	PBS	n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	0000 0020	0000 0003	0000 0000
			n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3433 3333	3000 0024	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 3: Raw data of PHAGORUB (50.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2421)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	50.0%	PBS	0.25	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	3332 4333	0400 0303	0000 0000	n.d.
			0.5	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	0400 3013	0000 0040	0000 0000	n.d.
			1.0	tttt tttt	4444 4444	4444 4444	4444 4444	2004 0030	4000 0000	0000 0000	0000 0000	n.d.
			5.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	50.0%	PBS	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.	PBS	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 2334	0400 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4333 3333	0003 0001	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 of PHAGORUB (10.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2449)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	10.0%	PBS	0.25	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3003 0001	0003 0000	n.d.
			0.5	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	0031 3330	0000 0000	n.d.
			1.0	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	3333 3243	4231 0000	0000 0000	n.d.
			5.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	10.0%	PBS	n.a.	0000 0000	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.	PBS	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	0000 0020	0000 0003	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	3323 0003	0101 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: Raw data of formaldehyde solution (0.7 %) tested against adenovirus type 5 (quantal test; 8 wells) (2449)

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	4444 4444	4444 4444	3333 3333	2000 1123	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	3443 4444	2212 3332	0001 1000	0002 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	2111 1022	0001 0000	0001 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	0000 0000	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	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	0000 0020	0000 0003	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3334	3323 0003	0101 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: Control of efficacy for suppression of disinfectant activity (100.0 %) (2449)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
product	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3333	3000 2010	0300 0000	n.d.
product	clean	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	dirty	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 7: Raw data (adenovirus type 5) for cell sensitivity to virus (100.0 %) (2449)

Product	Comparative virus titration with	Dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	PBS	without	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3333	3000 0300	0000 0000	n.d.
PBS	clean conditions	without	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS	dirty conditions	without	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	PBS	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3333 3344	3310 1330	0000 0000	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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 8: Results with PHAGORUB and adenovirus type 5 (summary)

Product	Con-centration	Interfering substance	Level of cytotoxicity	$\log_{10}$ TCID$_{50}$/ml after ....min										≥ 4 $\log_{10}$ reduction after ... min
				0	0.25	0.5	1.0	2.0	3.0	5.0	15.0	30.0	60.0	
product	100.0%	PBS	2.50	n.d.	≤2.50	≤2.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
product	100.0%	PBS	2.50	n.d.	≤2.50	≤2.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
product	50.0%	PBS	2.50	n.d.	7.88	7.13	6.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>1.0
product	10.0%	PBS	1.50	n.d.	7.63	7.50	7.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	>1.0
form-aldehyde	0.7% (m/V)	PBS	3.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.13	5.88	≤4.63	≤3.50	60
virus control	n.a.	PBS	n.a.	7.75 7.63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.38 7.88 7.75	n.a.
control of suppression	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.00	n.d.	n.a.
cell sens. (PBS)	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75	n.a.
cell sens. (disinfectant)	1:1000	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.25	n.a.

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

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TEST REPORT

18.11.2009

Test report no. P09ML928-1R

Evaluation of the effectiveness of

Gel Mains Desinfectant

Test virus: human rotavirus strain Wa

Method: following EN 14476:2007-02

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the hand disinfectant Gel Mains Desinfectant against human rotavirus strain Wa using a quantitative suspension assay following EN 14476:2007-02 (1).

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	LABORATOIRE PHAGOGENE
Name of product	Gel Mains Desinfectant
Application	hand hygiene
Batch number	-
Expiry date	-
Active compound (s)	-
Appearance and odour	clear, colourless, viscous liquid; alcoholic
pH-value (s)	undiluted: 6.74 (20°C)
Storage conditions	room temperature in the dark (area with limited access)
Date of arrival in the laboratory	12.10.2009

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)

4.2 Virus and cells

The human rotavirus strain Wa (serotype 1, subgroup II) was obtained from Prof. Dr. Holger Rabenau, Institute of Medical Virology of the Johann Wolfgang Goethe University of Frankfurt, D-60596 Frankfurt. Before the described tests, the virus had been passaged ten times in *MA-104* cells (embryonic rhesus monkey kidney cell line).

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)
- Transferpettor® (Brand GmbH & Co. KG, Wertheim, Germany)
- 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 ± 0.5°C
Concentration of test product	undiluted (80.0 %) and 10.0 % solutions (non-active range)
Contact times	15 and 30 seconds
Interfering substance	PBS
Diluent	water of standardised hardness (10.0 % solution)
Procedure to stop action of product	immediate dilution
Test virus strain	rotavirus strain Wa (VR-2018)
Test period	12.10.2009 – 18.11.2009
End of testing	18.11.2009

6. Methods

6.1 Preparation of test virus suspension

After three washings with serum-free Eagle`s Minimum Essential Medium (EMEM; Cambrex Bio Science Verviers s.p.r.l., B-4800 Verviers, Belgium) cells were incubated with EMEM without fetal calf serum (FCS , Biochrom AG, D-12247 Berlin, Germany) for three hours to eliminate all FCS. This was followed by the addition of virus (stock virus suspension) to MA-104 cells. After appearance of the cytopathic effect, cells were subjected to a rapid three-fold freeze-thawing procedure (-80°C for 20 min; 37°C for 10 min). The resulting fluid was centrifuged at 800 x g for 10 min at 4°C to eliminate cell debris. After aliquotation the supernatant was stored as test virus suspension at –80°C.

6.2 Disinfectant

The test product was evaluated undiluted. Due to the addition of test virus suspension and PBS an 80.0 % solution resulted. The product was also tested as 10.0 % solution (demonstration of non-active range).

The 10.0 % solution was prepared with water of standardised hardness immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM with 5 µg/mL trypsin and 100 µL of each dilution were placed after aspiration of the medium in eight wells of a sterile polystyrene flat bottom 96-well microtitre plate (Nunc A/S, DK-4000 Roskilde, Denmark) with a pre-formed *MA-104* monolayer. After one hour at 37°C, 100 µL EMEM with 5 µg/mL trypsin were added. Incubation was at 37°C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) was calculated according to 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 EN 14476:2007-02, 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 assays

Investigations for determination of virucidal activity followed to EN 6.6. The test product was examined undiluted (80.0 %) and as 10.0 % solution in hard water according to EN 5.2.2.2.
15 and 30 seconds were chosen as contact 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}.
A control of efficiency for suppression of disinfectant activity was included (EN 6.6.6).

Titration of the virus control was performed at contact times 0 min and 60 min (EN 6.6.8).

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 ± 0.5°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 EN 6.6.4.1 with 200 µl hard water 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, the disinfectant in a non-toxic dilution or PBS as a reference was added to the wells of the microtitre plates with a preformed monolayer of *Ma 104 cells*. After at least one hour, a comparative titration was performed on the cells treated in such a manner or treated with PBS only.

6.8 Control of efficacy for suppression of disinfectant activity

Furthermore, a control of efficiency for suppression of disinfectant activity was included (EN 6.6.6).

6.9 Reference virus inactivation test

0.7 (w/v) % formaldehyde was included as reference for test validation according to EN 6.6.7.1. Contact times were 5, 15, 30 and 60 minutes. In addition, cytotoxicity of formaldehyde test solution was determined following EN 6.6.7.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in EN 8.3 were fulfilled:

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

b) The test product (80.0 % solution) showed a cytotoxicity of two log_{10} steps.

c) The comparative titration on pretreated (disinfectant) and non pretreated (PBS) *Ma 104 cells* showed an acceptable difference (<1 log_{10}; EN 8.3) of virus titres: 7.63 (PBS) versus 7.75 (disinfectant) log_{10} $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant activity demonstrated no decrease in virus titre.

Since all criteria following EN 8.3 were fulfilled, examination with human rotavirus strain Wa following EN 14476:2007-02 is valid.

8. Results

Results of examination are shown in tables 1 to 6. Tables 1 to 5 demonstrate the raw data, whereas table 6 gives a summary of results.

The undiluted test product (80.0 %) was able to inactivate rotavirus after 15 seconds in this quantitative suspension test. At that time point, no human rotavirus was detectable. The reduction factors were ≥ 4.25 and ≥ 4.13. This corresponded to an inactivation of ≥ 99.99 %.

The 10.0 % solution showed no activity against human rotavirus after 30 seconds of exposure time.

9. Summary

In summary, a sufficient reduction of virus titre can be achieved by Gel Mains Desinfectant undiluted after an exposure time of 15 seconds. 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 hand disinfectant Gel Mains Desinfectant for inactivation of human rotavirus strain Wa as follows:

undiluted 15 seconds

Bremen, 18.11.2009

- 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 assays.

11. Recorders 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. EN 14476:2007-02: Chemical disinfectants and antiseptics – virucidal quantitative suspension test - Test method and requirements (phase 2, step 1)

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

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

Appendix:

Table 1: Raw data of Gel Mains Desinfectant (80.0 %) tested against human rotavirus strain Wa (1st assay)

Table 2: Raw data of Gel Mains Desinfectant (80.0 %) tested against human rotavirus strain Wa (2nd assay)

Table 3: Raw data of Gel Mains Desinfectant (10.0 %) tested against human rotavirus strain Wa

Table 4: Control of efficacy for suppression of disinfectant activity

Table 5: Raw data (human rotavirus strain Wa) for cell sensitivity

Table 6: Summary of results with Gel Mains Desinfectant and human rotavirus strain Wa

Table 1 (1st assay) : Raw data of Gel Mains Desinfectant (80.0 %) tested against human rotavirus strain Wa (quantal test; 8 wells) (2040)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	80.0%	PBS	0.25	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	80.0%	PBS	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	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4040 3000	0030 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	3333 3333	0130 3434	0030 0103	0000 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	3133 3333	3333 2311	3030 0030	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	3000 0100	0010 0000	0000 0000	0000 0000	0000 0000	n.d.	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2443 4444	0330 0400	4000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4434	0330 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 2 (2nd assay) : Raw data of Gel Mains Desinfectant (80.0 %) tested against human rotavirus strain Wa (quantal test; 8 wells) (2061)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	80.0%	PBS	0.25	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	80.0%	PBS	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	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4040 3000	0030 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	3333 3333	0130 3434	0030 0103	0000 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	3133 3333	3333 2311	3030 0030	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	3000 0100	0010 0000	0000 0000	0000 0000	0000 0000	n.d.	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	1000 0340	0000 0300	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3344 4443	0000 0400	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 3: Raw data of Gel Mains Desinfectant (10.0 %) tested against human rotavirus strain Wa (quantal test; 8 wells) (2040)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	10.0%	PBS	0.25	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0020 0000	0000 0000	n.d.
			0.5	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 2343	4033 0040	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	10.0%	PBS	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	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4040 3000	0030 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	4444 4444	3333 3333	0130 3434	0030 0103	0000 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	3133 3333	3333 2311	3030 0030	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	3000 0100	0010 0000	0000 0000	0000 0000	0000 0000	n.d.	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2443 4444	0330 0400	4000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4434	0330 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 4: Control of efficacy for suppression of disinfectant activity (80.0 %) (2061)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	PBS	tttt tttt	tttt tttt	4444 4444	4444 4433	4433 3320	3430 0010	0000 0020	0000 0000	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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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: Raw data (human rotavirus strain Wa) for cell sensitivity (80.0 %) (2061)

Product	Interfering substance	Product dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 3443	0300 0000	0000 0000	n.d.
PBS	clean conditions	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS	dirty conditions	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4043 0444	0003 3404	0000 0000	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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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: Summary of results with Gel Mains Desinfectant and human rotavirus strain Wa

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/mL after ....min								> 4 log_{10} reduction after ... min
				0	0.25	0.5	1.0	5.0	15.0	30.0	60.0	
product	80.0%	PBS	3.50	n.d.	≤ 3.50	≤ 3.50	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
product	80.0%	PBS	3.50	n.d.	≤ 3.50	≤ 3.50	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
product	10.0%	PBS	2.50	n.d.	7.63	8.00	n.d.	n.d.	n.d.	n.d.	n.d.	> 0.5
form-aldehyde	0.7% (m/V)	PBS	3.50	n.d.	n.d.	n.d.	n.d.	6.88	6.63	5.88	≤3.88	> 60
virus control	n.a.	PBS	n.a.	8.00 7.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75 7.62	n.a.
sens. PBS	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.63	n.a.
sens. product	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.75	n.a.

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

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30.12.2010
Dr St/NM

MikroLab GmbH, Norderoog 2, D-28259 Bremen

LABORATOIRE PHAGOGENE
B.P. 111
F-79103 THOUARS Cedex
FRANCE

Efficacité du PHAGORUB sur le norovirus murin de lors d'un essai de suspension quantitatif à 20°C

RAPPORT D'EXPERTISE

Le PHAGORUB, désinfectant hydro-alcoolique pour les mains du LABORATOIRE PHAGOGENE, a été testé sur son efficacité à inactiver le norovirus murin (MNV, virus de souris) selon la norme EN 14476 : 2007-02.

Le norovirus murin a alors servi de modèle au Norovirus humain (NoV), celui-ci ne pouvant être multiplié sur l'animal ou dans un système de culture de cellules. Le test sur ce virus modèle permet d'émettre des conclusions sur les recommandations d'utilisation du produit à tester en matière d'inactivation du NoV.

Dans la norme EN 14476 : 2007-02, 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 mains PHAGORUB a été utilisé non dilué, à une température de 20°C, en 15, 30 et 60 secondes de temps de contact. Après 15 secondes, une réduction du titre viral supérieure à quatre unités $\log_{10}$ a été constatée. L'efficacité du produit est donc démontrée selon les conditions suivantes:

Non dilué **15 secondes**

Dr. J. Steinmann

MIKROLAB GMBH
Laboratory for applied microbiology

Accredited by
Zentralstelle der Länder
für Gesundheitsschutz
bei Arzneimitteln
und Medizinprodukten
ZLG-P-429.08.10

TEST REPORT

30/12/2010

Test report no. P10ML1138M

Evaluation of the effectiveness of

PHAGORUB

Test virus: Murine norovirus (surrogate for human norovirus)

Method: following NF T72-185; NF EN 14476+A1:2007-01-01

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the hand disinfectant PHAGORUB against murine norovirus (MNV) as surrogate for human norovirus using a quantitative suspension assay following EN 14476:2007-02 (1).

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	LABORATOIRE PHAGOGENE
Name of product	PHAGORUB
Application	hand disinfectant
Lot number	JD0006.13
Expiry date	-
Active substance(s) and concentration(s) in 100 g	72 % ethanol
Appearance and odour	clear, colourless liquid alcoholic
pH-value (s) in SHW	undiluted: 7.75 (20°C)
Storage conditions	room temperature in the dark (area with limited access)
Date of receipt at laboratory	03/11/2010

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)

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 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 ± 1.0°C
Concentration of test product	undiluted (100.0 %) and 10.0 % solution (non-active range)
Contact times	15, 30 and 60 seconds
Interfering substance (s)	PBS
Diluent	water of standardised hardness (10.0 % solution)
Procedure to stop action of disinfectant	immediate dilution
Test virus	murine norovirus (Berlin 06 / 06 / DE Isolate S99)
Period of analysis	03/11/2010 – 30/12/2010
End of testing	30/12/2010

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. A sample was received from the company with an increase of the active ingredients (factor 1.25) resulting in a test concentration of 100.0 %.
The product was additionally tested as 10.0 % (non-active range) solution. The 10.0 % solution was prepared with water of standardised hardness immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined according to EN 6.5.1 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 EN 14476:2007-02, 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 EN 6.6. The test product PHAGORUB was examined undiluted (100.0 %) and as 10.0 % solution at 20°C. Contact times were 15, 30 and 60 seconds.

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 serial dilution.

Titration of the virus control was performed at contact times 0 min and 60 min (EN 6.6.8).

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 EN 6.6.4.1 with 200 µl standard hard water 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 one volume of the lowest apparently non-cytotoxic dilution of the test product or PBS as control were mixed with a volume of double concentrated cell suspension. After 1 h at 37°C the cells were centrifuged and resuspended in cell culture medium (EN 6.6.4.2b).
Finally, a comparative titration of the test virus suspension was performed on the pretreated (disinfectant) and non pretreated (PBS) cells as described above.

6.8 Control of efficacy for suppression of disinfectant activity

In accordance with EN 6.6.6, 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 EN 6.6.7.1. Contact times were 5, 15, 30 and 60 minutes. In addition, cytotoxicity of formaldehyde test solution was determined following EN 6.6.7.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in EN 8.3 were fulfilled:

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

b) There was a cytotoxicity of the test product in the 1:10 dilutions (100.0 %) thus allowing the demonstration of a 4 log_{10} reduction.

c) The comparative titration on pretreated (disinfectant in the lowest apparently non-cytotoxic dilution) and non pretreated (PBS) *RAW 264.7 cells* showed a difference < 1 log_{10} of virus titre: 7.88 (PBS) versus 8.00 (disinfectant) log_{10} $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant activity demonstrated no significant reduction of viral infectivity after 30 minutes of exposure time.

Since all criteria following EN 8.3 were fulfilled, examinations with MNV following EN 14476:2007-02 were valid.

8. Results

Results of examination are shown in tables 1 to 6. Tables 1 to 5 demonstrate the raw data, whereas table 6 gives a summary of results.
The undiluted test product (100.0 %) was able to inactivate MNV after 15 seconds exposure time in this quantitative suspension test. The following reduction factors were achieved at this time point: ≥ 5.13 and ≥ 5.63 (mean ≥ 5.38). This mean value corresponded to an inactivation of ≥ 99.999 % (table 6).

The 10.0 % solution of the test product was not able to inactivate MNV by 4 log_{10} steps after 60 seconds of exposure time (table 6).

