

SCIENTIFIC REPORT

PHAGO'RUB GEL SPS

For Sensitive Skin

Hydro-alcoholic gel for hand disinfection



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1. INTRODUCTION

To control infection is above all to control the spread of germs and bacteria.

Their circulation within the hospital, from one patient to another, from a caregiver to a patient, from a caregiver to another caregiver is done mainly through hand contact.

There are 2 types of flora found on hands:

- Resident flora
- Transient flora

The resident flora acts like a barrier against other microbes. Most often it has a harmonic relationship to people without hurting them.

The transient flora is a reflection of the environment, it varies during the day according to the activities and according to the variations of the environment. Often pathogenic, it causes severe disturbances and is responsible for many nosocomial infections.

These infections can be reduced by applying hygiene rules such as washing and / or disinfecting hands.

Hand hygiene therefore requires knowledge of these methods, awareness and training of teams, the provision of products and equipment adapted to the needs.

In order to address these concerns, the Phagogène Health Division of Christeyns France has developed the **Phago'rub Gel SPS** for the disinfection of hands.

Phago'rub Gel SPS is intended for hygienic and surgical disinfection of hands by friction. Frictional disinfection easily replaces simple, hygienic, or surgical washing for high-risk infectious acts through microbiological activity superior to disinfectant soaps.

Practicing hand hygiene by hydro-alcoholic friction is now a WHO recommended procedure (World Health Organization): it's faster, more effective and better tolerated than washing with water and antiseptic soap. It improves compliance by users and allows them to comply with recommendations for good hygiene practices.

2. COMPOSITION

- ✓ Ethyl alcohol (CAS N ° 64-17-5): 72% m / m
- ✓ Hydrating and softening glycerine
- ✓ Myristyl alcohol with emollient properties
- ✓ Gelling agent
- ✓ No colouring, phenoxyethanol-free, perfume-free, paraben-free

3. ORGANOLEPTIC AND PHYSICO-CHEMICAL CHARACTERISTICS

- ✓ Appearance: gel
- ✓ Colour: colourless
- ✓ Odour: alcoholic
- ✓ Density at 20 ° C (g / ml): 0.863 ± 0.009

4. DIRECTIONS FOR USE

Wearing gloves does not exclude hand disinfection.

In the case of visually soiled hands, the rubbing will be preceded by a simple hand washing with careful drying.

Phago'rub Gel SPS should be used pure on healthy, clean and dry skin. Do not rinse.

➤ Hygienic hand disinfection :

When to perform it:

- ✓ Before and after an intermediate level infectious risk act
- ✓ After contact with the healthy skin of an infected patient or with an injured skin or mucous membrane
- ✓ Between two patients
- ✓ Before any aseptic action
- ✓ In a controlled atmosphere zone.

Procedure

- ✓ Have short, unvarnished fingernails and short or folded sleeves
- ✓ Fill the palm of the hand with 3 ml of **Phago'rub Gel SPS**
- ✓ Rub hands and wrists for at least 30 seconds according to standardised procedure
- ✓ Hands should be completely covered in product for the duration of the application

➤ Surgical disinfection of hands :

When to perform it:

- ✓ Before any surgery, obstetrics, and interventional radiology
- ✓ Before any procedure for which a surgical type of aseptic technique is required: central catheter, spinal catheter, implantable venous access device, amniotic puncture....

Procedure

- ✓ Have short, unvarnished fingernails and short or folded sleeves
- ✓ Fill the palm of the hand with 3 ml of **Phago'rub Gel SPS**
- ✓ Rub hands, wrists, forearms and elbows until dry (time \geq 45 seconds)
- ✓ Rub up to sleeves according to the methodology followed for hygienic disinfection until skin is completely dry (time \geq 45 seconds)

5. TOLERANCE

DESIGNATION, REFERENCES AND DATE OF THE STUDY	COMPANY	RESULTS / CONCLUSIONS
Acute irritant / corrosive effect on rabbits' skin - OECD 404 - ref. Tp 285 / 09-1908, 28/07/09	Evic France	→ Product not classified as skin irritating
Verification in humans of cutaneous compatibility of a cosmetic product after open application - open test under dermatological control - Ref. Iq 051bis / 09, 3418, 15/09/10.	Evic France	→ Very good cutaneous compatibility.

Confirmation of cutaneous compatibility and lack of allergic potential of a product after repeated application under occlusive patch (HRIPT) - ref. EF Pq 034 / 09-3418 / ER 10 / 262-1 / 09-2358, 26/02/10.	Evic Romania	→ Very good cutaneous compatibility. → Induces no allergic reaction.
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The study reports are available as appendices.

6. ANTIMICROBIAL ACTIVITY

STANDARDS	CONCENTRATION	TIME	REPORT NUMBER AND DATE	LABORATORY OF EXPERTISE
<u>BACTERICIDAL</u>				
EN 1040 [2006] <i>(P.aeruginosa and S.aureus)</i>	80 %	15 s.	2012/02 13/02/2012	Laboratoire Rivadis
EN 13727 + A1 [2013] Clean conditions <i>(P.aeruginosa, S.aureus, E.hirae and E.coli)</i>	80 %	30 s.	3587-1 01/04/2015	LMH
EN 1500 [2013] <i>(E.coli)</i>	3 ml	30 s.	A15086 27/03/2015	Hygiène Nord GmbH
EN 12791 [2016] persistence 3 hours	2 x 3 ml	2 x 45 s.	B20234 17/07/2016	HygCen Austria GmbH
<u>MYCOBACTERICIDAL</u>				
EN 14348 [2005] Clean conditions <i>(M. avium)</i>	80 %	30 s	3712-1 13/10/15	LMH
EN 14348 [2005] Clean conditions <i>(M. terrae)</i>	80 %	30 s	BT-PHA-004 29/04/2011	BluTest
<u>FUNGICIDAL</u>				
EN 1275 [2006] <i>(C.albicans)</i>	80%	15s.	2012/001 07/02/2012	Laboratoire Rivadis
EN 1650 [2008] Clean conditions <i>(C.albicans)</i>	80%	15s.	2010/007 15/06/2010	Laboratoire Rivadis
EN 13624 [2013] Clean conditions <i>(C.albicans)</i>	80%	30s.	3588-1 01/04/2015	LMH
EN 13624 [2013] Clean conditions <i>(A. brasiliensis)</i>	97%	30s.	3714-1 14/09/2015	LMH

STANDARDS	CONCENTRATION	TIME	REPORT NUMBER AND DATE	LABORATORY OF EXPERTISE
<u>VIRUCIDAL</u>				
EN 14476 [2013] Clean conditions (Poliovirus type 1)	97%	30s.	C15L0111Po 18/06/2015	Dr. Brill + Partner GmbH
EN 14476 [2013] Clean conditions (Adenovirus type 5)	80%	30s.	C15L0111A 10/07/2015	Dr. Brill + Partner GmbH
EN 14476 [2013] Clean conditions (Norovirus)	97%	30s.	15/000189203 08/06/2015	Chellab Silliker
EN 14476 [2007] Clean conditions (Rotavirus)	80%	15s.	P09ML928-1R 18/11/2009	Mikrolab
EN 14476 [2007] Clean conditions (Influenza A/H1N1)	80%	15 s.	P09ML928-1SI 20/11/2009	Mikrolab
DVV-RKI Clean conditions (BVD)	80%	15s.	R09ML820aB 09/09/2009	Mikrolab
DVV-RKI Clean conditions (Vaccinia virus)	80%	15 s.	S09ML820V	Mikrolab
DVV-RKI Clean conditions (HBV, HCV, HIV, Herpes virus, Coronavirus (including RSV), Orthomyxovirus, Influenza A / H1N1 and Influenza A / H5N1)	80%	15 s.	See certificate	Mikrolab

The study reports are available as appendices.