9. Summary

The hand disinfectant PHAGORUB demonstrated effectiveness against MNV undiluted after a contact time of 15 seconds. 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 hand antiseptic PHAGORUB for inactivation of MNV (surrogate for human norovirus) as follows:

undiluted 15 seconds

Bremen, 30/12/2010

- 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. Recorders 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. EN 14476:2007-02: 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 Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix:

Table 1: Raw data for PHAGORUB (100.0 %) tested against MNV (1st assay)

Table 2: Raw data for PHAGORUB (100.0 %) tested against MNV (2nd assay)

Table 3: Raw data for PHAGORUB (10.0 %) tested against MNV

Table 4: Control of efficacy for suppression of disinfectant activity (100.0 %)

Table 5: Raw data (MNV) for cell sensitivity (100.0 %)

Table 6: Summary of results with PHAGORUB and MNV

Table 1 (1st assay) : Raw data for PHAGORUB (100.0 %) tested against MNV (quantal test; 8 wells) (2448)

Product	Concentration	Interfering substance	Contact time (min.)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	100.0%	PBS	0.25	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	100.0%	PBS	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (w/v)	PBS	5	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4440 4404	0000 0000	0000 0000	0000 0000
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0430 3000	0000 0000	0000 0000	0000 0000
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4042 3440	0000 0040	0000 0000	0000 0000	0000 0000
			60	tttt tttt	tttt tttt	tttt tttt	4444 4444	4040 0000	0000 0000	0000 0000	0000 0000	0000 0000
formaldehyde cytotoxicity	0.7% (w/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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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 2 (2nd assay) : Raw data for PHAGORUB (100.0 %) tested against MNV (quantal test; 8 wells) (2448)

Product	Concentration	Interfering substance	Contact time (min.)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	100.0%	PBS	0.25	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	100.0%	PBS	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (w/v)	PBS	5	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4440 4404	0000 0000	0000 0000	0000 0000
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0430 3000	0000 0000	0000 0000	0000 0000
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4042 3440	0000 0040	0000 0000	0000 0000	0000 0000
			60	tttt tttt	tttt tttt	tttt tttt	4444 4444	4040 0000	0000 0000	0000 0000	0000 0000	0000 0000
formaldehyde cytotoxicity	0.7% (w/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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4033 4004	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 3 : Raw data for PHAGORUB (10.0%) tested against MNV (quantal test; 8 wells) (2448)

Product	Concentration	Interfering substance	Contact time (min.)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0%	PBS	0.25	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0444	0004 0400	0000 0400	n.d.
			0.5	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 0400	0000 0000	n.d.
			1.0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0444 0444	4000 0000	0000 0040	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	10.0%	PBS	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (w/v)	PBS	5	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4440 4404	0000 0000	0000 0000	0000 0000
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0430 3000	0000 0000	0000 0000	0000 0000
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4042 3440	0000 0040	0000 0000	0000 0000	0000 0000
			60	tttt tttt	tttt tttt	tttt tttt	4444 4444	4040 0000	0000 0000	0000 0000	0000 0000	0000 0000
formaldehyde cytotoxicity	0.7% (w/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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 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 microrelates)

Table 4 : Control of efficacy for suppression of disinfectant activity (100.0 %) (2448)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0004 0400	0000 0000	0000 0040	0000 0000
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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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: Raw data (MNV) for cell sensitivity (100.0 %) (2448)

Product	Comparative virus titration with	Dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	PBS	without	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4040 0400	0000 0000	0000 0000
test product	PBS	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	PBS	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3240 0004	0000 0000	0000 0000
test product	PBS	1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

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

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

Table 6: Summary of results with PHAGORUB and MNV

Product	Concentr.	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min									> 4 log_{10} reduction after ... min
				0	0.25	0.5	1	2	5	15	30	60	
product	100.0%	PBS	2.50	n.d.	≤2.50	≤2.50	≤2.50	n.d.	n.d.	n.d.	n.d.	n.d.	0.25 (RF = ≥ 5.13)
product	100.0%	PBS	2.50	n.d.	≤2.50	≤2.50	≤2.50	n.d.	n.d.	n.d.	n.d.	n.d.	0.25 (RF = ≥ 5.63)
product	10.0%	PBS	1.50	n.d.	7.75	7.63	7.50	n.d.	n.d.	n.d.	n.d.	n.d.	> 1.0
form-aldehyde	0.7% (w/v)	PBS	4.50	n.d.	n.d.	n.d.	n.d.	n.d.	7.25	6.88	6.38	5.75	> 60
virus control	n.a.	PBS	n.a.	7.75	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.63 8.13	n.a.
suppression control	100.0%	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.75	n.d.	n.a.
cell sens. PBS	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.88	n.a.
cell sens. disinfectant	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.00	n.a.

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

Accredited by
Zentralstelle der Länder
für Gesundheitsschutz
bei Arzneimitteln
und Medizinprodukten
ZLG-P-429.08.10

Final test report #S09ML820V
submitted to

LABORATOIRES PHAGOGENE GROUPE RIVADIS
B.P. 111
F 79103 THOUARS Cedex

Evaluation of the effectiveness of

GEL MAINS DESINFECTANT

against

vaccinia virus strain Elstree

Test method according to the guideline of DVV and RKI
dating 01.08.2008

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14.08.2009

Test report number # P09ML820V

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	GEL MAINS DESINFECTANT
Manufacturer	LABORATOIRES PHAGOGENE GROUPE RIVADIS
Application	hand disinfectant
Lot no.	-
Expiry date	-
Active substance(s) and concentration(s) in 100 g	ethanol
Appearance and odour	clear, colourless, gelic liquid; product specific
pH-value (s) (in hard water)	undiluted: 8.16 (20°C)
Conditions of storage	room temperature in the dark (area with limited access)
Date of receipt at laboratory	08.07.2009

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Cambrex Bio Science Verviers s.p.r.l., catalogue no. 12-125F)
- fetal calf serum (Biochrom AG, article no. S 0115)
- formaldehyde (Riedel-de-Häen, article no. 33220)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)

3.2 Virus and cells

Vaccinia virus strain Elstree originated from the Institute of Medical Virology and Immunology of the University of Essen, D-45122 Essen. Before inactivation assays, virus had been

passaged 10 times in *GMK AH-1 cells* (green monkey kidney cell line) and three times in *HeLa cells*.

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.

3.3 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 and fixed-volume pipettes (Eppendorf AG)

4. Experimental conditions

Test temperature	20°C ± 0.5°C
Concentration of test product	undiluted (80.0 %) and 10.0 % (non active range)
Contact times	15 and 30 seconds
Interfering substance	fetal calf serum (FCS)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water of standardised hardness (10.0 %)
Virus strain	vaccinia virus strain Elstree
Date of testing	08.07.2009 – 14.08.2009
End of testing	14.08.2009

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension, *Vero cells* (ATCC CC81; permanent monkey kidney cells) were cultivated with Eagle's Minimum Essential Medium (EMEM, Cambrex Bio Science Verviers s.p.r.l., B-4800 Verviers, Belgium) and 10 % or 2 % fetal calf serum (FCS, Biochrom AG, D-12247 Berlin, Germany).
Vero cells were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a twofold freeze/thaw procedure 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.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated undiluted. Due to the addition of test virus suspension and the interfering substance (FCS) a test concentration of 80.0 % resulted. The 10.0 % solution was prepared with hard water.

5.3 Inactivation assays and controls

Tests were carried out in accordance with the DVV and RKI guideline (1). Eight parts by volume of the disinfectant were mixed with one part by volume of test virus suspension and one part by volume of Aqua bidest. In tests with interfering substance, instead of Aqua bidest., one part by volume of fetal calf serum was added. Immediately at the end of the chosen exposure time, activity of the disinfectant was stopped by serial dilutions.

Due to a more convenient handling and due to a limited amount of test virus suspension, the volumes in the inactivation assay were 0.1 mL test virus suspension, 0.1 mL interfering substance and 0.8 mL test product.

Virus controls were incorporated after the longest exposure time. One part by volume of test virus suspension was mixed with nine parts by volume of Aqua bidest. or with one part by volume of FCS and eight parts by volume of Aqua bidest.

A control was carried out with one part by volume of test virus suspension, four parts by volume of PBS (0.1 M, pH value 7.0) and five parts by volume of 1.4 % formaldehyde solution. 5, 15, 30 and 60 minutes were chosen as contact times.

For determination of cytotoxicity of the disinfectant, two parts by volume of Aqua bidest. were mixed with eight parts by volume of the disinfectant, diluted with ice-cold EMEM and inoculated onto permissive cells. Values are given as $log_{10}CD_{50}$/mL (in analogy to $log_{10}TCID_{50}$/mL).

For the control of cell sensitivity two parts by volume Aqua bidest. or one part by volume of FCS and one part by volume Aqua bidest were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product or PBS. This mixture was added to the permissive cell culture. After 1 h at 37°C the mixture was discharged and a comparative titration of the test virus suspension was performed on the pre-treated and non pre-treated (PBS) cells as described above.

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 0.5°C. Aliquots were retained after appropriate exposure times, and the residual infectivity was determined.
The inactivation experiments were run in two independent assays (two different days).

A control of efficiency for suppression of disinfectant activity was not included since at the end of the exposure time dilutions were done immediately.
Furthermore, a cell control was incorporated.

5.4 Determination of infectivity

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM with 2 % FCS and 100 µL of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed microtitre plate (Nunc A/S, DK-4000 Roskilde, Denmark). 100 µL of a fresh trypsinized *Vero cells* were added. Suspension was adjusted to reach approximately 10-15 x 10^3 cells per well. Incubation was at 37°C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) (with 95 % level of confidence) was calculated according to 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.

5.5 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 (virus control). The difference is given as reduction factor (RF).

According to the guideline (Leitlinie) of DVV/RKI, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four $\log_{10}$ steps.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of GEL MAINS DESINFECTANT (80.0 % and 10.0 %) and 0.7 % formaldehyde was measured. The formaldehyde solution was toxic for the *Vero cells* in the 1:100 dilutions. This corresponded to a $\log_{10}CD_{50}$/mL of 3.50 (Table 1).

Examinations also showed that the hand disinfectant GEL MAINS DESINFECTANT tested undiluted achieved a $\log_{10}CD_{50}$/mL of 2.50. The 10.0 % solution was not cytotoxic in the 1:10-dilutions. This corresponded to a $\log_{10}CD_{50}$/mL of 1.50 (Table 1).

These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated vaccinia virus could be determined.

6.2 Control of cell sensitivity

A non-cytotoxic concentration of the disinfectant might inhibit the virus replication. Therefore, the cell sensitivity in a non-cytotoxic concentration was evaluated by a comparative titration.

The comparative virus titration on cells treated with the disinfectant and PBS resulted in a difference of < 0.5 $\log_{10}$ of virus titre in the presence of the disinfectant demonstrating that virus replication was not inhibited (Table 3).

6.3 Virus-inactivating properties of formaldehyde control

Results of inactivation tests are found in table 3. Formaldehyde (0.7 %) reduced the vaccinia virus titre after five minutes by 0.88 ± 0.41 log_{10} steps. After 15 and 30 minutes reduction factors of 1.38 ± 0.51 and 2.25 ± 0.48 were measured. After 60 minutes the reduction of virus titre reached 4.13 ± 0.25 log_{10} steps (Table 3).

6.4 Virus-inactivating properties of disinfectant

Results of inactivation assays are demonstrated in tables 2 to 4.
The hand disinfectant GEL MAINS DESINFECTANT was examined undiluted (80.0 %) and as 10.0 % solution (demonstration of the non-active range). 15 and 30 seconds were chosen as exposure times.

GEL MAINS DESINFECTANT was already active against vaccinia virus undiluted in all assays after 15 seconds of exposure. The reduction factors were ≥ 5.38 ± 0.37 and ≥ 5.13 ± 0.25 (assays without soil load) and ≥ 5.25 ± 0.33 and ≥ 5.50 ± 0.44 (assays with FCS), respectively. The following mean values were calculated: ≥ 5.26 ± 0.25 (assay without soil load) and ≥ 5.38 ± 0.25 (assay with FCS).

The 10.0 % solution was chosen for testing the disinfectant in the non-active range. Table 4 shows that in the assay with FCS no efficacy was found after 30 seconds.

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 hand disinfectant GEL MAINS DESINFECTANT for inactivation of vaccinia virus as follows:

undiluted 15 s

- Dr. J. Steinmann -

7. 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.

8. Recorders 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.

9. Literature

1. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Institutes (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (in der Fassung vom 1. August 2008)
Bundesgesundheitsbl., 51, 2008, 936-445

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

table 1: cytotoxicity of GEL MAINS DESINFECTANT and 0.7% formaldehyde

	conc.	soil load	dilutions				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	10.0%	Aqua bidest.	-	-	-	-	-
product	10.0%	10.0% FCS	-	-	-	-	-
product	80.0%	Aqua bidest.	+	-	-	-	-
product	80.0%	10.0% FCS	+	-	-	-	-
formaldehyde	0.7%	PBS	+	+	-	-	-

table 2 (1st assay): inactivation of vaccinia virus by GEL MAINS DESINFECTANT (80.0%) and formaldehyde (0.7%) in a quantitative suspension test at 20°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s	
test product	80.0%	Aqua bid.	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥5.38±0.37	≥5.38±0.37	n.a.	n.a.	15 s
test product	80.0%	10.0% FCS	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥5.25±0.33	≥5.25±0.33	n.a.	n.a.	15 s
controls	**conc.**	**soil load**	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	7.88±0.37	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	7.75±0.33	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

table 3 (2nd assay): inactivation of vaccinia virus by GEL MAINS DESINFECTANT (80.0%) in a quantitative suspension test at 20°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s	
test product	80.0%	Aqua bid.	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥5.13±0.25	≥5.13±0.25	n.a.	n.a.	15 s
test product	80.0%	10.0% FCS	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥5.50±0.44	≥5.50±0.44	n.a.	n.a.	15 s
controls	**conc.**	**soil load**	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min.	
formaldehyde	0.7%	PBS	6.75±0.33	6.63±0.44	5.38±0.41	≤3.50±0.00	0.88±0.41	1.38±0.51	2.25±0.48	≥4.13±0.25	60 min
virus control	n.a.	Aqua bid.	n.d.	n.d.	n.d.	7.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	8.00±0.44	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	8.00±0.38	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	7.50±0.00	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	7.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.

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

table 4: inactivation of vaccinia virus by GEL MAINS DESINFECTANT (10.0%) in a quantitative suspension test at 20°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s	
test product	10.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	10.0%	10.0% FCS	7.50±0.00	7.88±0.37	n.d.	n.d.	0.50±0.44	0.13±0.58	n.a.	n.a.	> 30 s
controls	**conc.**	**soil load**	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	8.00±0.44	n.a	n.a.	n.a	n.a.	n.a
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Appendix table 1: Raw data (vaccinia virus) of Gel Mains Desinfectant (1st assay) (1913)

product	concentration	interfering substance	exposure time (min.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	Aqua bidest.	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	80.0%	Aqua bidest.	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4213	3004 0040	0000 0000	0000 0000
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3444 4444	4000 4000	0000 0000	0000 0000

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

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix table 2: Raw data (vaccinia virus) of Gel Mains Desinfectant (2nd assay) (1929)

product	concentration	interfering substance	exposure time (min.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	Aqua bidest.	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	80.0%	Aqua bidest.	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10,0% FCS	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4344 3434	0002 0000	0000 0000	0000 0000
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4343 2034	4300 4330	0000 0000	0000 0000

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

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix table 3: Raw data (vaccinia virus) of Gel Mains Desinfectant (1929)

product	concentration	interfering substance	Exposure time (min.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	10.0%	Aqua bidest.	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	0.25	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3424 3421	0000 0000	0000 0000	n.d.
			0.5	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4442 3444	0024 3000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	10.0%	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4344 3434	0002 0000	0000 0000	0000 0000
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4343 2034	4300 4330	0000 0000	0000 0000

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

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix table 4: Raw data (vaccinia virus) of formaldehyde control (20°C) (1929)

product	concentration	interfering substance	exposure time (min)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7% (m/V)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0310 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	1103 2100	0000 0020	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	2222 2223	1012 1012	0000 0010	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	0000 0000	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	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.

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

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 5: Raw data of cell sensitivity of Gel Mains Desinfectant (1782)

product	interfering substance	dilutions	dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	-	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2443 4433	0030 3204	0000 4000	n.d.
product	Aqua bidest.	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4433 4442	0000 0000	0000 0000	n.d.
		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.
product	FCS	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4343 1412	0000 0003	0000 0000	n.d.
		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.

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

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

MIKROLAB GMBH
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ZLG-P-429.08.10

TEST REPORT

09.09.2009

Test report no. R09ML820aB

Evaluation of the effectiveness of

Gel Mains Desinfectant

Testvirus:	Bovine Viral Diarrhea Virus (BVDV) (Surrogate of HCV)
Testmethod:	according to the guideline of DVV and RKI (dating 01.08.2008)

Client:
LABORATOIRES PHAGOGENE GROUPE RIVADIS
B.P. 111
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Test report number R09ML820aB

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	Gel Mains Desinfectant
Manufacturer	LABORATOIRE PHAGOGENE
Application	hand disinfection
Lot no.	-
Expiry date	-
substance(s) and concentration(s) in 100 g	-
Appearance and odour	clear, colourless gel; alcoholic
pH-value (s) (in hard water)	undiluted: 8.16 (20°C)
Conditions of storage	room temperature in the dark (area with limited access)
Date of receipt at laboratory	08.07.2009

3. Materials

3.1 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)

3.2 Virus and cells

BVDV strain NADL (VR-534) was obtained from Dr. Stephanie Bendtfeld, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, D-30559 Hannover). Prior to inactivation assays, the virus was passaged once in *primary bovine kidney cells* and five times in *KOP-R cells* (primary cells from bovine oropharyngeal tissue). *KOP-R cells* originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems) (Dr. R. Riebe, catalogue no. RIE 244). In the inactivation assays *ekl cells* (embryonal cells from bovine lung tissue) were used. These cells originated from Mrs. A. Kyas (Henkel KGaA, D-40191 Düsseldorf).