7. PRODUCT REGULATION – EMERGENCY PROCEDURES

Product regulation :

Phago'rub Gel SPS is formulated according to European Union and French regulations concerning biocidal products.

Emergency procedures:

- ✓ In case of contact of the product with the eyes, rinse carefully with water for several minutes. Remove contact lenses, if present and easy to do. Continue to rinse. If eye irritation persists: Get medical attention.
- ✓ Use biocides carefully. Read the label and product information before use.
- ✓ The safety data sheet is available to users at <http://www.quickfds.com>. The site allows you to be informed automatically by email of any update of the safety data sheets consulted.

8. CONSERVATION

- ✓ Store in closed original vials at room temperature, away from heat and frost.
- ✓ Expiration: 36 months from the date of manufacture.
- ✓ Period after opening (PAO) :
 - Flat dose of 5 ml: 1 to 2 application in one day then to be thrown away
 - For bottles of 100ml, 300ml, 500ml: 6 months after the first opening
 - 1000ml airless bottle: unopened but used, can be kept until the expiry date since it is equipped with a distribution system avoiding any entry of air and therefore any contamination by the external environment

Microbial air contamination study carried out on the packaging available on request.

9. PACKAGING

Type of container	Code	Number of units per carton
Dose of 5 ml	60676	250 units
Bottle of 100 ml	60673	30 units
Bottle of 300 ml with pump 1,5ml	60674	20 units
Bottle of 300 ml with pump 3ml	65024	20 units
Bottle of 500 ml with pump 1,5ml	65057	20 units
Bottle of 500 ml with pump 3ml	65058	20 units
Bottle of 1000 ml airless, teated 1,5ml	60672	12 units
Bottle of 1000 ml airless, teated 3ml	65056	12 units
Can of 5L	65054	2 units

10. APPENDICES



CHRISTEYNS

ATTESTATION

Vertou 29/10/2018

I hereby Olivier COTTRON, pharmacist responsible for the company Christeyns France that the products:

1592

And

PHAGORUB GEL SPS

And

SOLUTION HYDROALCOOLIQUE AL 3235

And

PHAGORUB

And

GEL DESINFECTANT MAINS

have a strictly identical formula and that disinfection tests were passed under this code.

Olivier COTTRON

Pharmacist responsible



CHRISTEYNS

ATTESTATION

Vertou 29/10/2018

I hereby Olivier COTTRON, pharmacist responsible for the company Christeyns France that the products:

PHAGORUB GEL SPS – AL3235

And

PHAGORUB SOLUTION SPS – DE528

have an identical rate of disinfectant active ingredient.

The efficacy and tolerance tests carried out on the Phagorub gel SPS product are therefore also valid for the Phagorub SPS solution.

Olivier COTTRON

Pharmacist responsible

LABORATOIRES : <input type="checkbox"/> PHAGOGENE <input checked="" type="checkbox"/> 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° 2012 /002

Norme : Antiseptiques et désinfectants chimiques.

Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide de base des antiseptiques et des désinfectants chimiques. Méthode d'essai et prescriptions (phase 1)

Identification de l'échantillon de désinfectant

Nom du produit : PHAGORUB solution

Numéro de 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, utilisé pur

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

Aspect du produit : liquide limpide fluide

Période d'essai : le jeudi 09/02/2012

Résultats : (voir page 3/3)

Conclusion :

Le produit PHAGORUB SOLUTION lot 20011 07.2013 utilisé pur (80%V/V au contact) est bactéricide en 15 secondes de contact vis-à-vis des souches de référence pseudomonas aeruginosa ATCC 15442 et staphylococcus aureus ATCC 6538 selon la méthodologie adaptée de la norme AFNOR NF EN 1040 2006.

SIGNATAIRES :

Alain LE BEC, Microbiologiste Laboratoire de Contrôle :

13/02/2012

Guillaume MACOUIN, Responsable Laboratoire de Contrôle :

13/02/2012



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

LABORATOIRES : PHAGOGENE RIVADIS

Réf. : F-278/A Page : 3/3

Fiche : Rapport d'essai de normes européennes sur les antiseptiques et désinfectants chimiques

Date d'application : 11 février 2009

RAPPORT D'ESSAI INTERNE N° 2012/002

Résultats d'essai :

selon l'instruction I-109/F
version du fichier : a
date de la dernière modification : 24/08/2010
résultats de rapport d'essai interne n° 2012/002

résultats de la norme AFNOR NF EN 1040 avril 2006

pseudomonas aeruginosa ATCC 15442

concentration=>	80		
Vc1	15		
Vc2	15		
Na	150	#DIV/0!	#DIV/0!
R	5,2	#DIV/0!	#DIV/0!

	Vc1	Vc2	moyenne
Nv			680
Nvo	67	69	68
A	199	224	212
B	220	222	221
C	57	74	66
N	210	239	2,25E+08

staphylococcus aureus ATCC 6538

concentration=>	80		
Vc1	15		
Vc2	15		
Na	150	#DIV/0!	#DIV/0!
R	5,1	#DIV/0!	#DIV/0!

	Vc1	Vc2	moyenne
Nv			860
Nvo	84	88	86
A	179	167	173
B	175	170	173
C	80	75	78
N	184	172	1,78E+08

pour chaque souche d'essai, vérifier que :

la suspension d'essai
le nombre d'UFC/ml dans le mélange à T0
la suspension de validation
le nombre d'UFC/ml dans les mélanges A,B,C
le témoin des conditions expérimentales
le témoin de toxicité du neutralisant
l'essai de validation du neutralisant
la réduction logarithmique de N/Na

N est comprise entre $1,5 \times 10^8$ et 5×10^8 UCF/ml
No est compris entre $1,5 \times 10^7$ et 5×10^7 UCF/ml (N/10)
Nv est compris entre 300 et 1600 UFC/ml
Nvo est compris entre 30 et 160
A doit être supérieur ou égal à $0,5 \times Nvo$
B doit être supérieur ou égal à $0,5 \times Nvo$
C doit être supérieur ou égal à $0,5 \times Nvo$
R est supérieur ou égal à 5 ($R = No/Na$)
Vc est le nombre de colonie par boîte de Pétri
Na est le nombre de colonie par boîte de Pétri
UFC/ml dans le mélange d'essai après le temps de contact

condition expérimentales :

date des essais : le jeudi 09/02/2012
produit testé : PHAGORUB solution lot 20011 07.2013
temps de contact en min : 15 secondes
température de contact en °C : 21°C
lot *pseudomonas aeruginosa* ATCC15442 693-50-2 13/04/2011
lot *staphylococcus aureus* ATCC 6538 485-106-2 2013/02
lot gélose TSA: 119301B 30/07/2014
lot tryptone sel: 201003 09/01/2013
lot neutralisant: N6 du 18/04/2011
analyste : Alain LE BEC

conclusion :

Le produit PHAGORUB SOLUTION lot 20011 07.2013 utilisé pur (80%V/V au contact) est bactéricide en 15 secondes de contact vis-à-vis des souches de référence *pseudomonas aeruginosa* ATCC 15442 et *staphylococcus aureus* ATCC 6538 selon la méthodologie adaptée de la norme AFNOR NF EN 1040 2006.

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N° 3587-1

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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

V_c : nombre de colonies comptées sur les boîtes,

N : nombre d'UFC / ml dans la suspension microbienne d'essai,

N₀ : nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de *N*,

N_v : nombre de cellules par ml de la suspension microbienne de validation,

N_{v0} : nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de *N_v*,

N_{vB} : 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 *N_v*,

N_a : 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 ($R=N_0/N_a$) 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)
<i>Enterococcus hirae</i> DSM 3320 Lot 568	10^{-6} : Vc1 : 286 Vc2 : 302 10^{-7} : Vc1 : 41 Vc2 : 42 N = $3,05 \cdot 10^8$ N ₀ = $3,05 \cdot 10^7$ Log N ₀ = 7,48	Vc1 : 68 Vc2 : 79 (dilution 10^{-1}) N _v = 735 N _{v0} = 74	Vc1 : 75 Vc2 : 88 (dilution 10^{-3}) N _{vB} = $8,15 \cdot 10^4$	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 $1,5 \cdot 10^8$ et $5 \cdot 10^8$ UFC/ml ($8,17 \leq \lg N \leq 8,70$)

N_0 est compris entre $1,5 \cdot 10^7$ et $5 \cdot 10^7$ UFC/ml ($7,17 \leq \lg N \leq 7,70$)

N_{v0} est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)

N_{vB} est compris entre $3,0 \cdot 10^4$ et $1,6 \cdot 10^5$

A, B et C sont supérieurs ou égaux à $0,5 \times N_{v0}$

B (dilution-neutralisation) est égal ou supérieur à $0,0005 \times N_{vB}$

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}
<i>Enterococcus hirae</i> DSM 3320 Lot 568	Vc1	>330	>330	>330	>330	>330	>330	0	0	0
	Vc2	>330	>330	>330	>330	>330	>330	0	0	0
	Na	> $3,3 \cdot 10^5$			> $3,3 \cdot 10^5$			<140		
	Ig 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)
<i>Staphylococcus aureus</i> DSM 799 Lot 567	10 ⁻⁶ : Vc1 : 263 Vc2 : 234	Vc1 : 52 Vc2 : 61 (dilution 10 ⁻¹)	Vc1 : 48 Vc2 : 59 (dilution 10 ⁻³)	Vc1 : 50 Vc2 : 54	Vc1 : 53 Vc2 : 49	Vc1 : 57 Vc2 : 61
	10 ⁻⁷ : Vc1 : 27 Vc2 : 29	Nv = 565 Nv ₀ = 57	NvB = 5,35.10 ⁴	A = 52	B = 51	C = 59
	N = 2,51.10 ⁸ N ₀ = 2,51.10 ⁷ Log N ₀ = 7,40					

L'essai est validé si :

N est compris entre $1,5.10^8$ et 5.10^8 UFC/ml ($8,17 \leq \lg N \leq 8,70$)

N_0 est compris entre $1,5.10^7$ et 5.10^7 UFC/ml ($7,17 \leq \lg N \leq 7,70$)

N_{v0} est compris entre 30 et 160 UFC/ml (N_v est compris entre 300 et 1600 UFC/ml)

N_{vB} est compris entre $3,0.10^4$ et $1,6.10^5$

A, B et C sont supérieurs ou égaux à $0,5 \times N_{v0}$

B (dilution-neutralisation) est égal ou supérieur à $0,0005 \times N_{vB}$

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
<i>Staphylococcus aureus</i> DSM 799 Lot 567	Vc1	>330	>330	>330	>330	>330	>330	0	0	0
	Vc2	>330	>330	>330	>330	>330	>330	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)
<i>Escherichia coli</i> CIP 54.117 Lot 323	10^{-6} : Vc1 : 224 Vc2 : 244 10^{-7} : Vc1 : 24 Vc2 : 20 N= $2,33 \cdot 10^8$ N ₀ = $2,33 \cdot 10^7$ 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= $5,65 \cdot 10^4$	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 $1,5 \cdot 10^8$ et $5 \cdot 10^8$ UFC/ml ($8,17 \leq \lg N \leq 8,70$)

*N*₀ est compris entre $1,5 \cdot 10^7$ et $5 \cdot 10^7$ UFC/ml ($7,17 \leq \lg N \leq 7,70$)

*N*_{v0} est compris entre 30 et 160 UFC/ml (*N*_v est compris entre 300 et 1600 UFC/ml)

*N*_{vB} est compris entre $3,0 \cdot 10^4$ et $1,6 \cdot 10^5$

A, B et *C* sont supérieurs ou égaux à $0,5 \times N_{v0}$

B (dilution-neutralisation) est égal ou supérieur à $0,0005 \times N_{vB}$

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}
<i>Escherichia coli</i> 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	> $3,3 \cdot 10^5$			$9,59 \cdot 10^3$			<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)
<i>Pseudomonas aeruginosa</i> DSM 939 Lot 556	10 ⁻⁶ : Vc1 : 281 Vc2 : 263	Vc1 : 89 Vc2 : 81 (dilution 10 ⁻¹)	Vc1 : 100 Vc2 : 107 (dilution 10 ⁻³)	Vc1 : 58 Vc2 : 73	Vc1 : 76 Vc2 : 88	Vc1 : 106 Vc2 : 118
	10 ⁻⁷ : Vc1 : 27 Vc2 : 32 N= 2,74.10 ⁸ N ₀ = 2,74.10 ⁷ Log N ₀ = 7,44	Nv= 850 Nv ₀ = 85	NvB= 1,04.10 ⁵	A= 66	B= 82	C= 112

L'essai est validé si :

N est compris entre 1,5.10⁸ et 5.10⁸ UFC/ml (8,17 ≤ lg N ≤ 8,70)

*N*₀ est compris entre 1,5.10⁷ et 5.10⁷ 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)

*N*_{vB} est compris entre 3,0.10⁴ et 1,6.10⁵

A, *B* et *C* sont supérieurs ou égaux à 0,5 × *N*_{v0}

B (dilution-neutralisation) est égal ou supérieur à 0,0005 × *N*_{vB}

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
<i>Pseudomonas aeruginosa</i> DSM 939 Lot 556	Vc1	>330	>330	>330	>330	>330	>330	0	0	0
	Vc2	>330	>330	>330	>330	>330	>330	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.