3.3 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 and fixed-volume pipettes (Eppendorf AG)

4. Experimental conditions

Test temperature	20°C ± 0.5°C
Concentration of test product	undiluted (80.0 %) and 10.0 % (demonstration of non-active range)
Contact times	15 and 30 seconds
Interfering substance	fetal calf serum (FCS)
Procedure to stop action of disinfectant	immediate dilution; assay following method of Lycke
Diluent	water of standardised hardness (10.0 % solution)
Virus strain	BVDV strain NADL
Date of testing	08.07.2009 – 09.09.2009
End of testing	09.09.2009

5. Methods

5.1 Preparation of test virus suspension

For the preparation of the test virus suspension, *KOP-R cells*, which were cultivated with Eagle's Minimum Essential Medium (EMEM) supplemented with L-glutamine, sodium pyruvate and 10 % or 2 % fetal calf serum (FCS), were infected with BVDV (stock virus suspension). As soon as cells showed a constant cytopathic effect, they were subjected to a rapid freeze/thawing procedure. This was followed by 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.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated undiluted. Due to the addition of test virus suspension and the interfering substance (FCS) a test concentration of 80.0 % resulted. The 10.0 % solution was prepared with hard water.

5.3 Inactivation assays and controls

Tests were carried out in accordance with the DVV and RKI guideline (1). Eight parts by volume of the disinfectant were mixed with one part by volume of test virus suspension and one part by volume of Aqua bidest. In tests with interfering substance, instead of Aqua bidest., one part by volume of fetal calf serum was added. Immediately at the end of the chosen exposure time, activity of the disinfectant was stopped by serial dilutions.

Due to a more convenient handling and due to a limited amount of test virus suspension, the volumes in the inactivation assay were 0.1 mL test virus suspension, 0.1 mL interfering substance (FCS) and 0.8 mL test product.

Virus controls were incorporated after the longest exposure time. One part by volume of test virus suspension was mixed with nine parts by volume of Aqua bidest. or with one part by volume of FCS and eight parts by volume of Aqua bidest.

A control was carried out with one part by volume of test virus suspension, four parts by volume of PBS (0.1 M, pH value 7.0) and five parts by volume of 1.4 % formaldehyde solution. 5, 15 and 30 minutes were chosen as contact times.

For determination of cytotoxicity of the disinfectant, two parts by volume of Aqua bidest. were mixed with eight parts by volume of the disinfectant, diluted with ice-cold EMEM and inoculated onto permissive cells. Values are given as $log_{10}CD_{50}$/ml (in analogy to $log_{10}TCID_{50}$/ml).

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 0.5°C. Aliquots were retained after appropriate exposure times, and the residual infectivity was determined.
The inactivation experiments were run in two independent assays (two different days).

A control of efficiency for suppression of disinfectant activity was not included since at the end of the exposure time dilutions were done immediately.

Furthermore, a cell control was incorporated.

5.4 Determination of infectivity

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM and 100 µl of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed microtitre plate (Nunc A/S, DK-4000 Roskilde, Denmark). 100 µl of *ekl cells* were added into the plates one day earlier. Suspension was adjusted to reach approximately 10-15 x 10^3 cells per well. Incubation was at 37°C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) (with 95 % level of confidence) was calculated according to 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.

5.5 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 (virus control). The difference is given as reduction factor (RF).

According to the guideline (Leitlinie) of DVV/RKI, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four $\log_{10}$ steps.

5.6 Inactivation assay following the method of Lycke

Following a modified procedure as described by Lycke (4), the test mixture was further diluted 1:1000 in EMEM and then the total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$-reduction of virus titre. This method is necessary for those products which demonstrate a great cytotoxicity.

100 µl of the test virus suspension and 100 µl Aqua bidest. and FCS, respectively were mixed with 800 µl disinfectant at 20°C. At the end of the exposure time (15 s) 62.5 µl of the mixture were immediately added to 62.5 ml EMEM (1:1000 dilutions) and then the volume was distributed in six microtitre plates (108 µl / well). After 10 days of inoculation cultures were observed for cytopathic effects. The calculation of virus titre followed the formula of Lycke:

$$-\log_{10} = [1.4 \times \ln (1\text{-}p)].$$

p is meaning the relation between the positive wells with virus detection in comparison to the total number of wells.

For the control of cell sensitivity 200 µl Aqua bidest. were mixed with 800 µl disinfectant (PBS as control). Then, an aliquot was diluted 1:1000 and 108 µl of this dilution were added to the wells of the microtitre plates with a preformed monolayer of *ekl cells*. After at least one hour, a comparative virus titration was performed on the cells treated in such a manner or treated with PBS only.

Determination of the initial virus titre was performed in a quantitative suspension tests by a fivefold assay (see 5.3). The virus-inactivating properties of the test product were calculated by subtracting the virus titre in the test mixture from the virus control.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of Gel Mains Desinfectant (80.0 % and 10.0 %) and 0.7 % formaldehyde was measured. The formaldehyde solution was toxic for the *ekl cells* in the 1:1000 dilutions. This corresponded to a $\log_{10}CD_{50}$/ml of 4.50 (Table 1).

Examinations also showed that the hand disinfectant Gel Mains Desinfectant tested undiluted achieved a $\log_{10}CD_{50}$/m- of 3.50 (cytotoxicity in the 1:100-dilutions). The 10.0 % solution showed no cytotoxicity in the 1:10-dilution, corresponding to a $\log_{10}CD_{50}$/ml of ≤ 1.50 (Table 1).

These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated BVDV could be determined.

6.2 Virus-inactivating properties of formaldehyde control

Formaldehyde (0.7 %) reduced the BVDV titre after five and 15 minutes by 0.75 ± 0.65 and ≥ 1.25 ± 0.48 $\log_{10}$ steps. After 30 minutes a reduction factor of ≥ 1.25 ± 0.48 was measured (Table 2).

6.3 Virus-inactivating properties of disinfectant

Results of inactivation assays (quantitative suspension test) are demonstrated in tables 2 to 3.

The hand disinfectant Gel Mains Desinfectant was examined undiluted (80.0 %) and as 10.0 % solution. 15 and 30 seconds were chosen as exposure times in these experiments.

Gel Mains Desinfectant was active against BVDV undiluted after 15 seconds of exposure time. The reduction factor was ≥ 2.25 ± 0.48 (assay without soil load) and ≥ 2.38 ± 0.37 (assay with soil load). Due to the cytotoxicity no reduction of 4 $\log_{10}$ steps could be demonstrated.

Additionally, the product was examined as 10.0 % solution in the presence of FCS for demonstrating the non-active range. After 30 seconds no sufficient reduction of was titre was detectable.

6.4 Virus-inactivating properties of the disinfectant following the method of Lycke*

As mentioned above, a 4 $\log_{10}$ reduction could not be measured in the quantitative suspension test due to high cytotoxicity. Therefore, the method of Lycke was introduced. The results are given in Appendix tables 5 and 6. The virus titres in the fivefold assay were $\log_{10}$ $TCID_{50}$ / ml = 6.25 (assay without soil load) and 6.25 (assay with FCS).

These values corresponded to the virus amount in the assays to 5.11 (assay without soil load) and 5.08 (assay with FCS) $\log_{10}$ $TCID_{50}$.
Since in 576 cell culture units in the assay without soil load no residual virus could be detected the result according to the formula of Lycke is $\log_{10}$ = 0. The reduction factor is therefore $\log_{10}$ 5.11 minus $\log_{10}$ 0 = 5.11.
In the presence of FCS the result is $\log_{10}$ = 0. The reduction factor is therefore $\log_{10}$ 5.08 minus $\log_{10}$ 0 = 5.08 after an exposure time of 15 seconds.

A control for cell sensitivity according to Lycke was evaluated by a comparative titration. The comparative virus titration on cells treated with the disinfectant (RF ≥ 6.63 ± 0.25) and PBS (RF ≥ 6.25 ± 0.44) resulted in a difference of ≤ 0.5 $\log_{10}$ of virus titre in the presence of the disinfectant demonstrating that virus replication was not inhibited (Appendix table 4).

In summary, Gel Mains Desinfectant was able to inactivate BVDV (surrogate of Hepatitis C Virus) as follows:

	undiluted	**15 sec**

- **B. Bischoff**

* Method not accredited

7. 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.

8. Recorders 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.

9. Literature

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Arch Exp Path Pharmak; 162, 1931, 480-487

4. Lycke, E.: Studies of the Inactivation of Poliomyelitis Virus by Formaldehyde.
Arch Ges Virusforsch; 7, 1957: 483-493

Table 1: cytotoxicity of Gel Mains Desinfectant and 0.7 % formaldehyde

	conc.	soil load	dilutions				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	80.0%	Aqua bidest.	+	+	-	-	-
product	80.0%	10.0% FCS	+	+	-	-	-
product	10.0%	Aqua bidest.	n.d.	n.d.	n.d.	n.d.	n.d.
product	10.0%	10.0% FCS	-	-	-	-	-
formaldehyde	0.7 %	PBS	+	+	+	-	-

Table 2: inactivation of BVDV by Gel Mains Desinfectant (80.0 %) and formaldehyde in a quantitative suspension test at 20°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s	
test product	80.0 %	Aqua bid.	≤3.50±0,00	≤3.50±0.00	n.d	n.d.	≥2.25±0.48	≥2.25±0.48	n.a.	n.a.	≥ 15 s
test product	80.0 %	10.0% FCS	≤3.50±0,00	≤3.50±0.00	n.d	n.d.	≥2.38±0.37	≥2.38±0.37	n.a.	n.a.	≥ 15 s
controls	**conc.**	**soil load**	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	≤5.00±0.44	≤4.50±0.00	≤4.50±0.00	n.d.	≥0.75±0.65	≥1.25±0.48	≥1.25±0.48	n.a.	≥ 15 min
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.75±0.48	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.88±0.37	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 3 : inactivation of BVDV by Gel Mains Desinfectant (10.0%) and formaldehyde in a quantitative suspension test at 20°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			15 s	30 s	60 s	120 s	15 s	30 s	60 s	120 s	
test product	10.0 %	Aqua bid.	n.d	n.d.	n.d	n.d.	n.a.	n.a.	n.a.	n.a.	n.d.
test product	10.0 %	10.0% FCS	6.38±0.25	6.13±0.45	n.d.	n.d.	0.00±0.45	0.00±0.59	n.a.	n.a.	> 30 s
controls	**conc.**	**soil load**	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
frmaldehyde	0.7%	PBS	n.d	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	6.00±0.38	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

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TEST REPORT

20.11.2009

Test report no. P09ML928-1SI

Evaluation of the effectiveness of

Gel Mains Desinfectant

Test virus: Influenza A virus H1N1 (swine)

Method: following EN 14476:2007-02

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the hand disinfectant Gel Mains Desinfectant against influenza A virus H1N1 (swine) using a quantitative suspension assay following EN 14476:2007-02 (1).

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	LABORATOIRE PHAGOGENE
Name of product	Gel Mains Desinfectant
Application	hand disinfection
Batch number	-
Expiry date	-
Active compound (s)	-
Appearance and odor	clear, colourless, viscous liquid; product specific
pH-value (s)	undiluted: 6.74 (20°C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	12.10.2009

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)

4.2 Virus and cells

The influenza A virus sw/Greven/IDT2889/2004 H1N1 virus was obtained from Prof. Dr. Georg Herrler, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, D-30559 Hannover). This virus strain was introduced in this study as surrogate of the pandemic strain influenza A virus /California/04/2009 H1N1 due to bio safety reasons.

The *MDCK cells* were obtained from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems) (Dr. R. Riebe, catalogue no. RIE 244).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations 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)
- Transferpettor® (Brand GmbH & Co. KG, Wertheim, Germany)
- 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 10.0 % solutions (non-active range)
Contact times	15 and 30 seconds
Interfering substance	PBS
Diluent	water of standardised hardness (10.0 % solution)
Procedure to stop action of product	immediate dilution
Test virus strain	influenza A virus sw/Greven/IDT2889/2004 H1N1
Test period	12.10.2009 – 20.11.2009
End of testing	20.11.2009

6. Methods

6.1 Preparation of test virus suspension

To prepare the test virus suspension, *MDCK cells* that had been cultured with Eagle´s minimum essential medium (EMEM) and 10 % or 2 % fetal calf serum (FCS) were inoculated with swine influenza A virus in 175 cm^2 cell culture flasks. Once a cytopathic effect had been induced (approx. 24 hours), freezing and thawing was carried out once. The cell debris was removed by centrifugation at 3.000 rpm for ten minutes (4°C) and the supernatant was recovered as test virus suspension and stored in aliquots at -80°C.

6.2 Disinfectant

The test product was evaluated undiluted. Due to the addition of test virus suspension and PBS an 80.0 % solution resulted. The product was also tested as 10.0 % solution (demonstration of non-active range).

The 10.0 % solution was prepared with water of standardised hardness immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined 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 EMEM, were transferred to eight cups of a sterile polystyrol 96-well microtitre plate with a

preformed monolayer of *MDCK cells* (placed in each well on the previous day; 100 µl aliquots with approx. 1.5 x 10^4 cells). 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 Kärber (2) and Spearman (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 EN 14476: 2007-02, 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 assays

Investigations for determination of virucidal activity followed to EN 6.6. The test product was examined undiluted (80.0 %) and 10.0 % solution in hard water according to EN 5.2.2.2.
15 and 30 seconds were chosen as contact 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 (EN 6.6.8).

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 Inactivation assay following the method of Lycke[1]

Following a modified procedure as described by Lycke (4), the test mixture was further diluted 1:1,000 in EMEM and then the total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$-reduction of virus titre. This method is necessary for those products which demonstrate a great cytotoxicity.

100 µl of the test virus suspension and 100 µl interfering substance (PBS) were mixed with 800 µl disinfectant at 20°C. At the end of the exposure time (15 s) 62.5 µl of the mixture were immediately added to 62.5 ml EMEM (1:1,000 dilution) and then the volume was distributed in six microtitre plates (108 µl / well). After 5 days p.i. cultures were observed for cytopathic effects. The calculation of virus titre followed the formula of Lycke:

$$-\log_{10} = [1.4 \times \ln (1-p)].$$

p is meaning the relation between the positive wells with virus detection in comparison to the total number of wells.

For the control of cell sensitivity 200 µl Aqua bidest. were mixed with 800 µl disinfectant (PBS as control). Then, an aliquot was diluted 1:1000 and 108 µl of this dilution were added to the 48 wells of a microtitre plate with a preformed monolayer of *MDCK cells*. After at least one hour, a comparative virus titration was performed on the cells treated in such a manner or treated with PBS only.

Determination of the initial virus titre was performed in a quantitative suspension test by a fivefold assay. The virus-inactivating properties of the test product were calculated by subtracting the virus titre in the test mixture from the virus control.

[1] Method not accredited

6.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 6.6.4.1 with 200 µl hard water and 800 µl test product.

Values are given as $log_{10}CD_{50}$/ml (in analogy to $log_{10}TCID_{50}$/ml).

6.8 Control of efficacy for suppression of disinfectant activity

Furthermore, a control of efficiency for suppression of disinfectant activity was included (EN 6.6.6).

6.9 Reference virus inactivation test

A 0.7 (w/v) % formaldehyde solution was included as reference for test validation according to EN 6.6.7.1. Contact times were 5 and 15 minutes. In addition, cytotoxicity of formaldehyde test solution was determined following EN 6.6.7.2 with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in EN 8.3 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a 4 log_{10} reduction with the assay following the method of Lycke.

b) The cytotoxicity of the test product (80.0 % solution) was two log_{10} steps.

c) The comparative titration on pretreated (disinfectant) and non pretreated (PBS) *MDCK* cells showed an acceptable difference (<1 log_{10}; EN 8.3) of virus titres: 7.13 (PBS) versus 6.50 (disinfectant) log_{10} $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant activity showed no decrease of virus titre.

Since all criteria following EN 8.3 were fulfilled, examination with influenza A virus H1N1 (swine) following EN 14476:2007-02 is valid.

8. Results

Results of examination 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 test product (80.0 %) was able to inactivate influenza A virus H1N1 (swine) after 15 seconds in this quantitative suspension test. The reduction factor was ≥ 3.13. Due to the cytotoxicity no reduction of 4 $\log_{10}$ steps could be demonstrated. Therefore, the method of Lycke was introduced. The results are given in table 6.
The virus titre in the fivefold assay was $\log_{10}$ $TCID_{50}$ / ml = 6.83. This value corresponded to the virus amount in the assays of 4.63 $\log_{10}$ $TCID_{50}$.
Since in 576 cell culture units no residual virus could be detected the result according to the formula of Lycke is $\log_{10}$ = 0. The reduction factor is therefore $\log_{10}$ 4.63 minus $\log_{10}$ 0 = 4.63 after 15 seconds of exposure time.

The 10.0 % solution was not active against H1N1 after 15 seconds of exposure time.