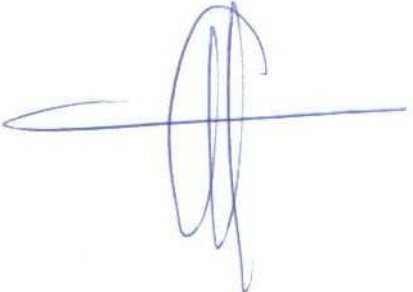

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VII-SIGNATURES

Fait à DINARD,

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

LMH
CMI – 18 rue Franklin Roosevelt
B.P. 80 139
35 401 Saint Malo cedex

Saint Malo le 12 mai 2016

Monsieur Dubourgeois

Vous nous avez interrogés sur les modifications apportées à la norme NF EN 13727 + A1 datant de décembre 2013 et sa nouvelle version NF EN 13727 + A2 sortie en décembre 2015.

En résumé, ces modifications sont dans l'ensemble essentiellement éditoriales et ne concerne sur le plan technique qu'une disposition mineure sur les produits destinés au lavage hygiénique des mains.

Il est précisé dans l'avant-propos de la norme que « les données obtenues avec la version précédente de l'EN 13727 peuvent être encore utilisées si un temps de neutralisation de 10 secondes s'est démontré suffisant pour tous les produits pour lesquels les temps de contact sont égaux ou inférieurs à 10 mn ».

C'est en effet le cas dans l'essai que nous avons réalisé sur votre produit dénommé 1592 et destiné quant à lui à l'application friction hygiénique et chirurgicale des mains. Il a été testé en 30 secondes et le temps de neutralisation de 10 secondes qui a été appliqué a été validé.

Donc en fonction de tous ces éléments, je vous confirme qu'il n'est pas nécessaire pour vous de tester de nouveau ce produit selon la nouvelle version et que les résultats obtenus sont parfaitement valables en regard de la version NF EN 13 727 + A2 de décembre 2015.

En espérant avoir répondu à votre interrogation. Je vous prie de croire monsieur Dubourgeois en l'expression de nos salutations les plus respectueuses.

Martine SESQUES
Docteur en microbiologie
Directeur opérationnel et technique



Christeyns France
31 rue de la Maladie
44120 VERTOU
France

CUSTOMER NUMBER
815

DATE
March 27, 2015

REPORT A 15086
PHAGORUB GEL SPS
HYGIENIC HANDRUB (EN 1500)

Purpose

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

Test Description

Manufacturer:	Christeyns France, Vertou, France			
Product:	Phagorub Gel SPS Hygienic hand antiseptic			
Sample number:	P 151168			
Batch number:	PR195-F14			
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:	<i>Escherichia coli</i> 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 %: 9.02	80 %: 8.42	10 %: 7.26	WSH: 7.20
pH – value (pH-stripes)	100 %: 5 -6	10 %: 6-7		
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 Gel 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 Gel 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: $4.80 \cdot 10^8$ 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 Gel 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 Gel SPS** at a contact time of **30 s** are:


prevalue	5.92
postvalue	1.62
reduction factor (RF)	4.30

The mean log reduction (RF) for the test product (**Phagorub Gel 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 = 0.49).

The test product formulation **Phagorub Gel 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. Kobörger
- General Manager -


AKKREDITIERTES PRÜFLABOR
HYGIENE NORD GMBH
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17489 GREIFSWALD
DIN EN ISO/IEC 17025
DAK-ES-18411-0

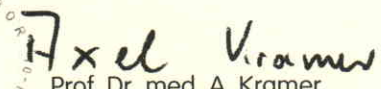

Prof. Dr. med. A. Kramer
MD for Hygiene and Environmental Medicine -

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

Date:	March 27, 2015	Order number:	A 15086
Product:	Phagorub Gel SPS	Sample number:	P 151168
Test organism:	<i>E. coli</i>	Batch number:	PR195-F14
Interfering substance:	none	Neutralizer:	XXIV
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h – 48 h
Test suspension (N):	4.80*10 ⁸ cfu / ml	Test temperature:	20 ± 1 °C
Test suspension (N _v):	1.25*10 ⁵ cfu / ml		
Validation Suspension (N _v):	1.58*10 ³ cfu / ml		

Neutralization – control and validation:

Validation suspension (N _{v0})				Neutralizer control (control B)				Method validation (Control C) 80 %			
cfu / Plate 1 & 2		V _c	\bar{x} [cfu / ml]	cfu / Plate 1 & 2		V _c	\bar{x} [cfu / ml]	cfu / Plate 1 & 2		V _c	\bar{x} [cfu / ml]
V _{c1}	86	75	161	V _{c1}	69	76	145	V _{c1}	76	54	130
V _{c2}	72	83	155	V _{c2}	70	68	138	V _{c2}	72	63	135
158				141.5				132.5			
30 ≤ \bar{x} of N _{v0} ≤ 160?				\bar{x} of B is ≥ 0.0005* \bar{x} of N _{v0} ?				\bar{x} of C is ≥ 0.5* \bar{x} of N _{v0} ?			
<input checked="" type="checkbox"/> Yes* <input type="checkbox"/> No				<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No				<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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}	95	80	175								
V _{c2}	83	92	175								
175											
\bar{x} of C is ≥ 0.5* \bar{x} of N _{v0} ?											
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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	abs (0.00 - 0.38) = 0.39
RP	PP		RP	PP		
4.52	4.52	0.00	4.47	4.09	0.38	
result < 2.00 ?						
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No						

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

test date: March 26, 2015
 reference: 60 % propan-2-ol
 test product: Phagorub Gel SPS
 neutralizer: XXIV

order number: A 15086
 batch number: 093192228
 batch number: PR195-F14
 sample number: P 151168