9. Summary

In summary, a sufficient reduction of virus titre can be achieved by Gel Mains Desinfectant undiluted after an exposure time of 15 seconds. 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 hand disinfectant Gel Mains Desinfectant for inactivation of influenza A virus H1N1 (swine) as follows:

undiluted 15 seconds

Bremen, 20.11.2009

- 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. Recorders 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. EN 14476:2007-02: Chemical disinfectants and antiseptics – virucidal quantitative suspension test - Test method and requirements (phase 2, step 1)

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

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

Appendix:

Table 1: Raw data of Gel Mains Desinfectant (80.0 %) tested against influenza A virus H1N1 (swine)

Table 2: Raw data of Gel Mains Desinfectant (10.0 %) tested against influenza A virus H1N1 (swine)

Table 3: Control of efficacy for suppression of disinfectant activity (80.0 %)

Table 4: Raw data (influenza A virus H1N1 swine) for cell sensitivity (80.0 %)

Table 5: Determination of virus titre (assay following method of Lycke)

Table 6: Inactivation of influenza A virus H1N1 by Gel Mains Desinfectant (80.0%) in the assay following the method of Lycke (15 seconds exposure time)

Table 7a: Summary of results (suspension test) with Gel Mains Desinfectant and influenza A virus H1N1 (swine)

Table 7b: Summary of results (assay following Lycke) with Gel Mains Desinfectant and influenza A virus H1N1 (swine)

Table 1 : Raw data of Gel Mains Desinfectant (80.0 %) tested against influenza A virus H1N1 (quantal test; 8 wells) (2051)

Product	Concentration	Interfering Substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	80.0%	PBS	0.25	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	80.0%	PBS	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	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.	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 0044	4004 0000	0000 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 2: Raw data of Gel Mains Desinfectant (10.0 %) tested against influenza A virus H1N1 (quantal test; 8 wells) (2051)

Product	Concentration	Interfering Substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	10.0%	PBS	0.25	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	0014 0001	0000 0000	0000 0000	n.d.
			0.5	tttt tttt	4444 4444	4444 4444	4444 4444	3444 4424	0000 4001	0000 0000	0000 0000	n.d.
			1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product cytotoxicity	10.0%	PBS	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	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.	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 0044	4004 0000	0000 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 3: Control of efficacy for suppression of disinfectant activity (80.0 %) (2067)

Product	Interfering substance	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
Test product	PBS	tttt tttt	tttt tttt	4444 4444	4344 1431	0000 0040	0000 0000	0000 0000	0000 0000	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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 (influenza A virus H1N1) for cell sensitivity (80.0 %) (2067)

Product	Interfering substance	Dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	PBS	-	4444 4444	4444 4444	4444 4444	4444 4334	4144 1411	4140 0201	0000 0000	0000 0000	n.d.
PBS	clean conditions	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBS	dirty conditions	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product	PBS	1:1,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4044	2000 0000	0000 0000	0000 0000	n.d.
		1:2,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product	clean conditions	1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:2,000	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	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:2,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 : Determination of virus titre (assay following method of Lycke) (2067)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4431 4444	0044 2204	0000 0000	0000 0000	n.d.
2nd assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4444 4422	0030 0140	0000 0000	0000 0000	n.d.
3rd assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	3444 4444	0004 0000	0000 0000	0000 0000	n.d.
4th assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4442 4444	0000 0000	0000 0000	0000 0000	n.d.
5th assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4402 4144	0001 0044	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: Inactivation of influenza A virus H1N1 (swine) by Gel Mains Desinfectant (80.0 %) in the assay following the method of Lycke (15 seconds exposure time) (2067)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
PBS	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 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 7a: Summary of results (suspension test) with Gel Mains Desinfectant and influenza A virus H1N1 (swine)

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min								> 4 log_{10} reduction after ... min
				0	0.25	0.5	1.0	5.0	15.0	30.0	60.0	
Product	80.0%	PBS	3.50	n.d.	≤ 3.50	≤ 3.50	n.d.	n.d.	n.d.	n.d.	n.d.	≥ 0.25
Product	10.0%	PBS	2.50	n.d.	6.88	6.75	n.d.	n.d.	n.d.	n.d.	n.d.	> 0.5
Form-aldehyde	0.7% (m/V)	PBS	3.50	n.d.	n.d.	n.d.	n.d.	≤ 3.50	≤ 3.50	n.d.	n.d.	≥ 5.0
Virus control	n.a.	PBS	n.a.	6.88*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.63	n.a.
Sens. PBS	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
Sens. product	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.

n.a. = not applicable n.d. = not done * raw data not shown

Table 7b: Summary of results (assay following Lycke) with Gel Mains Desinfectant and influenza A virus H1N1 (swine)

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min								> 4 log_{10} reduction after ... min
				0	0.25	0.5	1.0	5.0	15.0	30.0	60.0	
Product	80.0%	PBS	3.50	n.d.	0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
Virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.13 6.88 6.63 6.50 6.75	n.a.
Sens. PBS	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.13	n.a.
Sens. product	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.50	n.a.

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

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Ihre Zeichen, Ihre Nachrichten vom | Unsere Zeichen, unsere Nachricht vom | Bremen ,den 06.03.2014

Résumé des propriétés actives contre virus du produit GEL MAINS DESINFECTANT fabriqué par LABORATOIRES PHAGOGENE GROUPE RIVADIS au cours de l'essai de suspension quantitatif

Les documents suivants (rapport d'essai et d'expertise) exposés par MikroLab GmbH pour GEL MAINS DESINFECTANT (produit pour la désinfection des mains) fabriqué par LABORATOIRES PHAGOGENE GROUPE RIVADIS basent sur cette prise de position:

expertise du BVDV du 08 septembre 2009

expertise relative à virus vaccinia du 14 aout 2009

En considération de ces deux virus testés, la concentration suivante aux temps de contact suivant résulte:

sans dilution 15 secondes

afin de réaliser une réduction de titres de > quatre $\log_{10}$ degrés (réduction de titres > 99,99 %) dans l'essai de suspension quantitatif suivant la directive de l'association allemande de lutte contre les maladies virales (DVV) et du « Robert Koch-Institut » (RKI).
Par conséquent, GEL MAINS DESINFECTANT satisfait les conditions afin de pouvoir être dénoté « virucide de manière étroite » ce qui est formulé dans une prise de position du groupe « virucidie » auprès du RKI (Bundesgesundheitsbl. 2004, 47: 62-66) et le produit est donc efficace contre tous les virus enveloppés. De cette manière, ces essais impliquent une efficacité de GEL MAINS DESINFECTANT contre les soi-disant « blood-borne viruses » dont HBV, HCV et HIV font partie, contre HSV et coronavirus et contre les orthomyxovirus de type A comme H5N1 et H1N1.

Dr. Jochen Steinmann

REFERENCES ETUDE/ELEMENT D'ESSAI : Tp 285 / 09-1908

DONNEUR D'ORDRE : PHAGOGENE
Impasse du petit rosé
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EFFET IRRITANT/CORROSIF AIGU SUR LA PEAU CHEZ LE LAPIN – OCDE 404 -

ELEMENT D'ESSAI :

GEL HYDRO-ALCOOLIQUE - REF. AL3235

RAPPORT FINAL

Blanquefort, le 28 juillet 2009

Ce rapport comporte 13 pages dont 3 d'Annexes

SF

LABORATOIRES DE RECHERCHE ET D'EXPÉRIMENTATION

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EVALUATION DE L'EFFET IRRITANT/CORROSIF AIGU SUR LA PEAU CHEZ LE LAPIN

SOMMAIRE DU RAPPORT FINAL

DECLARATION DU DIRECTEUR DE L'ETUDE ET BPL 3

DECLARATION DE L'ASSURANCE QUALITE 4

RESUME 5

1. OBJECTIF ET PRINCIPE DE L'ETUDE 6

2. REFERENCES 6

3. INSTALLATION D'ESSAI 6

4. ARCHIVAGE 7

5. ELEMENT D'ESSAI 7

6. SYSTEME D'ESSAI 7

7. MODE OPERATOIRE 8

8. RESULTATS 10

9. CONCLUSION 10

ANNEXES 11-13

DECLARATION DU DIRECTEUR DE L'ETUDE ET BPL

REFERENCES ETUDE / ELEMENT D'ESSAI : **Tp 285 / 09-1908**

ELEMENT D'ESSAI : **GEL HYDRO-ALCOOLIQUE - REF. AL3235 – lot JD0006.07**

TITRE DE L'ETUDE : **Effet irritant/corrosif aigu sur la peau chez le Lapin – Ligne directrice 404 de l'OCDE (24/04/2002)**

Je soussignée, **Sophie FAGETTE, Directeur d'étude,** déclare que :

- l'étude décrite a été réalisée sous ma responsabilité et conformément :

  - aux principes des Bonnes Pratiques de Laboratoires (BPL) du Groupe Interministériel des Produits Chimiques (GIPC) (décret n° 2006-1523 du 04 décembre 2006 du Ministère Français de l'Economie, des Finances et de l'Industrie publié dans le Journal Officiel de la République française du 06 décembre 2006)

  - à la norme européenne NF EN ISO 9001 – décembre 2000 qui reproduit intégralement la norme internationale ISO 9001 : 2000.

Ces textes sont acceptés par les agences réglementaires des pays suivants: Australie, Autriche, Belgique, Canada, République tchèque, Danemark, Finlande, France, Allemagne, Grèce, Hongrie, Islande, Irlande, Israël, Italie, Japon, République de Corée, Luxembourg, Mexique, Pays bas, Nouvelle Zélande, Norvège, Pologne, Portugal, Slovénie, Afrique du sud, Espagne, Suède, Suisse, Turquie, Royaume Uni et les Etats Unis d'Amérique.

- le rapport final reflète de façon exacte et complète les données brutes de l'étude.

Date : 29 juillet 2009 Signature

DECLARATION DE L'ASSURANCE QUALITE

REFERENCES ETUDE / ELEMENT D'ESSAI : **Tp 285 / 09-1908**

ELEMENT D'ESSAI : **GEL HYDRO-ALCOOLIQUE - REF. AL3235 – lot JD0006.07**

TITRE DE L'ETUDE : **Effet irritant/corrosif aigu sur la peau chez le Lapin – Ligne directrice 404 de l'OCDE (24/04/2002)**

Je soussignée, **Michèle DARRICAU**, pour le service Assurance Qualité, déclare que :

- le service Assurance Qualité s'est assuré du respect des procédures de travail relatives à ce type d'étude.

Des inspections portant sur l'Installation d'Essai ont été réalisées annuellement. Ces inspections ont permis de vérifier que l'Installation d'Essai répondait aux exigences des autorités.

Les inspections d'étude (documentation et techniques appropriées à ce type d'étude) ont été réalisés ; le résultat des évaluations de l'Assurance Qualité a fait l'objet de comptes rendus qui ont été transmis au Directeur d'étude et à la Direction générale comme suit :

Dates d'inspections	Phases inspectées	Dates de transmission des rapports d'inspections
16 juin 2009	Conduite d'étude : J1 : traitement	22 juin 2009
29 juillet 2009	Rapport final	31 juillet 2009

- le rapport final reflète de façon précise et complète les données brutes de l'étude.

Date : 29 juillet 2009 Signature

EFFET IRRITANT/CORROSIF AIGU SUR LA PEAU CHEZ LE LAPIN - LIGNE DIRECTRICE 404 DE L'OCDE (24/04/2002)

ACUTE DERMAL IRRITATION/CORROSION TEST IN THE RABBIT - OECD GUIDELINE 404 (24/04/2002)

- **Élément d'essai / *Test element* :**

GEL HYDRO-ALCOOLIQUE - REF. AL3235 – lot/ *batch* JD0006.07

- **Résumé de l'essai/ *Summary of the study***

L'objectif de l'étude a été d'apprécier qualitativement et quantitativement l'effet irritant ou corrosif et son délai d'apparition après application cutanée unique de 0,5 ml de l'élément d'essai testé tel quel sous pansement semi-occlusif pendant 4 heures chez 3 lapins.

Les réactions cutanées (érythème et oedème) ont été lues 1h, 24h, 48h et 72 heures après enlèvement du pansement.
L'élément d'essai a été classé selon les critères définis dans l'arrêté du 20/04/1994 pris en application de la Directive de base 67/548/CEE du Journal Officiel des Communautés européennes du 27 juin 1967 et ses amendements successifs.

The aim of the study was to assess qualitatively and quantitatively irritancy or corrosion and the delay of appearance after single skin application of 0.5 ml of test element undiluted, under semi-occlusive dressing for 4 hours, in 3 rabbits.

The cutaneous reactions (erythema and oedema) were scored 1h and then 24, 48 and 72 hours after patch removal.
The test element was classified in accordance with the criteria defined in the decree of 20/04/1994 taken in enforcement from the basic Directive 67/548/EEC of the Official Journal of the European Communities of June 27, 1967 and its successive amendments.

- **Dates de l'expérimentation / *Experimental dates* :** du/*from* 20/07/09 au/*to* 24/07/09

- **Résultats/ *Results* :**

Animaux/ *Animals*	**Score moyen 24h, 48h et 72h après application/ *Mean score 24h, 48h and72h after application***	
	Erythème/ *Erythema*	**Oedème/ *Oedema***
3849	0.0	0.0
3851	0.0	0.0
3861	0.0	0.0

- **Conclusion/ *Conclusion* :**

L'élément d'essai **n'a pas été classé** parmi les produits chimiques irritants pour la peau.
The test element ***was unclassified*** *among the chemicals irritating to skin.*

1. OBJECTIF ET PRINCIPE DE L'ETUDE

L'objectif de l'étude a été d'apprécier qualitativement et quantitativement l'effet irritant ou corrosif et son délai d'apparition après application unique sur la peau, chez le Lapin, d'un élément d'essai.

L'élément d'essai a été appliqué à une dose, sous pansement semi-occlusif, à plusieurs animaux, une zone de peau non traitée servant de contrôle.

Le degré d'irritation a été lu et noté à des intervalles de temps déterminés.

La durée de l'étude a été suffisamment longue pour évaluer complètement la réversibilité des effets observés.

Le Lapin albinos est l'espèce animale communément utilisée et recommandée par les autorités officielles pour l'évaluation de la tolérance cutanée des éléments d'essai chimiques par ce type de méthode.

Cette étude a permis de classer l'élément d'essai dans une des catégories définies dans l'arrêté du 20 avril 1994 pris en application de la Directive de base 67/548/CEE et ses amendements successifs.

2. REFERENCES

- Arrêté du 20 avril 1994 pris en application de la Directive de base 67/548/CEE publiée au Journal Officiel des Communautés européennes du 27 juin 1967 et ses amendements successifs.
- Annexe V partie B4 de la Directive Européenne 92/69/CEE du 31 juillet 1992, publiée au Journal Officiel des Communautés Européennes du 29 décembre 1992 (L383A).
- Ligne directrice 404 de l'OCDE du 24 avril 2002, concernant les essais de produits chimiques, qui définit l'irritation cutanée comme la production sur la peau de réactions inflammatoires réversibles et la corrosion cutanée comme la production de lésions tissulaires irréversibles après application de l'élément d'essai.

3. INSTALLATION D'ESSAI

3.1 Installation d'Essai et équipe technique

EVIC France – Division Evic-Tox
48 rue Jean Duvert
33290 Blanquefort
05 56 95 59 95

Directeur de l'étude : Sophie FAGETTE
Techniciens responsables : Mouctar BARRY

3.2. Ethique et agréments de l'Installation d'Essai

L'Installation d'Essai réalise les études selon les règles d'éthique animale indiquées dans la **Directive européenne 86/609/CEE** du 24 novembre 1986 après avoir soumis les plans d'étude à l'avis préalable du Comité d'Ethique Animale interne à l'Installation d'Essai.

L'Installation d'Essai EVIC-TOX est reconnue conforme aux BPL par l'**AFSSaPS** (arrêté du 14 mars 2000 publié au Journal Officiel de la République Française du 23 mars 2000 et arrêté du 10 août 2004 publié au Journal Officiel de la République Française du 18 septembre 2004) et le **GIPC** (décret n° 2006-1523 du 04 décembre 2006 publié au Journal Officiel de la République Française du 06 décembre 2006) et de la norme européenne NF EN ISO 9001 v. 2000.

4. ARCHIVAGE

En fin d'étude, les documents de travail ont été archivés avec le rapport final et seront conservés pendant 10 ans dans la salle d'archives de l'Installation d'Essai.

A l'issue de cette période, l'Installation d'Essai définit avec le donneur d'ordre la poursuite de l'archivage, la restitution ou la destruction des données.

5. ELEMENT D'ESSAI

5.1. Référence de l'élément d'essai

Nom de l'élément d'essai	GEL HYDRO-ALCOOLIQUE
Référence	AL3235
Numéro de lots	JD0006.07
Aspect physique	Gel incolore transparent
Nombre et contenance des conditionnements	1 flacon en verre blanc de 125 ml
Référence EVIC-France	09-1908

Le donneur d'ordre a certifié que les informations fournies concernant la caractérisation de l'élément d'essai correspondaient bien à cet élément d'essai et que l'échantillon transmis était représentatif du lot indiqué.

5.2. Stockage

L'élément d'essai a été stocké à température ambiante et à l'abri de la lumière dans un local spécialement aménagé à cet effet.

Un échantillon de l'élément d'essai a été classé dans l'échantillothèque de l'Installation d'Essai à titre de référence ; il y sera conservé, au plus, pendant 10 ans.

6. SYSTEME D'ESSAI

Espèce : Lapins albinos Néo-zélandais conventionnels

Origine : Elevage GRIMAUD (49450 Roussay, France)

Poids : de 2.92 à 3.33 kg, la veille de l'application de l'élément d'essai

Nombre et sexe : 3 mâles

Acclimatation : pendant 5 jours au moins avant le début de l'expérimentation

Identification : chaque animal a été identifié par marquage auriculaire avec un clip métallique numéroté et le numéro correspondant a été reporté sur une étiquette apposée sur sa cage.

Hébergement : les animaux ont été hébergés à raison de 1 par cage, dans des cages inox sur grille (60 cm x 45 cm x 32 cm).

Les cages ont été placées dans un local d'accès limité, de 7 m x 4 m x 3 m, maintenu en légère surpression (10 mm d'eau minimum), sous air régulé en température (t = 19 ± 2°C) et humidité relative contrôlée (HR = 50 ± 20 %) excepté pendant les cycles de lavage et dont le renouvellement en air non recyclé filtré (sur filtre 99 %) s'est fait à raison de 10 cycles par heure environ.
Les écarts de température et d'humidité relative relevés au cours de la réalisation de l'étude ont été jugés par le directeur de l'étude mineurs et sans influence sur les résultats.

L'éclairage artificiel a assuré 12 heures de lumière par jour et 12 heures d'obscurité.

Nourriture : l'aliment complet a été fourni sous forme de granulé STARLAP UNIC ROBE, livré par EVIALIS (37018 Tours, France).