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	162	25	26	1	0
	left	187	28	78	4	0	151	19	> 330	33	4
2	right	28	6	16	1	0	41	4	28	1	0
	left	55	9	10	0	0	57	5	14	1	0
3	right	84	22	130	17	0	97	7	60	6	0
	left	23	5	38	6	1	60	8	4	0	0
4	right	16	1	216	24	4	8	0	180	7	0
	left	20	1	20	1	0	21	2	30	2	0
5	right	76	14	2	0	0	68	11	0	0	0
	left	58	8	6	1	0	65	6	2	0	0
6	right	174	43	0	0	0	> 330	42	0	0	0
	left	146	15	0	0	0	194	25	0	0	0
7	right	115	12	0	0	0	118	14	0	0	0
	left	105	23	0	0	0	89	10	0	0	0
8	right	97	5	74	6	0	139	20	26	3	0
	left	58	5	0	0	0	131	16	2	0	0
9	right	54	8	224	23	2	93	12	> 330	150	25
	left	72	6	26	1	0	85	11	> 330	171	33
10	right	12	1	> 330	62	4	50	4	> 330	168	33
	left	9	0	> 330	59	8	36	4	> 330	102	15
11	right	171	32	2	0	0	> 330	34	> 330	50	2
	left	127	21	22	1	0	> 330	32	> 330	36	0
12	right	65	10	8	0	0	82	16	100	11	0
	left	70	4	16	1	0	95	11	46	4	0
13	right	132	27	0	0	0	76	12	26	2	0
	left	120	22	12	0	0	63	10	12	0	0
14	right	64	10	26	6	0	92	12	10	1	0
	left	69	11	62	5	1	70	12	12	1	0
15	right	35	2	26	2	0	155	24	> 330	106	14
	left	51	4	8	1	0	155	24	30	4	0
16	right	50	8	10	2	0	49	12	26	5	0
	left	44	8	42	3	0	38	8	16	5	0
17	right	105	25	> 330	38	2	110	18	> 330	91	14
	left	133	8	> 330	129	21	144	16	> 330	> 330	38
18	right	> 330	40	92	11	0	170	31	> 330	36	0
	left	141	19	24	2	0	139	27	40	5	0
19	right	59	8	6	0	0	64	9	14	0	0
	left	45	5	2	0	0	57	7	2	0	0
20	right	20	1	140	18	1	32	6	76	11	1
	left	3	0	108	10	1	16	1	> 330	48	8

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

test product: Phagorub Gel SPS
reference: 60 % propan-2-ol

sample number: P 151168
order number: A 15086

subject	hand	reference product					test product				
		prevalue log		postvalue log		reduction-factors	prevalue log		postvalue log		reduction-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	6.23	6.21	1.41	1.97	4.24
	L	6.29		1.89			6.19		2.52		
2	R	5.45	5.59	1.20	1.10	4.49	5.61	5.68	1.45	1.30	4.39
	L	5.74		1.00			5.76		1.15		
3	R	5.98	5.67	2.13	1.85	3.82	5.99	5.88	1.78	1.19	4.69
	L	5.36		1.58			5.78		0.60		
4	R	5.20	5.25	2.34	1.82	3.43	4.90	5.11	2.26	1.87	3.25
	L	5.30		1.30			5.32		1.48		
5	R	5.91	5.84	0.30	0.54	5.30	5.83	5.82	0.00	0.15	5.67
	L	5.76		0.78			5.81		0.30		
6	R	6.30	6.23	0.00	0.00	6.23	6.62	6.46	0.00	0.00	6.46
	L	6.17		0.00			6.30		0.00		
7	R	6.06	6.06	0.00	0.00	6.06	6.08	6.01	0.00	0.00	6.01
	L	6.07		0.00			5.95		0.00		
8	R	5.99	5.88	1.87	0.93	4.94	6.16	6.14	1.41	0.86	5.28
	L	5.76		0.00			6.13		0.30		
9	R	5.73	5.79	2.35	1.88	3.91	5.97	5.95	3.20	3.23	2.71
	L	5.86		1.41			5.93		3.27		
10	R	5.08	5.02	2.79	2.78	2.24	5.70	5.63	3.26	3.14	2.48
	L	4.95		2.77			5.56		3.03		
11	R	6.27	6.20	0.30	0.82	5.38	6.53	6.52	2.70	2.63	3.89
	L	6.13		1.34			6.51		2.56		
12	R	5.81	5.83	0.90	1.05	4.78	5.95	5.96	2.00	1.83	4.13
	L	5.85		1.20			5.98		1.66		
13	R	6.16	6.14	0.00	0.54	5.60	5.88	5.84	1.41	1.25	4.59
	L	6.11		1.08			5.80		1.08		
14	R	5.81	5.82	1.41	1.60	4.22	5.96	5.90	1.00	1.04	4.86
	L	5.84		1.79			5.85		1.08		
15	R	5.54	5.63	1.41	1.16	4.47	6.21	6.21	3.04	2.26	3.95
	L	5.71		0.90			6.21		1.48		
16	R	5.70	5.67	1.00	1.31	4.36	5.69	5.63	1.41	1.31	4.33
	L	5.64		1.62			5.58		1.20		
17	R	6.07	6.10	2.58	2.86	3.24	6.07	6.11	2.98	3.28	2.83
	L	6.12		3.13			6.16		3.58		
18	R	6.60	6.38	1.96	1.67	4.71	6.26	6.22	2.56	2.08	4.14
	L	6.16		1.38			6.18		1.60		
19	R	5.77	5.71	0.78	0.54	5.17	5.81	5.78	1.15	0.72	5.06
	L	5.65		0.30			5.76		0.30		
20	R	5.30	4.89	2.16	2.10	2.79	5.51	5.35	1.88	2.28	3.07
	L	4.48		2.03			5.20		2.68		
arithm. mean		5.80		1.30		4.49	5.92		1.62		4.30
absolute SD		0.40		0.80		1.04	0.34		1.02		1.09
relative SD		6.88		61.09		23.14	5.80		62.78		25.30

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 Gel SPS

order number: A 15086
 sample number: P 151168

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: 0.11

Subject	RP-PP	Mean pairwise differences $(d_i+d_{ii})/2$ (no duplicates. only values \geq median)																			
		11	9	13	12	18	1	15	17	4	19	2									
11	1.49	1.49																			
9	1.20	1.35	1.20																		
13	1.01	1.25	1.11	1.01																	
12	0.65	1.07	0.93	0.83	0.65																
18	0.57	1.03	0.89	0.79	0.61	0.57															
1	0.52	1.01	0.86	0.77	0.59	0.55	0.52														
15	0.52	1.01	0.86	0.77	0.59	0.55	0.52	0.52													
17	0.41	0.95	0.81	0.71	0.53	0.49⁵³	0.47	0.47	0.41												
4	0.18	0.84	0.69	0.60	0.42	0.38	0.35	0.35	0.30	0.18											
19	0.11	0.80	0.66	0.56	0.38	0.34	0.32	0.32	0.26	0.15	0.11										
2	0.10	0.80	0.65	0.56	0.38	0.34	0.31	0.31	0.26	0.14	0.11										
7	0.05	0.77	0.63	0.53	0.35	0.31	0.29	0.29	0.23	0.12											
16	0.03	0.76	0.62	0.52	0.34	0.30	0.28	0.28	0.22	0.11											
6	-0.23	0.63	0.49	0.39	0.21	0.17	0.15	0.15													
10	-0.24	0.63	0.48	0.39	0.21	0.17	0.14	0.14													
20	-0.28	0.61	0.46	0.37	0.19	0.15	0.12	0.12													
8	-0.34	0.58	0.43	0.34	0.16	0.12															
5	-0.37	0.56	0.42	0.32	0.14																
14	-0.64	0.43	0.28	0.19																	
3	-0.87	0.31	0.17																		

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 **0.49**, 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
> 300	=	not countable
n.d.	=	not determined
WSH	=	hard water (water of standardized hardness)
DGHM	=	Deutsche Gesellschaft für Hygiene und Mikrobiologie
cfu	=	colony forming unit

Christeyns France
31 rue de la maladie
BP 2421
F -44124 Vertou cedex

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 Nv ₀ : 52.5 Nv: 525	Vc: 57 53 NvB: 55	Vc: 39 40 B: 39.5	Vc: 28 25 C: 26.5	Vc: 57 56 C: 56.5
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 Nv ₀ : 56.5 Nv: 565	Vc: 47 52 NvB: 49.5	Vc: 34 42 B: 38	Vc: 48 52 C: 49	Vc: 65 55 C: 60
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 Nv ₀ : 55.5 Nv: 555	Vc: 38 37 NvB: 37.5	Vc: 33 38 B: 35.5	Vc: 58 59 C: 58.5	Vc: 66 62 C: 64
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 Nv ₀ : 48 Nv: 480	Vc: 53 51 Nv _B : 52	Vc: 46 42 B: 44	Vc: 52 45 C: 48.5	Vc: 51 45 C: 48
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 Nv ₀ : 41 Nv: 410	Vc: 56 53 Nv _B : 54.5	Vc: 36 42 B: 39	Vc: 42 34 C: 38	Vc: 35 43 C: 39
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

Nv₀ 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 Nv₀

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 Validierungssuspension / number of cfu/ml in the validation suspension

Nv₀ = 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

Nv_B = Im Falle der Neutralisationskontrolle B (Verdünnungs-Neutralisation) die Anzahl der KBE/ml nach 100-facher Verdünnung. Nv₀ 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. Nv₀ is one-tenth of the mean of the Vc values of validation suspension 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 to 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 Methodvalidierung 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: 4/4/3/4/3/4/3/4/3/4/3/3/3/3/3/3/5/3/3/4/3/3/3

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					
		-1	-2	-3	Sofortwirkung / immediate			3-Stundenwirkung / 3-hours		
					0	-1	-2	0	-1	-2
1	links / left	>330	>330	<u>137</u>	>330	<u>162</u>	<u>17</u>			
	rechts / right	>330	>330	<u>296</u>				>330	>330	<u>53</u>
2	links / left	>330	>330	<u>228</u>	<u>51</u>	5	0			
	rechts / right	>330	>330	<u>85</u>				<u>49</u>	4	0
3	links / left	>330	>330	<u>33</u>	>330	<u>63</u>	6			
	rechts / right	>330	<u>197</u>	<u>20</u>				>330	>330	<u>84</u>
4	links / left	>330	>330	<u>272</u>				>330	>330	<u>66</u>
	rechts / right	>330	>330	<u>59</u>	>330	<u>73</u>	7			
5	links / left	>330	>330	<u>128</u>	>330	<u>83</u>	8			
	rechts / right	>330	>330	<u>70</u>				>330	<u>213</u>	<u>21</u>
6	links / left	>330	>330	<u>265</u>				>330	>330	<u>104</u>
	rechts / right	>330	>330	<u>229</u>	>330	<u>49</u>	4			
7	links / left	>330	>330	<u>219</u>	>330	>330	<u>120</u>			
	rechts / right	>330	>330	<u>149</u>				>330	>330	<u>89</u>
8	links / left	>330	>330	<u>259</u>				>330	>330	<u>70</u>
	rechts / right	>330	>330	<u>237</u>	>330	>330	<u>142</u>			
9	links / left	>330	<u>170</u>	<u>17</u>	>330	<u>81</u>	8			
	rechts / right	>330	>330	<u>84</u>				>330	>330	<u>58</u>
10	links / left	>330	>330	<u>77</u>				>330	>330	<u>49</u>
	rechts / right	>330	>330	<u>61</u>	>330	<u>116</u>	11			
11	links / left	>330	>330	<u>166</u>				>330	>330	<u>69</u>
	rechts / right	>330	>330	<u>159</u>	<u>144</u>	<u>15</u>	1			
12	links / left	>330	>330	<u>292</u>	>330	<u>112</u>	12			
	rechts / right	>330	>330	<u>289</u>				<u>92</u>	9	0
13	links / left	>330	>330	<u>59</u>				>330	<u>44</u>	4
	rechts / right	>330	>330	<u>95</u>	<u>185</u>	<u>18</u>	1			
14	links / left	>330	>330	<u>68</u>	<u>273</u>	<u>28</u>	2			
	rechts / right	>330	>330	<u>55</u>				<u>23</u>	2	0
15	links / left	>330	<u>161</u>	<u>16</u>				>330	>330	<u>68</u>
	rechts / right	>330	<u>230</u>	<u>23</u>	<u>75</u>	8	1			
16	links / left	320	<u>243</u>	<u>24</u>	<u>4</u>	0	0			
	rechts / right	>330	<u>223</u>	<u>23</u>				<u>88</u>	9	1
17	links / left	>330	>330	<u>53</u>				<u>89</u>	9	1
	rechts / right	>330	>330	<u>37</u>	<u>121</u>	12	1			
18	links / left	>330	>330	<u>84</u>	>330	>330	<u>106</u>			
	rechts / right	>330	>330	<u>119</u>				>330	>330	<u>266</u>
19	links / left	>330	>330	<u>280</u>	>330	>330	<u>58</u>			