Boisson : l'eau d'adduction a été distribuée en biberon en polypropylène munis d'une tétine en inox, un échantillon d'eau étant prélevé au minimum tous les 6 mois et envoyé pour analyse physico-chimique et bactériologique à un organisme de contrôle spécialisé.

Préparation des animaux et sélection : 24 heures environ avant l'application de l'élément d'essai, la fourrure de la région dorsale du tronc des animaux a été tondue avec précaution, en évitant toute abrasion de la peau. Seuls les animaux présentant une peau saine et intacte ont été retenus pour l'essai.
Si possible, les régions de forte pilosité n'ont pas été utilisées comme zones d'essai.

7. MODE OPERATOIRE

7.1. Préparation de l'élément d'essai

L'élément d'essai (liquide) a été appliqué tel quel, non dilué.

7.2. Chronologie expérimentale

L'élément d'essai étant supposé ne provoquer aucun effet irritant grave ou corrosif, l'essai a démarré avec trois animaux, chacun d'eux recevant un seul pansement pendant une période d'exposition de quatre heures.

7.3. Application de l'élément d'essai

Pour chaque animal, l'élément d'essai a été déposé à raison de 0,5 ml sur un carré de gaze hydrophile de 2,5 cm x 2,5 cm (soit 6 cm^2 environ) lui-même posé sur un carré d'adhésif Micropore® de 5 cm x 5 cm (soit 25 cm^2).

Le pansement semi-occlusif a été appliqué sur la peau tondue et maintenu en place au moyen d'une bande extensible (type bande à varices) qui a entouré le tronc de l'animal et qui a été fixée par du Micropore®.

La bande a été placée de manière à ne pas gêner les mouvements respiratoires et à empêcher l'animal d'avoir accès au pansement et donc d'ingérer ou d'inhaler l'élément d'essai.

Les lapins ont été remis dans leur cage pendant la période d'exposition de 4 heures, au terme de laquelle les pansements ont été enlevés. L'élément d'essai résiduel a été éliminé à l'aide d'un coton imbibé d'eau distillée.

7.4. Observations cliniques et notation des réactions cutanées

L'observation des signes d'érythème et d'œdème chez les animaux et la notation des réponses ont été effectuées 1 heure puis 24, 48 et 72 heures après l'enlèvement du pansement. Les réactions cutanées ont été notées selon l'échelle numérique figurant ci-dessous et enregistrées sur le document de travail réservé à cet effet.

Outre les observations concernant l'irritation, toutes les lésions et tous les autres effets toxiques ont été notés et décrits de façon complète sur le document de travail réservé à cet effet.

Notation des réactions cutanées

Formation d'érythème et d'escarres*

Pas d'érythème 0
Erythème très léger (à peine perceptible) 1
Erythème bien défini 2
Erythème modéré à grave 3
Erythème grave (couleur rouge violacé) à formation d'escarres empêchant la cotation de l'érythème 4

** Erythème : rougeur de la peau provoquée par une congestion vasculaire ou une perfusion accrue. Escarre (croûte) = croûte sèche superficielle au site d'une brûlure thermique ou caustique, et qui contient des débris cellulaires, un exsudat tissulaire séché et recouvre la peau en cicatrisation.*

Formation d'œdème*

Pas d'œdème 0
Oedème très léger (à peine perceptible) 1
Oedème léger (pourtour de la zone oedémateuse bien défini par un gonflement net) 2
Oedème modéré (gonflement d'environ 1 mm) 3
Oedème grave (gonflement de plus d'1 mm s'étendant au-delà de la région exposée) 4

** Oedème = présence de quantités anormalement importantes de liquide dans les espaces intercellulaires des tissus de l'épiderme, du derme et des tissus sous-cutanés.*

7.5. Interprétation des résultats

L'élément d'essai a été classé en fonction des critères de classification et d'étiquetage des produits chimiques dangereux définis dans la Directive de base 67/548/CEE et ses amendements successifs.

Critères de classification	Phrases de risque
Eléments d'essai classés corrosifs s'ils produisent des destructions tissulaires *sur toute la profondeur de la peau, chez un animal au moins, lorsqu'elles sont appliquées sur la peau saine et intacte, au cours de l'essai, ou bien si le résultat peut être prédit : réaction fortement acide (pH ≤ 2) ou fortement alcaline (pH ≥ 11,5).*	
• Ces destructions tissulaires apparaissent après un temps d'exposition ne dépassant pas 3 minutes ; ou un tel résultat est prévisible.	R 35 Provoque de graves brûlures
• Ces destructions tissulaires apparaissent après un temps d'exposition ne dépassant pas 4 heures ; ou un tel résultat est prévisible.	R 34 Provoque des brûlures
Eléments d'essai non corrosifs classés irritants s'ils produisent *une inflammation de la peau, présente au moins 24h après une période d'exposition ne dépassant pas 4 heures,* **jugée importante à partir des indices observés lors de l'essai :*	
• Si essai réalisé sur 1 animal : valeur moyenne des scores pour la formation d'érythème et d'escarres ou la formation d'œdème calculée pour les 3 temps d'observation (24, 48 et 72h) ≥ 2	R 38 Irritant pour la peau
• Si essai réalisé sur 3 animaux : scores individuels moyens pour la formation d'érythème et d'escarres ou la formation d'œdème calculés sur les 3 temps d'observation (24, 48 et 72h) chez au moins 2 animaux ≥ 2	
**ou en cas de persistance à la fin de la période d'observation chez 2 animaux au moins*	

8. RESULTATS

Les poids individuels des animaux au début (J-1) et au terme de l'essai (J_f) sont fournis en Annexe 1.
La croissance pondérale des animaux a été normale pendant l'expérimentation.

Les résultats des observations cliniques sont résumés dans le tableau joint en Annexe 2.
Aucune symptomatologie significative n'a été observée.

Les résultats d'irritation cutanée, résumés sous forme de tableaux faisant apparaître, pour chaque animal, le score d'irritation pour l'érythème et l'œdème relevé 1, 24, 48 et 72 heures après l'enlèvement du pansement, sont joints en Annexe 3.

9. CONCLUSION

Compte tenu des critères définis par la Directive 67/548/CEE et ses amendements successifs, l'élément d'essai **GEL HYDRO-ALCOOLIQUE – REF. AL3235 n'a pas été classé** parmi les produits chimiques irritants pour la peau.

Annexe 1

Poids corporel - Valeurs individuelles

N° des animaux	Poids (en kg)	
	J-1	J_f
3849	2.920	2.950
3851	3.330	3.360
3861	3.220	3.260

Annexe 2

Observations cliniques

Temps de lecture	Commentaires		
	Animal n° 3849	**Animal n° 3851**	**Animal n° 3861**
J-1	RAS	RAS	RAS
J1 (1h)	RAS	RAS	RAS
J2	RAS	RAS	RAS
J3	RAS	RAS	RAS
J4	RAS	RAS	RAS
J5	/	/	/
J6	/	/	/
J7	/	/	/
J8	/	/	/
J9	/	/	/
J10	/	/	/
J11	/	/	/
J12	/	/	/
J13	/	/	/
J14	/	/	/

/ : pas de lecture
RAS : rien à signaler

Annexe 3

Réactions cutanées - Elément d'essai

Erythème

N° des animaux	Temps de lecture (après enlèvement du pansement)													
	1h	24h	48h	72h	M*	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
3849	1	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/
3851	1	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/
3861	1	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/

Oedème

N° des animaux	Temps de lecture (après enlèvement du pansement)													
	1h	24h	48h	72h	M*	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
3849	0	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/
3851	0	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/
3861	0	0	0	0	**0.0**	/	/	/	/	/	/	/	/	/	/

/ : pas de lecture

M : moyenne des valeurs observées 24h, 48h et 72h après enlèvement du pansement*

EVIC france

Etude : Iq 051bis / 09.3418 – RESUME DU RAPPORT D'ETUDE

Référence de la proposition acceptée : 10-1030/0

TITRE DE L'ETUDE : VERIFICATION CHEZ L'HOMME DE LA COMPATIBILITE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION EN OUVERT – Open test sous contrôle dermatologique -

OBJECTIF DE L'ETUDE : Vérification de la compatibilité cutanée (en particulier l'absence de sensation d'inconfort significative) du produit **PHAGORUB GEL SPS – Code AL3235**, après application quotidienne à l'Institut et à domicile (4 fois par jour), au niveau des mains, pendant 5 jours consécutifs, dans des conditions contrôlées et très proches des conditions normales d'emploi.

Contrôle clinique à chaque passage avant et dans l'heure suivant l'application.

DATES DE L'ETUDE : du 4 au 8 janvier 2010

EFFECTIF DE VOLONTAIRES dont les données sont exploitables : **10** femmes et **1** homme (11 inclus, aucun abandon et aucune sortie d'essai).

RESULTATS

Compatibilité

Aucun signe clinique imputable au produit d'investigation n'a été constaté par l'investigateur.

Aucune sensation d'inconfort n'a été rapportée par les volontaires.

CONCLUSION

Très bonne compatibilité ☒

SIGNATURE ET DATE

Directeur du centre d'investigation : Dominique MASSON

16/09/10

REFERENCES ETUDE/PRODUIT : Iq 051bis / 09.3418

VERIFICATION CHEZ L'HOMME DE LA COMPATIBILITE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION EN OUVERT

Open test sous contrôle dermatologique

PROMOTEUR : PHAGOGENE

PRODUIT TESTE : PHAGORUB GEL SPS – Code AL3235

Rapport d'étude

Bordeaux, le 15/09/2010

Ce rapport comporte 15 pages dont 6 pages d'Annexes

VERIFICATION CHEZ L'HOMME DE LA COMPATIBILITE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION EN OUVERT

Open test sous contrôle dermatologique

SOMMAIRE DU RAPPORT D'ETUDE

I	OBJECTIF ET PRINCIPE DE L'ETUDE	3
II	PERTINENCE DE L'ETUDE	3
III	TYPE DE L'ETUDE	4
IV	CENTRE INVESTIGATEUR ET EQUIPE TECHNIQUE	4
V	DATES DE REALISATION DE L'ETUDE	4
VI	PRODUIT TESTE	4
VII	VOLONTAIRES	5
VIII	METHODOLOGIE	6
IX	RESULTATS	9
X	CONCLUSION	9

ANNEXE 1	11
ANNEXES 2	12-13
ANNEXES 3	14-15

I . OBJECTIF ET PRINCIPE DE L'ETUDE

Cette étude avait pour but de vérifier la compatibilité cutanée (en particulier l'absence de sensation d'inconfort significative) du produit **PHAGORUB GEL SPS – Code AL3235,** après application quotidienne à l'Institut et à domicile (4 fois par jour), au niveau des mains, pendant 5 jours consécutifs, dans des conditions contrôlées et très proches des conditions normales d'emploi.

La compatibilité a été :

- contrôlée à chaque passage, après examen visuel des zones expérimentales, par le dermatologue ou par le technicien responsable, et interrogatoire des volontaires,

- vérifiée au jour le jour, par les volontaires eux-mêmes à domicile.

II . PERTINENCE DE L'ETUDE

Ethique

L'étude visant à une meilleure connaissance de la compatibilité cutanée du produit testé et le risque prévisible encouru par les personnes qui se sont prêtées à l'étude étant infime, il y a eu adéquation entre l'objectif de l'étude, ses risques potentiels et les désagréments éventuels liés aux modalités prévues par le protocole.

Approche méthodologique

La compatibilité cutanée du produit a été vérifiée par le dermatologue qui justifie d'une expérience appropriée.

La méthodologie choisie a été établie sur la base de nombreuses publications. Parmi celles-ci :

- Matthies W., Test strategies for development of cosmetic products using dermatological test models, Seifen-Öle-Fette-Wachse, 1991, 117, pp.42-43

- Frosch P.J., Kurte A., Pilz B., Efficacy of skin barrier creams (III). The repetitive irritation test (RIT) in humans, Contact Dermatitis, 1993, 29, pp. 113-118

- Strube D.D., Koontz S.W., Murahata R.I., Theiler R.F., The flex wash test. A method for evaluating the mildness of personal washing products, J. Soc. Cosm. Chem., 1989, 40, pp. 297-306

La zone expérimentale a été choisie en fonction de la nature du produit testé et de ses conditions normales d'emploi.

Les conditions d'application ont été très proches des conditions normales d'emploi.

Résultats

Les résultats ont été exprimés principalement sous forme de données descriptives et n'ont pas justifié un traitement statistique.

III . TYPE DE L'ETUDE

Cette étude mono-centrique a été réalisée en ouvert.

Chaque volontaire y participant, a été son propre témoin.

Elle a été réalisée selon les conditions générales d'Evic France – division Idec, établies pour la réalisation d'un projet d'étude chez l'Homme.

Le projet d'étude a été soumis à l'avis du comité interne d'Evic France qui a donné un avis favorable à sa réalisation (avis n° 09/961 du 23 décembre 2009).

IV . CENTRE INVESTIGATEUR ET EQUIPE TECHNIQUE

IV.1 . Centre investigateur

Evic France – division Idec
57, rue Ulysse Gayon
33 000 Bordeaux – France

tél : 05 57 14 00 80

IV.2. Equipe technique

Investigateur : Docteur Françoise MAGNE (dermatologue)

Co-investigateur : Docteur Clotilde TRARIEUX-FOURAULT (médecin généraliste)

Technicien responsable : Paméla SOM

V . DATES DE REALISATION DE L'ETUDE

Début le : 4 janvier 2010

Fin le : 8 janvier 2010

VI . PRODUIT TESTE

VI.1 . Identification du produit testé

Dénomination	PHAGORUB GEL SPS
Référence	AL3235
Numéro de lot	JD0006.11
Référence Evic France	09.3418
Forme galénique et caractères organoleptiques	Gel incolore
Nombre et type de conditionnements	1 bidon en plastique
Contenance des conditionnements	1L (reconditionné en 15 flacons de 125 ml)

VI.2 . Informations concernant le produit testé

Les documents relatifs au produit testé accompagnant les échantillons ont été la formule quali-quantitative et la lettre d'engagement du Promoteur concernant en particulier, la conformité de la formule aux réglementations en vigueur et sa sécurité, ainsi que les pré-requis toxicologiques (Tp 285).

VII . VOLONTAIRES

VII.1 . Effectif

Le nombre de volontaires dont les données devaient être exploitables en fin d'essai était de 10.

11 volontaires ont été inclus dans l'étude.
Aucun abandon n'a été déploré et aucune sortie d'essai n'a été décidée par l'investigateur.

La compatibilité du produit testé a donc été appréciée chez 11 volontaires.

VII.2 . Critères d'inclusion spécifiques

Les critères d'inclusion spécifiques, définis au protocole, étaient les suivants :

- âge : 18 à 70 ans,
- sexe : féminin et/ou masculin,
- phototype (Fitzpatrick) : I à V,
- ayant tous types de peau au niveau des mains.

Tous les volontaires ont répondu à ces critères d'inclusion spécifiques. Leurs caractéristiques typologiques sont définies en **Annexe 1.**

VII.3 . Critères de non inclusion spécifiques

Les critères de non inclusion spécifiques, définis au protocole, étaient les suivants :

- marques cutanées au niveau des zones expérimentales pouvant interférer avec l'évaluation des réactions de la peau (troubles de la pigmentation, éléments cicatriciels, éphélides et naevi en trop grande quantité, coup de soleil....),
- allergie ou réactivité particulière aux gels hydro alcooliques,
- hyper-réactivité cutanée,
- prévision d'exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude,
- intention de se baigner en baignoire, en mer ou en piscine, de faire du sauna ou du hammam pendant la période du test,
- arrêt de traitement à base de vitamine A acide ou de ses dérivés depuis moins de 3 mois avant le début de l'étude,

- arrêt de traitement par corticoïdes topiques sur la zone expérimentale depuis moins de 8 jours avant l'étude,

- prévision de vaccination pendant la période de l'essai, dernière vaccination dans les 3 semaines précédant l'étude.

Tous les volontaires ont répondu à ces critères de non inclusion spécifiques.

VIII . METHODOLOGIE

VIII.1 . Conditions expérimentales d'utilisation du produit testé

Les conditions expérimentales définies au protocole étaient les suivantes :

Mode d'emploi du produit quantité à appliquer	**Zones d'application Localisation/surface**	**Applications à l'Institut Fréquence/durée**	**Applications à domicile Fréquence/durée**
Verser une noisette de gel dans le creux de la main et frotter jusqu'au séchage complet. Ne pas rincer.	Les mains (environ 600 cm^2)	1 fois par jour de J1 à J5	4 fois par jour de J1 à J4

Toutes les conditions expérimentales d'application à l'Institut, définies au protocole, ont été respectées.

VIII.2 . Contraintes de l'étude

Les contraintes imposées aux volontaires étaient les suivantes :

- pas d'application de produits similaires au produit d'investigation (gel ou lotion hydro alcoolique) sur les mains,

- pas de changement des habitudes d'hygiène (savon) et de soin au niveau des mains,

- pas d'activité qui pourrait être agressive pour les mains sans utiliser des gants adaptés (ménage, jardinage, bricolage),

- pas de soin beauté au niveau des mains (manucure, pose d'ongle), ou maquillage des ongles (exemple vernis à ongles),

- pas de bain (en baignoire ou en piscine ou en mer) et pas de hammam ou de sauna pendant l'étude,

- pas d'exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude,

- pas de traitement anti-allergique, anti-inflammatoire ou par des spécialités à base de vitamine A acide ou de ses dérivés pendant l'étude (si nécessité thérapeutique : sortie d'essai envisagée),

- pas de vaccination pendant l'étude.

VIII.3 . Contrôle de l'observance des modalités du protocole

L'investigateur a vérifié si les **contraintes** avaient été respectées.

Les volontaires ont été questionnés en fin d'étude, sur les **modalités suivies pour l'application du produit à domicile.** L'investigateur a apprécié l'importance des déviations éventuelles par rapport aux conditions expérimentales imposées au démarrage de l'étude.