	rechts / right	>330	>330	<u>269</u>				>330	<u>258</u>	<u>25</u>
20	links / left	>330	>330	<u>276</u>				>330	<u>81</u>	8
	rechts / right	>330	>330	<u>284</u>	>330	>330	<u>96</u>			
21	links / left	>330	<u>203</u>	<u>20</u>	>330	<u>166</u>	<u>17</u>			
	rechts / right	>330	<u>130</u>	13				>330	>330	<u>33</u>
22	links / left	>330	>330	<u>64</u>				>330	>330	<u>149</u>
	rechts / right	>330	>330	<u>48</u>	>330	>330	<u>208</u>			
23	links / left	<u>178</u>	<u>18</u>	1	<u>13</u>	1	0			
	rechts / right	>330	>330	<u>143</u>				>330	<u>91</u>	9
24	links / left	>330	>330	<u>97</u>				<u>271</u>	<u>27</u>	2
	rechts / right	>330	>330	<u>136</u>	<u>271</u>	<u>27</u>	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					
		-1	-2	-3	Sofortwirkung / immediate			3-Stundenwirkung / 3-hours		
					0	-1	-2	0	-1	-2
1	links / left	>330	>330	<u>87</u>				>330	>330	<u>48</u>
	rechts / right	>330	>330	<u>107</u>	>330	>330	<u>34</u>			
2	links / left	>330	>330	<u>67</u>	<u>23</u>	2	0			
	rechts / right	>330	<u>287</u>	<u>29</u>				<u>36</u>	3	0
3	links / left	>330	>330	<u>138</u>				>330	<u>108</u>	10
	rechts / right	>330	>330	<u>96</u>	>330	>330	<u>50</u>			
4	links / left	>330	>330	<u>283</u>	>330	<u>70</u>	7			
	rechts / right	>330	>330	<u>272</u>				>330	<u>34</u>	3
5	links / left	>330	<u>155</u>	<u>15</u>				<u>153</u>	<u>15</u>	1
	rechts / right	>330	<u>294</u>	<u>31</u>	>330	<u>34</u>	3			
6	links / left	>330	>330	<u>124</u>	>330	<u>34</u>	3			
	rechts / right	>330	>330	<u>77</u>				>330	<u>32</u>	3
7	links / left	>330	>330	<u>282</u>				n z	<u>75</u>	7
	rechts / right	>330	>330	<u>297</u>	>330	>330	<u>79</u>			
8	links / left	>330	>330	<u>251</u>	>330	>330	<u>81</u>			
	rechts / right	>330	>330	<u>257</u>				>330	>330	<u>60</u>
9	links / left	>330	<u>136</u>	<u>14</u>				>330	<u>71</u>	7
	rechts / right	>330	<u>128</u>	13	<u>64</u>	6	0			
10	links / left	>330	>330	<u>74</u>	>330	<u>35</u>	3			
	rechts / right	>330	>330	<u>63</u>				>330	>330	<u>32</u>
11	links / left	>330	>330	<u>168</u>	<u>70</u>	7	0			
	rechts / right	>330	>330	<u>80</u>				>330	<u>38</u>	3
12	links / left	>330	>330	<u>247</u>				<u>67</u>	6	0
	rechts / right	>330	>330	<u>272</u>	<u>37</u>	3	0			
13	links / left	>330	>330	<u>59</u>	<u>99</u>	10	1			
	rechts / right	>330	>330	<u>65</u>				<u>89</u>	8	0
14	links / left	>330	>330	<u>103</u>				<u>25</u>	2	0
	rechts / right	>330	>330	<u>138</u>	<u>224</u>	<u>22</u>	2			
15	links / left	>330	<u>100</u>	9	<u>47</u>	4	0			
	rechts / right	>330	<u>142</u>	<u>14</u>				>330	<u>81</u>	8
16	links / left	>330	>330	<u>151</u>				<u>68</u>	6	0
	rechts / right	>330	>330	<u>100</u>	<u>3</u>	0	0			
17	links / left	>330	<u>116</u>	11	<u>52</u>	5	0			
	rechts / right	>330	>330	<u>96</u>				<u>47</u>	4	0

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

Ig R Dezimale log Reduktion / decimal Ig 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 / <i>complete set of results available:</i> von 24 Personen / <i>from 24 volunteers</i>	Somit mehr als das Minimum von 23 / <i>hence. more than the minimum of 23</i>
b)	Die mittleren Ig der Vorwerte des RP (Sofortwirkung / 3-h Wirkung) / <i>Mean of Ig prevalues for RP (immediate / 3-h effect) = 4.85 / 4.84</i> und für PP (Sofortwirkung / 3-h Wirkung) / <i>and for PP (immediate / 3-h effect) = 4.85 / 4.84</i>	Somit alle min. 3.5 / <i>hence all min. 3.5</i>
c)	Die absolute Differenz der mittleren Differenzen zwischen RP und PP / <i>Absolute difference of mean differences between RP and PP</i> d1) zwischen den Gruppen RP => PP und PP => RP (Sofortwirkung) / <i>between the groups RP => PP and PP => RP (immediate effect): 0.83</i> d2) zwischen den Gruppen RP => PP und PP => RP (3-h Wirkung) / <i>between the groups RP => PP and PP => RP (3-h effect): 0.15</i>	Somit < 2 / <i>hence < 2</i> Somit < 2 / <i>hence < 2</i>
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 / <i>Results in tables page 6-9 which were used for weighted mean counts; all quotions of weighted mean counts between 5 and 15.</i>	

Alle Akzeptanz- Kriterien erfüllt / All acceptance criteria are fulfilled

Legende:

- RF = Reduktionsfaktor / *reduction factor*
- RP = Referenzprodukt / *reference product*
- PP = Prüfprodukt / *test product*
- Ig = \log_{10} / *Ig₁₀*

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	0.44	0.43	0.25	0.18	0.15	0.15	0.15	0.11	-0.06	-0.27	-0.31	-0.34
of RP-PP	Mittlere paarweise Differenzen / Mean pairwise differences (d _i + d _{ii}) / 2											
0.44	1											
0.43	2	2										
0.34	4	4	14									
0.18	6	7	15	19								
0.15	8	8	16	22	24							
0.15	8	8	16	22	24	24						
0.11	12	12	19	27	28	28	30					
-0.06	18	19	31	34	38	38	41	48				
-0.27	32	32	44	46	48	48	55	71	74			
-0.31	34	34	45	48	55	55	63	74	74			
-0.34	37	38	46	55	61	61	64	77	77			
-0.40	41	43	52	64	66	66	70					
-0.58	52	52	71	77	82	82						
-0.60	55	55	73	81								
-0.69	66	69										
-0.80	74	74										
-0.84	77	77										
-0.98												
-1.11												
-1.41												
-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 ($d_i + d_{ij}$) / 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														
0.49	2	5													
0.34	3	11	13												
0.00	4	17	23	39											
-0.06	5	18	26	42	45										
-0.07	6	18	27	42	45	50									
-0.09	7	21	29	44	50	53									
-0.12	8	22	31	45	55	58									
-0.16	9	24	32	45	55	58	63								
-0.16	10	24	32	45	55	58	63	67							
-0.23	11	27	34	60	67	70	72	75	77						
-0.47	14	38	45	82	-0.26	-0.27	-0.28	-0.29	-0.31	-0.16	-0.19	-0.23			
-0.49	15	39	50	-0.24	-0.27	-0.28	-0.29	-0.30	-0.32	-0.35	-0.36	-0.38	-0.47		
-0.54	16	41	58	-0.27	-0.30	-0.30	-0.31	-0.33	-0.35	-0.36	-0.38	-0.42	-0.48		
-0.62	18	45	67	-0.31	-0.34	-0.34	-0.35	-0.37	-0.39	-0.42	-0.44	-0.48	-0.50		
-0.73	24	63	77	-0.36	-0.39	-0.40	-0.41	-0.42	-0.44	-0.48	-0.48	-0.51			
-0.81	29	72	81	-0.40	-0.43	-0.44	-0.45	-0.46	-0.48	-0.48	-0.51				
-0.86	32	76		-0.43	-0.46	-0.46	-0.47	-0.49	-0.49	-0.51					
-0.94	34	79		-0.47	-0.50	-0.50	-0.51								
-0.94	34	79		-0.47	-0.50	-0.50	-0.51								
-0.98	37	79		-0.47	-0.50	-0.50	-0.51								
-1.29	63			-0.32	-0.47	-0.47	-0.49								
-1.35	70			-0.47	-0.50	-0.50	-0.51								
-1.55				-0.50	-0.50	-0.50	-0.51								
-1.73				-0.50	-0.50	-0.50	-0.51								