La synthèse des réponses aux différentes questions posées est jointe en **Annexes 2/1 et 2/2.**

En cas de déviations au protocole, celles-ci ont été analysées et l'investigateur a apprécié leur incidence sur la validité des résultats. **Toutes les contraintes** de l'étude, définies au protocole, ont été respectées par les volontaires.

Une déviation au protocole concernant les conditions expérimentales d'application à domicile a été notée par l'investigateur et est reportée en **Annexe 2/1**. Compte tenu de son caractère ponctuel, elle a été jugée acceptable par l'Investigateur.

VIII.4 . Vérification de la compatibilité cutanée

VIII.4.1 . Fréquence des contrôles

L'examen de la peau, au niveau des mains, devait être effectué, par le dermatologue ou par le technicien responsable.

Cet examen devait être réalisé, visuellement, sous éclairage standardisé type « lumière du jour », avant puis dans l'heure suivant chaque application.

Parallèlement aux examens cliniques réalisés après application du produit, chaque volontaire devait être questionné sur les éventuelles sensations d'inconfort ressenties.

Tous les contrôles ont été réalisés conformément aux conditions définies au protocole.

VIII.4.2 . Expression des résultats de l'examen cutané et de l'interrogatoire

L'expression des résultats de l'examen cutané et de l'interrogatoire a été celle définie pour ce type d'étude conformément à la procédure correspondante.

En cas de réactivité :

- **les principaux signes décelables visuellement ont été notés, à savoir :**

Erythème, Œdème, Vésicule, Bulle, Papule, Macule, Croutelle, Dessèchement, Coloration.

L'intensité de **l'érythème et de l'œdème** a été évaluée selon l'échelle ordinale en 4 points : très légère, légère, modérée, sévère.

L'aspect de **l'érythème** a été précisé : diffus, ponctué.

L'importance du nombre des **vésicules** a été évaluée selon une échelle ordinale en 2 points : 1 à 2 vésicules, vésicules en nombre >2.

Les bulles, papules et macules ont été dénombrées.

Les croutelles, dessèchement et coloration ont été décrits.

L'importance du **dessèchement et de la coloration** a été évaluée selon l'échelle ordinale en 4 points : très légère, légère, modérée, sévère.

- **les principales sensations d'inconfort ont été décrites, à savoir :**

Echauffement, Picotement, Prurit (démangeaison), Tiraillement, Brûlure.

L'intensité **des sensations d'inconfort** a été évaluée selon l'échelle ordinale en 4 points : très légère, légère, modérée, sévère.

Le dermatologue ou le technicien responsable a noté pour tout signe clinique ou toute sensation d'inconfort décrite, sa localisation, sa durée, son délai d'apparition par rapport à l'application du produit sa fréquence, son intensité, son évolution, le traitement médicamenteux éventuellement entrepris, l'imputabilité du produit **: exclue, douteuse, possible, vraisemblable très vraisemblable**.

Il a noté également le caractère habituel ou inhabituel du signe clinique ou de la sensation d'inconfort décrite, en questionnant le volontaire sur ce qu'il observait dans la vie courante, lors de l'utilisation de produits similaires.

Les résultats ont été exprimés de façon descriptive pour les signes décelables visuellement ou les sensations d'inconfort et le pourcentage de volontaires réactifs a été calculé.

VIII.4.3 . Interprétation des résultats de l'examen cutané et de l'interrogatoire

Ont été pris en compte pour vérification de la compatibilité cutanée du produit testé, tous les volontaires inclus pour autant qu'ils aient fait l'objet au moins d'un examen post application, à la date prévue ou en dehors de celle-ci.

L'interprétation des résultats de l'examen cutané et de l'interrogatoire a été celle définie pour ce type d'étude conformément à la procédure correspondante.

Elle a été faite par le dermatologue de façon absolue, en se basant sur une grille d'évaluation établie par le centre investigateur, qui tient compte des réactions d'irritation observables visuellement (signes cliniques) et des sensations d'inconfort décrites par les volontaires.

L'investigateur a conclu en terme de **très bonne, bonne, moyenne ou mauvaise compatibilité cutanée**, selon la grille d'évaluation suivante :

Compatibilité cutanée	% de volontaires présentant des signes cliniques imputables au produit à tester	% de volontaires présentant des sensations d'inconfort imputables au produit à tester
Très bonne	0 %	0 %
Bonne	0 %	< 25 %
Moyenne	< 10 %	+/-
	0 %	25 à 50 %
Mauvaise	≥ 10%	+/-
	0 %	> 50 %

En fonction de la nature et de l'importance des signes cliniques ou des sensations d'inconfort observés, l'investigateur a pu cependant être amené à déclasser ou à surclasser le produit par rapport à la présente grille d'évaluation.

IX . RESULTATS

Les données individuelles de l'examen cutané et de l'interrogatoire des volontaires sont jointes en **Annexes 3.**

En résumé :

Zones expérimentales : les mains			
Types de réactions cutanées observées imputables au produit testé	**% de volontaires présentant des signes cliniques imputables au produit testé**	**Types de sensations d'inconfort ressenties imputables au produit testé**	**% de volontaires présentant des sensations d'inconfort imputables au produit testé**
Aucune	0 %	Aucune	0 %

X . CONCLUSION

Dans les conditions expérimentales adoptées et compte tenu de la grille d'évaluation établie par le centre investigateur, le produit **PHAGORUB GEL SPS – Code AL3235 a une très bonne compatibilité cutanée.**

SIGNATURE ET DATE

Directeur du centre d'investigation : Dominique MASSON

16/09/10

ANNEXES

Annexe 1

CARACTERISTIQUES TYPOLOGIQUES DES VOLONTAIRES

Volontaires		Age (ans)	Sexe F=féminin M=masculin	Phototype *	Types de peau au niveau des mains	Peau saine au niveau des mains
Réf.	Nom prénom					
1	ANCE. AN	63	F	III	Sèche	X
2	FAUC. MA	53	F	III	Normale	X
3	LARR. CY	36	F	III	Sèche	X
4	DELA. JO	61	F	IV	Sèche	X
5	DAGU. JO	69	F	IV	Sèche	X
6	ESTE. SY	55	F	II	Normale	X
7	MONT. ST	46	M	II	Sèche	X
8	PASC. SU	63	F	III	Sèche	X
9	GOUB. MA	62	F	III	Normale	X
10	BERT. MA	54	F	II	Normale	X
11	BOUT. CL	63	F	III	Sèche	X

Légendes : / = non x = oui

***phototype selon Fitzpatrick,** établi sur le principe d'une première exposition de 30 à 40 minutes au soleil après l'hiver ou une période sans exposition d'une durée équivalente :*

Type I *: Brûle toujours facilement, ne bronze jamais*
Type II *: Brûle toujours facilement, bronze légèrement*
Type III *: Brûle modérément, bronze progressivement*
Type IV *: Brûle faiblement, bronze toujours facilement*
Type V *: Brûle rarement, bronze intensément*
Type VI *: Ne brûle jamais, fortement pigmenté*

Annexe 2/1

CONTROLE DE L'OBSERVANCE
Modalités d'utilisation à domicile

Modalités d'utilisation (11 résultats exploitables)	**Nombre** de volontaires ayant respecté les modalités	**Pourcentage** de volontaires ayant respecté les modalités
Zones d'application : les mains Déviation : aucune	11	100 %
Mode d'utilisation du produit : Verser une noisette de gel dans le creux de la main et frotter jusqu'au séchage complet. Ne pas rincer. Déviation : aucune	11	100 %
Fréquence d'application à domicile : 4 applications par jour Déviation : 1 volontaire (réf. 9) a appliqué le produit seulement 2 fois à J1.	10	91%
Durée d'application à domicile : de J1 à J4 Déviation : aucune	11	100 %

Annexe 2/2

CONTROLE DE L'OBSERVANCE
Contraintes

Contraintes **(11 résultats exploitables)**	**Nombre** de volontaires ayant respecté les contraintes	**Pourcentage** de volontaires ayant respecté les contraintes
Pas d'application de produits similaires au produit d'investigation (gel ou lotion hydro alcoolique) sur les mains Déviation : aucune	11	100 %
Pas de changement des habitudes d'hygiène (savon) et de soin au niveau des mains Déviation : aucune	11	100 %
Pas d'activité qui pourrait être agressive pour les mains sans utiliser des gants adaptés (ménage, jardinage, bricolage) Déviation : aucune	11	100 %
Pas de soin beauté au niveau des mains (manucure, pose d'ongle), ou maquillage des ongles (exemple vernis à ongles) Déviation : aucune	11	100 %
Pas de bain (en baignoire ou en piscine ou en mer) et pas de hammam ou de sauna pendant l'étude Déviation : aucune	11	100 %
Pas d'exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude Déviation : aucune	11	100 %
Pas de traitement anti-allergique, anti-inflammatoire ou par des spécialités à base de vitamine A acide ou de ses dérivés pendant l'étude – pas de médication pouvant interférer avec l'étude Déviation : aucune	11	100 %
Pas de vaccination pendant l'étude Déviation : aucune	11	100 %

Annexe 3/1

EXAMEN CUTANE : SIGNES CLINIQUES

Volontaires		J1		J2		J3		J4		J5	
Réf.	Nom prénom	T0*	T1h	T0	T1h	T0	T1h	T0	T1h	T0	T1h
1	ANCE. AN	/	/	/	/	/	/	/	/	/	/
2	FAUC. MA	/	/	/	/	/	/	/	/	/	/
3	LARR. CY	/	/	/	/	/	/	/	/	/	/
4	DELA. JO	/	/	/	/	/	/	/	/	/	/
5	DAGU. JO	/	/	/	/	/	/	/	/	/	/
6	ESTE. SY	/	/	/	/	/	/	/	/	/	/
7	MONT. ST	/	/	/	/	/	/	/	/	/	/
8	PASC. SU	/	/	/	/	/	/	/	/	/	/
9	GOUB. MA	/	/	/	/	/	/	/	/	/	/
10	BERT. MA	/	/	/	/	/	/	/	/	/	/
11	BOUT. CL	/	/	/	/	/	/	/	/	/	/

**Les signes cliniques compatibles avec les critères d'inclusion ne sont pas reportés dans ce tableau*
Les réactions non imputables au produit testé ne sont pas reportées dans ce tableau

Légendes :

/ : Rien à signaler
E : Erythème
Oe : Œdème
V : Vésicule
D : Dessèchement

Bu : Bulle
Pa : Papule
Cr : Croutelle
C : Coloration
M : Macule

Vésicules

0,5 : Intensité très légère
1 : Intensité légère
2 : Intensité modérée
3 : Intensité sévère
d : diffus
p : ponctué

1 : nb = 1 ou 2
2 : nb > 2

Annexe 3/2

INTERROGATOIRE DES VOLONTAIRES

Volontaires		Réactions d'intolérance et sensations d'inconfort ressenties en cours d'étude
Réf.	**Nom prénom**	
1	ANCE. AN	/
2	FAUC. MA	/
3	LARR. CY	/
4	DELA. JO	/
5	DAGU. JO	/
6	ESTE. SY	/
7	MONT. ST	/
8	PASC. SU	/
9	GOUB. MA	/
10	BERT. MA	/
11	BOUT. CL	/

Les sensations non imputables au produit testé ne sont pas reportées dans ce tableau

Légendes :

/ : Rien à signaler
Ech : Echauffement
Pi : Picotement
Pr : Prurit (démangeaison)
Br : Brûlure
Ti : Tiraillement

0,5 : Intensité très légère
1 : Intensité légère
2 : Intensité modérée
3 : Intensité sévère

SPONSOR: **PHAGOGENE**
Impasse du petit rosé
Z.I.
79100 LOUZY

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE PRODUCT AFTER REPEATED APPLICATION UNDER PATCH

Human repeated insult patch test

SUMMARY OF THE STUDY REPORT

AIM AND PRINCIPLE OF THE STUDY

This study intended to confirm the skin compatibility and the absence of allergenic potential of the product **PHAGORUB GEL SPS - Code AL3235,** after repeated application to the skin under exaggerated experimental conditions.

The product was applied under patch for a defined time. The applications were repeated 9 times over a period of 3 consecutive weeks, period necessary for the possible induction of an allergy.

After a minimal 2-week rest period, with no treatment, a single application of the product under patch, to the induction site and to a virgin site and for a defined time, enabled to reveal a possible induced allergy.

DATES OF PERFORMANCE OF THE STUDY: from January 11th to February 19th, 2010.

VOLUNTEERS

- **Number of volunteers defined in the protocol: 100**
- **Number of volunteers whose data are exploitable: 106** (108 volunteers included, 2 volunteers (ref. 36 and 74) discontinued for personal reasons independent of the study and no exclusion was decided by the investigator).
- **Specific inclusion criteria:**

- age: 18 to 70 years old,
- sex: male and/or female,
- phototype (Fitzpatrick): I to V,
- all types of skin on body.

SC BIO HIGH TECH SRL – 15, Constantin Bosianu Street, Bucharest, Romania
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RO 16679189, J40/13128/2004

SUMMARY OF THE METHODOLOGY

The experimental conditions defined in the protocol were the following ones:

Patch material	Experimental conditions of use	Quantity applied
Finn Chamber standard®	As it is Evaporation for at least 15 minutes on the patch before application	20 µl

The applications of the test product, the removal of the patches and the controls were performed by the dermatologist or the technician in charge of the study.

- **Induction phase:** 3 consecutive weeks.

  * application of the product to a perfectly delimited site, under patch on D1, D3, D5, D8, D10, D12, D15, D17, D19.

  * patch removal
    - after 48 h of contact on D3, D5, D10, D12, D17, D19.
    - after 72 h of contact on D8, D15, D22.

  * controls: skin examination and questioning before patching on D1 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D3, D5, D8, D10, D12, D15, D17, D19, D22.

- **Rest period:** 2 consecutive weeks at least (4 weeks at the most).

  * no application of product.

- **Challenge:** 1 week.

  * application of the product to a perfectly delimited virgin site and to the site defined for the induction phase, under patch on D36.

  * patch removal after 48 h of contact on D38.

  * controls: skin examination and questioning before patching on D36 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D38, D39, D40 (48, 72, 96 h after application).

RESULTS

Induction phase	
Type of reactivity on the induction site	**Number and percentage of reactive volunteers**
None	0 / 0 %

Challenge	
Type of reactivity on the induction site and virgin site	**Number and percentage of reactive volunteers**
None	0 / 0 %

CONCLUSION

Under the experimental conditions adopted the repeated applications of the product **PHAGORUB GEL SPS - Code AL3235,** under occlusive patch induced no reaction of irritation and the product **has a very good skin compatibility.**

Moreover, the repeated applications **induced no allergic reaction.**

Signatures and dates

Investigator: Doctor Rozalia OLSAVSZKY (dermatologist) 09/07/2010

Quality Assurance Personnel: Lucia CHIRITA 09.07.2010

Head manager of the investigator centre: Alina NANU 09/07/2010

STUDY/PRODUCT REFERENCES: EF Pq 034/09-3418/ER 10/262-1/09-2358

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE PRODUCT AFTER REPEATED APPLICATION UNDER PATCH

Human Repeated Insult Patch Test (HRIPT)

SPONSOR: PHAGOGENE
Impasse du petit rosé
Z.I.
79100 LOUZY

For: Mr Jérôme DUBOURGEOIS

TEST PRODUCT: PHAGORUB GEL SPS - Code AL3235

Study report

Bucharest, February 26th, 2010

34 pages in this report including 21 in Appendices

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RO 16679189, J40/13128/2004

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE PRODUCT AFTER REPEATED APPLICATION UNDER PATCH

Repeated Insult Patch Test (HRIPT)

TABLE OF CONTENTS

I	AIM AND PRINCIPLE OF THE STUDY	3
II	RELEVANCE OF THE STUDY	3
III	TYPE OF THE STUDY	4
IV	INVESTIGATOR CENTRE AND TECHNICAL STAFF	4
V	COORDINATOR CENTRE AND TECHNICAL STAFF	5
VI	DATES OF PERFORMANCE OF THE STUDY	5
VII	TEST PRODUCTS	5
VIII	VOLUNTEERS	6
IX	METHODOLOGY	8
X	RESULTS	12
XI	CONCLUSION	13
APPENDICES 1		15-20
APPENDICES 2		21-22
APPENDICES 3		23-28
APPENDICES 4		29-34

I. AIM AND PRINCIPLE OF THE STUDY

This study intended to confirm the skin compatibility and the absence of allergenic potential of the product **PHAGORUB GEL SPS - Code AL3235,** after repeated application to the skin under exaggerated experimental conditions.

The product was applied under patch for a defined time. The applications were repeated 9 times over a period of 3 consecutive weeks, period necessary for the possible induction of an allergy.

After a minimal 2-week rest period, with no treatment, a single application of the product under patch, to the induction site and to a virgin site and for a defined time, enabled to reveal a possible induced allergy.

II. RELEVANCE OF THE STUDY

Ethics

The study aiming at confirming the skin compatibility of the test product and the foreseeable risk incurred by the volunteers who took part in the study being minor, there was a suitability between the aim of the study, its possible risks and the potential troubles related to the modalities planned in the protocol.

All the volunteers were included on the study in the same day, taking into account their number.

The applications were performed at the Institute by the dermatologist helped by the technician in charge of the study.

A clinical examination by the dermatologist helped by the technician in charge of the study was performed after each passage at the Institute.

In case of important reactivity to the product, the applications could be interrupted in the volunteers concerned.

Methodological approach

The skin compatibility of the product was confirmed by the dermatologist who has an appropriate experience.

The experimental conditions adopted created a certain occlusion and favoured the penetration of the ingredients through the skin. If some of them had an allergenic potential, this one could be more easily proved by this kind of approach.

The methodology used was an adaptation from that described by **Marzulli and Maibach** (Human Repeated Insult Patch Test for delayed contact hypersensitivity: HRIPT)

- Marzulli F.N., Maibach H.I., Contact allergy : predictive testing in man, Contact Dermatitis, 1976, 2, pp. 1-17.

The patch material and the conditions of use of the product were adapted to the type of test product in accordance with the corresponding procedure.

Several products were tested in parallel. The experimental area chosen (back) enabled to test easily the products. The sites of application of the products were chosen at random to get rid of the variability of the skin reactivity according to the site.

A control site (without test products) served as control to avoid the possible intercurrent effects not directly related to the test product.

Panel

Referring to the experience acquired in the field of contact allergy to cosmetic product and to the accurate knowledge of the ingredients incorporated into the test product, the number of volunteers, defined in the protocol, was acceptable to confirm, in first approach, the absence of allergenic potential of the test product.

Results

The results were mainly expressed as descriptive data and did not require a statistical treatment.

III. TYPE OF THE STUDY

This mono-centric study was performed in open.

The subject was used as own control.

It was performed according to the general conditions of Evic Romania, established for the performance of Human test project.

The test project was submitted to the previous agreement of the internal committee of the coordinator centre, Evic France, before its performance (opinion n° 10/995 of January 08th, 2010) and of the investigator centre, Evic Romania (opinion n° 737/10 of January 08th 2010).

IV. INVESTIGATOR CENTRE AND TECHNICAL STAFF

The Investigator centre, Evic Romania, is equipped with material and technical means suitable for the research and compatible with the safety requirements for human subjects.

IV.1. Investigator Centre

EVIC Romania – S.C. BIO HIGH TECH S.R.L.
15, Constantin Bosianu Street,
040505 – Bucharest – Romania

Tel: 0040 21 335 70 90
Fax: 0040 21 335 70 91
E-mail: evicromania@evic.ro

IV.2. Technical staff

The study is performed by a technical staff having the required qualifications and experience, under the responsibility of a competent investigator.

Investigator: Rozalia Olsavszky (dermatologist)

Responsible Technician: Nicoleta Dumitru

Quality Assurance: Lucia Chirita

V. COORDINATOR CENTRE AND TECHNICAL STAFF

V.1. Coordinator Centre

Evic France
48 rue Jean Duvert
ZI – 33295 – Blanquefort Cedex
France

Tel: 33(0) 5 56 95 59 95
Fax: 33(0) 5 56 95 05 22

Evic France – Idec department
57, rue Ulysse Gayon
33000 – Bordeaux
France

Tel: 33 (0)5 57 14 00 80
Tel: 33 (0)5 56 48 72 49

The Institute of Dermo-Cosmetic (Idec), department of Evic France, located 57, rue Ulysse Gayon – 33 000 Bordeaux – is equipped with material and technical means suitable for the research and compatible with the safety requirements for human subjects and allowed by the "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS): decision of 17/7/2000, place nb 02128M.

V.2. Technical staff

Administrative coordinator: Sylvie Pazzini

Clinical coordinator: Clotilde Trarieux-Fourault (general practitioner)

Quality Assurance: Joëlle Mimiague

VI. DATES OF PERFORMANCE OF THE STUDY

Beginning on: January 11th, 2010

End on: February 19th, 2010

VII. TEST PRODUCTS

VII.1. Total number of products simultaneously tested in the study

The number of test products was 15.

This number complied with the corresponding procedure which defines the maximal number of test products according to the chosen experimental area and patch material.

This report concerns only the product **PHAGORUB GEL SPS - Code AL3235.**

One control patch, corresponding to the type of patch material used, containing an ad hoc quantity of distilled water was applied at the same time.

VII.2. Identification of the test product

Denomination of the test product provided	PHAGOBIOL GEL
Denomination in the final report	PHAGORUB GEL SPS
Reference	AL3235
Batch number	JD0006.11
Evic France / Evic Romania reference	09-3418/09-2358
Galenic form and organoleptic characteristics	Colourless gel
Number and type of samples	10 glass flasks
Content of the samples	125 ml

VII.3. Information concerning the test product

The documents relating to the test product supplied with the samples were the qualitative formula, the Sponsor's letter of agreement particularly concerning the conformity of the formula to the regulations in force and its safety and the results of the toxicological pre-requisite (Tq 285) and of the clinical study previously performed on the product (Iq 051).

VIII . VOLUNTEERS

VIII.1 . Number

The number of volunteers whose data had to be exploitable at the end of the study was 100, with a lower acceptable limit of 97, in accordance with the corresponding procedure.

In order to compensate for the possible withdrawals during the study and to obtain this quota of volunteers at the end of the study, about 8% of extra people were recruited.

*The volunteers whose data were exploitable at the end of the study:

- to check the skin compatibility of the test product, corresponded to all the volunteers included as long as they were submitted at least to one post application examination at the defined time or else,

- to check the absence of allergenic potential of the test product (in absence of allergic reaction during the induction phase), corresponded to all the volunteers included as long as they were submitted to the challenge.

108 volunteers were included in the study.

2 volunteers discontinued (ref. 36 and 74) for personal reasons independent of the study and no exclusion was decided by the investigator.

The compatibility of the test product was therefore assessed in 106 volunteers.

The confirmation of the absence of allergenic potential of the test product was assessed in 106 volunteers.

VIII.2. Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were the following ones:

- age: 18 to 70 years old,
- sex: male and/or female,
- phototype (Fitzpatrick): I to V,
- all types of skin on body.

All the volunteers corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendices 1/1 to 1/6.**

VIII.3. Specific non inclusion criteria

The specific non inclusion criteria were the following ones:

- cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn.....),
- eczematoid reaction still visible, scar or pigmentary sequelae of previous tests on the experimental area,
- allergy to colophony, to nickel,
- allergy or reactivity to the type of test product,
- skin hyper-reactivity,
- reactivity to adhesive plaster,
- participation in more than 5 tests under exaggerated use conditions (under patch) within 12 months before the study, including 3 hypoallergenicity tests at the most,
- intensive sun exposure within the month before the study,
- forecast of intensive sun or UVA exposure (UV lamps) during the test period,
- forecast of bath (bathtub, sea or swimming-pool), sauna or hammam sessions during the test period,
- intensive or regular practice of one or several sports whose temporary interruption created difficulties,
- treatment with Vitamin A acid or its derivatives within 3 months before the beginning of the study,
- treatment with topical corticoids on the experimental area within 8 days before the study,
- treatment with PUVA or UVB within 1 month before the study,

- forecast of vaccination during the test period or last vaccination within 3 weeks before the study.

All the volunteers corresponded to these specific non inclusion criteria.

IX. METHODOLOGY

IX.1. Experimental area and sites of application of the test product

The chosen experimental area was the back.

The site of application of the product was chosen by the dermatologist or the technician in charge of the study. Skin appearance was taken into account and the areas of friction with clothes were avoided.

The product was applied by the technician in charge of the study, to one of the sites localized by a clockwise distribution, altering of one rank from a subject to another.

IX.2. Experimental conditions of application of the test product

The experimental conditions defined in the protocol were the following ones:

Patch material	Experimental conditions of use	Quantity applied
Finn Chamber standard®	As it is Evaporation for at least 15 minutes on the patch before application	20 µl

Occlusive patch

-Finn Chamber standard®: aluminium cupula in which the product was put down (20 µl or approximately 20 mg), kept in position by an hypoallergenic adhesive: Scanpor® (inner diameter: 8 mm, surface: 50 mm^2).

The quantities of product had to be measured with a single use syringe.

All the experimental conditions of application at the Institute, defined in the protocol, were respected.

IX.3. Chronology of the study

The applications of the test product, the removal of the patches and the controls were performed by the dermatologist or the technician in charge of the study.

- **Induction phase:** 3 consecutive weeks.

  * application of the product to a perfectly delimited site, under patch on D1, D3, D5, D8, D10, D12, D15, D17, D19.

  * patch removal
    - after 48 h of contact on D3, D5, D10, D12, D17, D19.

    - after 72 h of contact on D8, D15, D22.

* controls: skin examination and questioning (paragraph IX.6) before patching on D1 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D3, D5, D8, D10, D12, D15, D17, D19, D22.

- **Rest period:** 2 consecutive weeks at least (4 weeks at the most).

  * no application of product.

- **Challenge:** 1 week.

  * application of the product to a perfectly delimited virgin site and to the site defined for the induction phase, under patch on D36.

  * patch removal after 48 h of contact on D38.

  * controls: skin examination and questioning (paragraph IX.6) before patching on D36 and about 15 minutes (or more, if redness appeared after removal of the adhesive), after patch removal on D38, D39, D40 (48, 72, 96 h after application).

All the experimental conditions of application, defined in the protocol, were respected.

IX.4. Constraints of the study

The constraints imposed on the volunteers were the following ones:

- no application of other products (than the tested ones) to the experimental area,
- no wearing of too thigh or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patches,
- no bath (bathtub or swimming-pool or sea), no hammam or sauna sessions during the study,
- if shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary,
- no excessive sweating and no intensive sport liable to cause unsticking of the patches,
- no intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal,
- neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen),
- no vaccination during the study,
- at least 14 passages at the Institute (15 if a pre-inclusion visit was necessary).

IX.5. Control of the observance of the modalities of the protocol

The dermatologist checked the respect of the **constraints**.

The volunteers were questioned at the end of the study. The dermatologist assessed the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study.

The synthesis of the answers obtained is enclosed in **Appendices 2/1 and 2/2**.

All the deviations from the protocol were analysed and the dermatologist assessed their effect on the validity of the results.

All the constraints of the study, defined in the protocol, were respected by the volunteers.

IX.6. Confirmation of the compatibility (absence of irritant effect) and absence of allergenic potential

IX.6.1. Frequency of the examinations

The skin examination and joint questioning had to be performed by the dermatologist helped by the technician in charge of the study.

The examination had to be performed, visually under standard "daylight", according to the frequency mentioned on paragraph IX.3.

All the examinations were performed in accordance with the conditions defined in the protocol.

IX.6.2. Expression of the results of the skin examination and questioning

The expression of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

In case of reactivity:

- **the main visible signs were noted, i.e. :**

Erythema, Œdema, Vesicle, Bulla, Papule, Scab, Dryness, Coloration, Soap effect.

The intensity of the **erythema and oedema** was assessed according to an ordinal scale: very slight, slight, moderate, severe.

The appearance of the **erythema** was specified: diffuse, punctuated, peripheral (around the application site).

The importance of the number of **vesicles and papules** was assessed according to an ordinal scale: 1 to 2 vesicles or papules, more than 2 vesicles or papules.

Bulla, scab, dryness, coloration and soap effect were described.

The importance of the **dryness and coloration** was assessed according to an ordinal scale: slight, moderate, severe.

- **the main sensations of discomfort were described, i.e. :**

Heating, Stinging, Pruritus (itching).

The results were expressed:

- **in percentage of reactive volunteers** : for this calculation only the visible signs of reactivity were taken into account : erythema, oedema, vesicle, bulla, papule, scab.

- **in a descriptive manner** for the other visible signs or for the sensations of discomfort : when the frequency of appearance of these signs justified it, the percentage of reactive volunteers was possibly calculated.

IX.6.3. Interpretation of the results of the skin examination and questioning

All the volunteers included in the study were taken into account to confirm the skin compatibility of the test product as long as they were submitted at least to one post application examination at the defined time or else.

All the volunteers included in the study were taken into account to confirm the absence of allergenic potential of the test product (in absence of allergic reaction during the induction phase) as long as they were submitted to the challenge.

The interpretation of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

The possible reactions observed during the induction phase were either **irritation reactions** or **revelation of an allergy previously contracted or revelation of an allergy precociously induced** by the test product.

The possible reactions observed during the challenge on the "virgin" site were compared to those observed on the "induction" site at the same times. They were either **irritation reactions** or **revelation of an allergy induced during the induction phase** by the test product.

The natures, intensity, time of appearance, time of disappearance, location (induction site and/or virgin site) of the skin reaction were taken into account for the interpretation of the results.

To appreciate the skin compatibility and possible irritation reactions, the interpretation of the results, performed by the dermatologist, was absolute (referring to **the experience of the investigator centre** in this field and especially to the **data acquired** on products of same cosmetic category tested under similar conditions). The test product could therefore have a **very good, good, moderate or bad skin compatibility**.

To appreciate the allergenic potential, the interpretation of the results was partly based on the allergenicity evaluation scale established by the **ICDRG** (International Contact Dermatitis Research Group) and took into account the visible reactions (clinical signs) and the possible reactions appeared on the control site:

NT	: non tested
?+	: doubtful reaction, only slight erythema
+	: positive reaction (with no vesicle): erythema, infiltration, sometimes some papules
++	: strong positive reaction: presence of erythema, papules, vesicles
+++	: violent positive reaction: with presence of bullae
-	: negative reaction
IR	: irritation reaction

X. RESULTS

The individual data of the skin examination and questioning of the volunteers are enclosed in **Appendices 3/1 to 3/6 and 4/1 to 4/6**.

In brief:

Induction phase	
Type of reactivity on the induction site	**Number and percentage of reactive volunteers**
None	0 / 0%

Challenge	
Type of reactivity on the induction site and virgin site	**Number and percentage of reactive volunteers**
None	0 / 0 %

XI. CONCLUSION

Under the experimental conditions adopted the repeated applications of the product **PHAGORUB GEL SPS - Code AL3235** under occlusive patch induced no reaction of irritation and the product **has a very good skin compatibility.**

Moreover, the repeated applications **induced no allergic reaction.**

Signatures and dates

Investigator: Doctor Rozalia OLSAVSZKY (dermatologist) 09/07/2010

I the undersigned, Rozalia OLSAVSZKY declare that the overall conduct of the study was carried out under my responsibility and in spirit with the principles of Good Clinical Practices for cosmetics (International recommendations ICH E 6, step 4, of 1/5/1996).

Quality Assurance Personnel: Lucia CHIRITA 09.07.2010

I the undersigned, Lucia CHIRITA, declare that:
- this type of study was audited according to the procedure of the investigator centre on February 15th, 2010,
- the final report was examined on July 09th, 2010,
- the results reported accurately and completely reflect the raw data of the study.

Head manager of the investigator centre: Alina NANU 09/07/2010

I the undersigned, Alina NANU, declare to have designated Rozalia OLSAVSZKY as investigator and ensured that she approved the study protocol with full knowledge of the facts and made it available to the Quality Assurance personnel.

APPENDICES

Appendix 1/1

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype*
Ref.	Name Surname			
1	COSM. E	63	F	III
2	CUCU. P	37	F	II
3	CIOC. R	62	F	III
4	BELC. I	70	M	III
5	DIMO. N	42	F	III
6	COST. A	55	F	II
7	FILI. F	56	F	III
8	DEJA. A	62	F	III
9	PAUN. L	50	F	III
10	DEFT. T	41	F	III
11	HERE. I	58	F	II
12	FERA. C	51	F	II
13	COJO. A	69	F	III
14	TUDO. G	34	F	III
15	CIUF. I	63	M	III
16	HODO. M	53	M	III
17	COZA. A	58	F	III
18	CIUF. P	56	F	III
19	SCHI. L	40	F	IV
20	ENAC. M	49	F	IV

Appendix 1/2

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype*
Ref.	Name Surname			
21	IRUC. S	68	F	III
22	DOTE. V	63	F	III
23	CIOB. A	20	M	IV
24	CONS. F	53	F	III
25	DUTU. V	65	F	IV
26	CRIS. A	54	M	III
27	ENES. E	39	F	III
28	CORD. I	61	F	III
29	CORB. A	55	F	III
30	CORN. E	55	F	II
31	BACA. D	63	M	III
32	BACA. A	67	F	III
33	CIRL. M	41	F	III
34	CUIB. A	49	F	III
35	FILI. M	21	M	II
36	**DUMI. I**	**66**	**F**	**III**
37	SARB. M	50	F	III
38	DINC. A	35	F	III
39	CHIO. V	44	F	III
40	CHIR. V	50	M	III

Appendix 1/3

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype*
Ref.	Name Surname			
41	DUMI. P	57	F	II
42	CORN. D	43	F	II
43	ILIE. M	25	F	III
44	CORN. N	66	M	IV
45	CIOB. AN	46	F	IV
46	CIOC. L	42	F	III
47	CHIR. A	54	F	III
48	ILIN. D	23	F	III
49	CRIS. M	49	F	II
50	CHIR. VI	51	F	III
51	CHIR. AN	41	F	IV
52	COVA. I	59	F	II
53	SAND. E	19	F	IV
54	LUCA. A	23	F	III
55	PAVE. A	60	F	III
56	RADU. I	56	M	III
57	ABUZ. M	24	M	III
58	CORB. C	56	M	II
59	GURG. I	61	F	II
60	MOGI. M	41	F	III

Appendix 1/4

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype *
Ref.	Name Surname			
61	DINC. A	66	F	III
62	PANA. O	58	F	III
63	SLAV. I	48	M	II
64	SMEU. E	67	F	III
65	MIHA. L	66	F	III
66	NITA. G	59	F	II
67	GING. D	49	F	II
68	MIEL. S	19	F	III
69	DOBR. A	62	F	III
70	NANE. R	18	F	III
71	BRAT. I	19	M	III
72	IONE. S	64	F	IV
73	ANDR. E	50	F	III
74	DOBR. I	55	M	III
75	LUNC. A	57	F	III
76	GHEO. I	63	F	III
77	BERC. V	59	F	III
78	SIMI. D	45	F	II
79	BOER. M	55	F	III
80	DRAG. D	25	F	III

Appendix 1/5

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype *
Ref.	Name Surname			
81	RATU. E	22	F	III
82	BARB. M	56	F	III
83	OBOR. R	56	F	III
84	DANI. T	48	F	II
85	RAIC. E	43	F	IV
86	CONS. G	57	F	III
87	DIAC. P	39	F	III
88	COLT. E	66	F	III
89	CHIR. G	37	F	III
90	BURC. M	53	F	III
91	PAUN. C	36	F	III
92	POPE. M	59	M	III
93	SIMO. I	31	F	II
94	DRAG. S	34	M	III
95	DIMA. M	53	F	III
96	COCA. V	58	F	III
97	CIUR. D	47	F	II
98	BRAN. M	47	F	II
99	SFIC. V	49	F	III
100	COCO. L	44	F	III

Appendix 1/6

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female M=male	Phototype*
Ref.	Name Surname			
101	ALBU. I	52	F	III
102	GHEO. M	43	F	III
103	PREC. E	65	F	II
104	PETR. G	43	M	III
105	POP. A	52	F	III
106	BISU. C	37	F	III
107	COLI. S	32	F	III
108	DAMI. G	47	F	III

Legends: ▭ *Withdrawal*

**__phototype according to Fitzpatrick__, established on the principle of a first 30 to 40-minute sun exposure after the winter or a period without exposure of an equivalent duration:*

Type I *: Always burns easily, never tans*
Type II *: Always burns easily, tans minimally*
Type III *: Burns moderately, tans gradually*
Type IV *: Burns slightly, always tans easily*
Type V *: Burns rarely, tans intensely*
Type VI *: Never burns, strongly pigmented*

Appendix 2/1

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(106 exploitable results)**	**Number** of volunteers who respected the constraints	**Percentage** of volunteers who respected the constraints
No application of other products than the tested ones to the experimental area Deviation: none	106	100 %
No wearing of too tight or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patch Deviation: none	106	100 %
No bath (bathtub, swimming pool or sea), no hammam or sauna sessions during the study Deviation: none	106	100 %
If shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary Deviation: none	106	100 %
No excessive sweating and no intensive sport liable to cause unsticking of the patch Deviation: none	106	100 %

Appendix 2/2

CONTROL OF THE OBSERVANCE
Constraints

Constraints **(106 exploitable results)**	**Number** of volunteers who respected the constraints	**Percentage** of volunteers who respected the constraints
No intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal Deviation: none	106	100 %
Neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen) – no medical treatment which could interfere with the study Deviation: none	106	100 %
No vaccination during the study Deviation: none	106	100 %
At least 14 passages at the Institute (15 if a pre-inclusion visit was necessary) Deviation: none	106	100 %

Appendix 3/1

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
1	COSM. E	/	/	/	/	/	/	/	/	/
2	CUCU. P	/	/	/	/	/	/	/	/	/
3	CIOC. R	/	/	/	/	/	/	/	/	/
4	BELC. I	/	/	/	/	/	/	/	/	/
5	DIMO. N	/	/	/	/	/	/	/	/	/
6	COST. A	/	/	/	/	/	/	/	/	/
7	FILI. F	/	/	/	/	/	/	/	/	/
8	DEJA. A	/	/	/	/	/	/	/	/	/
9	PAUN. L	/	/	/	/	/	/	/	/	/
10	DEFT. T	/	/	/	/	/	/	/	/	/
11	HERE. I	/	/	/	/	/	/	/	/	/
12	FERA. C	/	/	/	/	/	/	/	/	/
13	COJO. A	/	/	/	/	/	/	/	/	/
14	TUDO. G	/	/	/	/	/	/	/	/	/
15	CIUF. I	/	/	/	/	/	/	/	/	/
16	HODO. M	/	/	/	/	/	/	/	/	/
17	COZA. A	/	/	/	/	/	/	/	/	/
18	CIUF. P	/	/	/	/	/	/	/	/	/
19	SCHI. L	/	/	/	/	/	/	/	/	/
20	ENAC. M	/	/	/	/	/	/	/	/	/

Appendix 3/2

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
21	IRUC. S	/	/	/	/	/	/	/	/	/
22	DOTE. V	/	/	/	/	/	/	/	/	/
23	CIOB. A	/	/	/	/	/	/	/	/	/
24	CONS. F	/	/	/	/	/	/	/	/	/
25	DUTU. V	/	/	/	/	/	/	/	/	/
26	CRIS. A	/	/	/	/	/	/	/	/	/
27	ENES. E	/	/	/	/	/	/	/	/	/
28	CORD. I	/	/	/	/	/	/	/	/	/
29	CORB. A	/	/	/	/	/	/	/	/	/
30	CORN. E	/	/	/	/	/	/	/	/	/
31	BACA. D	/	/	/	/	/	/	/	/	/
32	BACA. A	/	/	/	/	/	/	/	/	/
33	CIRL. M	/	/	/	/	/	/	/	/	/
34	CUIB. A	/	/	/	/	/	/	/	/	/
35	FILI. M	/	/	/	/	/	/	/	/	/
36	DUMI. I	**WITHDRAWAL**								
37	SARB. M	/	/	/	/	/	/	/	/	/
38	DINC. A	/	/	/	/	/	/	/	/	/
39	CHIO. V	/	/	/	/	/	/	/	/	/
40	CHIR. V	/	/	/	/	/	/	/	/	/

Appendix 3/3

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
41	DUMI. P	/	/	/	/	/	/	/	/	/
42	CORN. D	/	/	/	/	/	/	/	/	/
43	ILIE. M	/	/	/	/	/	/	/	/	/
44	CORN. N	/	/	/	/	/	/	/	/	/
45	CIOB. A	/	/	/	/	/	/	/	/	/
46	CIOC. L	/	/	/	/	/	/	/	/	/
47	CHIR. A	/	/	/	/	/	/	/	/	/
48	ILIN. D	/	/	/	/	/	/	/	/	/
49	CRIS. M	/	/	/	/	/	/	/	/	/
50	CHIR. V	/	/	/	/	/	/	/	/	/
51	CHIR. A	/	/	/	/	/	/	/	/	/
52	COVA. I	/	/	/	/	/	/	/	/	/
53	SAND. E	/	/	/	/	/	/	/	/	/
54	LUCA. A	/	/	/	/	/	/	/	/	/
55	PAVE. A	/	/	/	/	/	/	/	/	/
56	RADU. I	/	/	/	/	/	/	/	/	/
57	ABUZ. M	/	/	/	/	/	/	/	/	/
58	CORB. C	/	/	/	/	/	/	/	/	/
59	GURG. I	/	/	/	/	/	/	/	/	/
60	MOGI. M	/	/	/	/	/	/	/	/	/

Appendix 3/4

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
61	DINC. A	/	/	/	/	/	/	/	/	/
62	PANA. O	/	/	/	/	/	/	/	/	/
63	SLAV. I	/	/	/	/	/	/	/	/	/
64	SMEU. E	/	/	/	/	/	/	/	/	/
65	MIHA. L	/	/	/	/	/	/	/	/	/
66	NITA. G	/	/	/	/	/	/	/	/	/
67	GING. D	/	/	/	/	/	/	/	/	/
68	MIEL. S	/	/	/	/	/	/	/	/	/
69	DOBR. A	/	/	/	/	/	/	/	/	/
70	NANE. R	/	/	/	/	/	/	/	/	/
71	BRAT. I	/	/	/	/	/	/	/	/	/
72	IONE. S	/	/	/	/	/	/	/	/	/
73	ANDR. E	/	/	/	/	/	/	/	/	/
74	DOBR. I	**WITHDRAWAL**								
75	LUNC. A	/	/	/	/	/	/	/	/	/
76	GHEO. I	/	/	/	/	/	/	/	/	/
77	BERC. V	/	/	/	/	/	/	/	/	/
78	SIMI. D	/	/	/	/	/	/	/	/	/
79	BOER. M	/	/	/	/	/	/	/	/	/
80	DRAG. D	/	/	/	/	/	/	/	/	/

Appendix 3/5

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
81	RATU. E	/	/	/	/	/	/	/	/	/
82	BARB. M	/	/	/	/	/	/	/	/	/
83	OBOR. R	/	/	/	/	/	/	/	/	/
84	DANI. T	/	/	/	/	/	/	/	/	/
85	RAIC. E	/	/	/	/	/	/	/	/	/
86	CONS. G	/	/	/	/	/	/	/	/	/
87	DIAC. P	/	/	/	/	/	/	/	/	/
88	COLT. E	/	/	/	/	/	/	/	/	/
89	CHIR. G	/	/	/	/	/	/	/	/	/
90	BURC. M	/	/	/	/	/	/	/	/	/
91	PAUN. C	/	/	/	/	/	/	/	/	/
92	POPE. M	/	/	/	/	/	/	/	/	/
93	SIMO. I	/	/	/	/	/	/	/	/	/
94	DRAG. S	/	/	/	/	/	/	/	/	/
95	DIMA. M	/	/	/	/	/	/	/	/	/
96	COCA. V	/	/	/	/	/	/	/	/	/
97	CIUR. D	/	/	/	/	/	/	/	/	/
98	BRAN. M	/	/	/	/	/	/	/	/	/
99	SFIC. V	/	/	/	/	/	/	/	/	/
100	COCO. L	/	/	/	/	/	/	/	/	/

Appendix 3/6

SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Volunteers		Reactivity								
Ref.	**Name Surname**	**D3**	**D5**	**D8**	**D10**	**D12**	**D15**	**D17**	**D19**	**D22**
101	ALBU. I	/	/	/	/	/	/	/	/	/
102	GHEO. M	/	/	/	/	/	/	/	/	/
103	PREC. E	/	/	/	/	/	/	/	/	/
104	PETR. G	/	/	/	/	/	/	/	/	/
105	POP. A	/	/	/	/	/	/	/	/	/
106	BISU. C	/	/	/	/	/	/	/	/	/
107	COLI. S	/	/	/	/	/	/	/	/	/
108	DAMI. G	/	/	/	/	/	/	/	/	/

Legends:

/: nothing to report
E: Erythema
Oe: Œdema
V: Vesicle
D: Dryness
S: Soap effect

Bu: Bulla
Pa: Papule
Sc: Scab
C: Coloration
Pr: Pruritus
Hea: Heating
Sti: Stinging

0.5: Very slight intensity
1: Slight intensity
2: Moderate intensity
3: Severe intensity
d: diffuse
p: punctuated
peri: peripheral
Vesicles or papules
1: nb = 1 or 2
2: nb > 2

Appendix 4/1

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
1	COSM. E	/	/	/	/	/	/	/	/	-
2	CUCU. P	/	/	/	/	/	/	/	/	-
3	CIOC. R	/	/	/	/	/	/	/	/	-
4	BELC. I	/	/	/	/	/	/	/	/	-
5	DIMO. N	/	/	/	/	/	/	/	/	-
6	COST. A	/	/	/	/	/	/	/	/	-
7	FILI. F	/	/	/	/	/	/	/	/	-
8	DEJA. A	/	/	/	/	/	/	/	/	-
9	PAUN. L	/	/	/	/	/	/	/	/	-
10	DEFT. T	/	/	/	/	/	/	/	/	-
11	HERE. I	/	/	/	/	/	/	/	/	-
12	FERA. C	/	/	/	/	/	/	/	/	-
13	COJO. A	/	/	/	/	/	/	/	/	-
14	TUDO. G	/	/	/	/	/	/	/	/	-
15	CIUF. I	/	/	/	/	/	/	/	/	-
16	HODO. M	/	/	/	/	/	/	/	/	-
17	COZA. A	/	/	/	/	/	/	/	/	-
18	CIUF. P	/	/	/	/	/	/	/	/	-
19	SCHI. L	/	/	/	/	/	/	/	/	-
20	ENAC. M	/	/	/	/	/	/	/	/	-

Appendix 4/2

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
21	IRUC. S	/	/	/	/	/	/	/	/	-
22	DOTE. V	/	/	/	/	/	/	/	/	-
23	CIOB. A	/	/	/	/	/	/	/	/	-
24	CONS. F	/	/	/	/	/	/	/	/	-
25	DUTU. V	/	/	/	/	/	/	/	/	-
26	CRIS. A	/	/	/	/	/	/	/	/	-
27	ENES. E	/	/	/	/	/	/	/	/	-
28	CORD. I	/	/	/	/	/	/	/	/	-
29	CORB. A	/	/	/	/	/	/	/	/	-
30	CORN. E	/	/	/	/	/	/	/	/	-
31	BACA. D	/	/	/	/	/	/	/	/	-
32	BACA. A	/	/	/	/	/	/	/	/	-
33	CIRL. M	/	/	/	/	/	/	/	/	-
34	CUIB. A	/	/	/	/	/	/	/	/	-
35	FILI. M	/	/	/	/	/	/	/	/	-
36	DUMI. I	WITHDRAWAL								NT
37	SARB. M	/	/	/	/	/	/	/	/	-
38	DINC. A	/	/	/	/	/	/	/	/	-
39	CHIO. V	/	/	/	/	/	/	/	/	-
40	CHIR. V	/	/	/	/	/	/	/	/	-

Appendix 4/3

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
41	DUMI. P	/	/	/	/	/	/	/	/	-
42	CORN. D	/	/	/	/	/	/	/	/	-
43	ILIE. M	/	/	/	/	/	/	/	/	-
44	CORN. N	/	/	/	/	/	/	/	/	-
45	CIOB. A	/	/	/	/	/	/	/	/	-
46	CIOC. L	/	/	/	/	/	/	/	/	-
47	CHIR. A	/	/	/	/	/	/	/	/	-
48	ILIN. D	/	/	/	/	/	/	/	/	-
49	CRIS. M	/	/	/	/	/	/	/	/	-
50	CHIR. V	/	/	/	/	/	/	/	/	-
51	CHIR. A	/	/	/	/	/	/	/	/	-
52	COVA. I	/	/	/	/	/	/	/	/	-
53	SAND. E	/	/	/	/	/	/	/	/	-
54	LUCA. A	/	/	/	/	/	/	/	/	-
55	PAVE. A	/	/	/	/	/	/	/	/	-
56	RADU. I	/	/	/	/	/	/	/	/	-
57	ABUZ. M	/	/	/	/	/	/	/	/	-
58	CORB. C	/	/	/	/	/	/	/	/	-
59	GURG. I	/	/	/	/	/	/	/	/	-
60	MOGI. M	/	/	/	/	/	/	/	/	-

Appendix 4/4

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
61	DINC. A	/	/	/	/	/	/	/	/	-
62	PANA. O	/	/	/	/	/	/	/	/	-
63	SLAV. I	/	/	/	/	/	/	/	/	-
64	SMEU. E	/	/	/	/	/	/	/	/	-
65	MIHA. L	/	/	/	/	/	/	/	/	-
66	NITA. G	/	/	/	/	/	/	/	/	-
67	GING. D	/	/	/	/	/	/	/	/	-
68	MIEL. S	/	/	/	/	/	/	/	/	-
69	DOBR. A	/	/	/	/	/	/	/	/	-
70	NANE. R	/	/	/	/	/	/	/	/	-
71	BRAT. I	/	/	/	/	/	/	/	/	-
72	IONE. S	/	/	/	/	/	/	/	/	-
73	ANDR. E	/	/	/	/	/	/	/	/	-
74	DOBR. I	WITHDRAWAL								NT
75	LUNC. A	/	/	/	/	/	/	/	/	-
76	GHEO. I	/	/	/	/	/	/	/	/	-
77	BERC. V	/	/	/	/	/	/	/	/	-
78	SIMI. D	/	/	/	/	/	/	/	/	-
79	BOER. M	/	/	/	/	/	/	/	/	-
80	DRAG. D	/	/	/	/	/	/	/	/	-

Appendix 4/5

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
81	RATU. E	/	/	/	/	/	/	/	/	-
82	BARB. M	/	/	/	/	/	/	/	/	-
83	OBOR. R	/	/	/	/	/	/	/	/	-
84	DANI. T	/	/	/	/	/	/	/	/	-
85	RAIC. E	/	/	/	/	/	/	/	/	-
86	CONS. G	/	/	/	/	/	/	/	/	-
87	DIAC. P	/	/	/	/	/	/	/	/	-
88	COLT. E	/	/	/	/	/	/	/	/	-
89	CHIR. G	/	/	/	/	/	/	/	/	-
90	BURC. M	/	/	/	/	/	/	/	/	-
91	PAUN. C	/	/	/	/	/	/	/	/	-
92	POPE. M	/	/	/	/	/	/	/	/	-
93	SIMO. I	/	/	/	/	/	/	/	/	-
94	DRAG. S	/	/	/	/	/	/	/	/	-
95	DIMA. M	/	/	/	/	/	/	/	/	-
96	COCA. V	/	/	/	/	/	/	/	/	-
97	CIUR. D	/	/	/	/	/	/	/	/	-
98	BRAN. M	/	/	/	/	/	/	/	/	-
99	SFIC. V	/	/	/	/	/	/	/	/	-
100	COCO. L	/	/	/	/	/	/	/	/	-

Appendix 4/6

SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE

Volunteers		Reactivity								According to the ICDRG criteria
		Induction site				Virgin site				
Ref.	Name Surname	D36	D38	D39	D40	D36	D38	D39	D40	
101	ALBU. I	/	/	/	/	/	/	/	/	-
102	GHEO. M	/	/	/	/	/	/	/	/	-
103	PREC. E	/	/	/	/	/	/	/	/	-
104	PETR. G	/	/	/	/	/	/	/	/	-
105	POP. A	/	/	/	/	/	/	/	/	-
106	BISU. C	/	/	/	/	/	/	/	/	-
107	COLI. S	/	/	/	/	/	/	/	/	-
108	DAMI. G	/	/	/	/	/	/	/	/	-

Legends:

/: nothing to report
E: Erythema
Oe: Œdema
V: Vesicle
D: Dryness
S: Soap effect

Bu: Bulla
Pa: Papule
Sc: Scab
C: Coloration
Pr: Pruritus
Hea: Heating
Sti: Stinging

0.5: Very slight intensity
1: Slight intensity
2: Moderate intensity
3: Severe intensity
d: diffuse
p: punctuated
peri: peripheral
Vesicles or papules
1: nb = 1 or 2
2: nb > 2

ICDRG

NT	*:*	*non tested*
?+	*:*	*uncertain reaction, only slight erythema*
+	*:*	*positive reaction (with no vesicle): erythema, infiltration, sometimes some papules*
++	*:*	*strong positive reaction: presence of erythema, papules, vesicles*
+++	*:*	*violent positive reaction: with presence of bullae*
-	*:*	*negative reaction*
IR	*:*	*irritation reaction*