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	ohne VZ / without 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 $\neq 0$ / number of pairs with difference $\neq 0$	Signifikanzniveau / level of significance		
	0.025 einseitig / one sided	0.01 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	s ($P \leq 0.01$)
3-Stunden-Wirkung - Langzeitwirkung / 3-hours effect - sustained effect	2.26	1.76	
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).

Der vorliegende Prüfbericht bezieht sich ausschließlich auf die dem Labor vorliegenden Prüfgegenstände. Jede auszugsweise Vervielfältigung bedarf der schriftlichen Genehmigung durch das Prüflabor

The test results in this test report relate only to the items tested. This test report shall not be reproduced except in complete text without the written approval of the testing laboratory.

A handwritten signature in blue ink, appearing to read "Prof. Dr. med. H.-P. Werner", is written over the printed name and title.

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

RAPPORT D'ESSAI
N°3712-1

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

Imprimé le : 13/10/15
Date de 1^{ère} impression : 13/10/15
Page 1 sur 5

Test d'efficacité mycobactéricide
selon la norme NF EN 14348 (Juin 2005)

Produit 1592

Essai partiel sur *Mycobacterium avium* DSM 44157
(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 : 20/07/15
- Date de péremption : 20/07/18
- Date de réception au laboratoire : 28/07/15
- Aspect du produit : liquide visqueux limpide incolore
- Conditions de stockage : à température ambiante et à l'abri de la lumière
- Diluant du produit recommandé par le fabricant : non concerné
- Matière(s) active(s) : Non communiquées

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III- METHODE D'ESSAI

Norme NF EN 14348 (Juin 2005) : Essai quantitatif de suspension pour l'évaluation de l'activité mycobactéricide des désinfectants chimiques utilisés en médecine, y compris les désinfectants pour instrument.

Réduction logarithmique au moins égale à 4 log décimaux dans les conditions de l'essai.

IV- CONDITIONS EXPERIMENTALES

- Période d'analyse : du 17/09/15 au 08/10/15
- Analyse réalisée par : AF. GABILLET
- Diluant du produit utilisé au cours de l'essai : eau distillée
- Concentrations de produit testé (V/V) : 20-40 et 80%
- Technique d'essai : dilution/neutralisation
- Aspect des dilutions : opaques pour 20% et 40% et limpide pour 80%
- Stabilité du mélange substance interférente/dilutions du produit/suspension microbienne : absence de précipité au cours de l'essai
- Temps de contact : 30 secondes (+/-5 secondes)
- Température d'essai : 20°C (+/-1°C)
- Substance interférente : Albumine bovine 0,3 g/l (conditions de propreté)
- Température d'incubation : 37°C (+/-1°C)
- Identification de la souche utilisée :
 - *Mycobacterium avium* DSM 44157
- Neutralisant (m/V) : 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).

RAPPORT D'ESSAI

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V- RESULTATS D'ESSAI

V_c : nombre de colonies comptées sur les boîtes,

N : nombre d'UFC / ml dans la suspension microbienne d'essai,

N₀ : nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de N,

N_v : Nombre de cellules par ml de la suspension microbienne de validation. Il est 10 fois supérieur aux dénombrements en termes de valeurs de V_c en raison de l'étape de dilution à 10⁻¹,

N_{v0} : Nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de N_v,

N_a : Nombre de survivants par ml dans le mélange d'essai à l'issue du temps de contact et avant neutralisation ou filtration sur membrane,

A : nombre de survivants dans le témoin des conditions expérimentales,

B : nombre de survivants dans le témoin du neutralisant ou du témoin de filtration,

C : nombre de survivants dans le témoin de validation de la méthode,

R : réduction du nombre de cellules viables ($R=N_0/N_a$ ou $\text{Log}R=\text{Log}N_0-\text{Log}N_a$).

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Essai sur *Mycobacterium avium* DSM 44157

Souche testée	Suspension microbienne d'essai	Essai de validation			
		Suspension microbienne	Conditions expérimentales (A)	Témoin du neutralisant ou du témoin de filtration (B)	Inactivation par neutralisation ou par filtration sur membrane (C)
<i>Mycobacterium avium</i> DSM 44157 Lot 558	10^{-7} : Vc1 : 365 Vc2 : 340 10^{-8} : Vc1 : 64 Vc2 : 65 N= $3,8 \cdot 10^9$ N ₀ = $3,8 \cdot 10^8$ Log N ₀ = 8,58	Vc1 : 100 Vc2 : 119 Nv= $1,1 \cdot 10^3$ Nv ₀ = $1,1 \cdot 10^2$	Vc1 : 146 Vc2 : 137 A= $1,4 \cdot 10^2$	Vc1 : 138 Vc2 : 114 B= $1,3 \cdot 10^2$	80% Vc1 : 104 Vc2 : 130 C = $1,2 \cdot 10^2$
<p><u>L'essai est validé si :</u> <i>N</i> est compris entre $1,5 \cdot 10^9$ et $5 \cdot 10^9$ UFC/ml ($9,17 \leq \lg \leq 9,70$) <i>N</i>₀ est compris entre $1,5 \cdot 10^8$ et $5 \cdot 10^8$ UFC/ml ($8,17 \leq \lg \leq 8,70$) <i>N</i>_{v0} est compris entre 30 et 160 UFC/ml (<i>N</i>_v est compris entre 300 et 1600 UFC/ml) <i>A</i>, <i>B</i>, <i>C</i> est supérieur ou égal à $0,5 \times N_{v0}$ Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15</p>					

Souche testée	Concentrations testées % (V/V)												
	20%				40%				80%				
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	
<i>Mycobacterium avium</i> DSM 44157 Lot 558	Vc1	>660	>660	>660	>660	>660	>660	>660	336	365	85	16	3
	Vc2	>660	>660	>660	>660	>660	>660	>660	352	361	78	17	4
	Na	>6,6.10 ⁶				3,4.10 ⁶				4,0.10 ³			
	lg Na	>6,82				6,53				3,60			
	Lg R	<1,76				2,05				4,98			

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VI- CONCLUSION

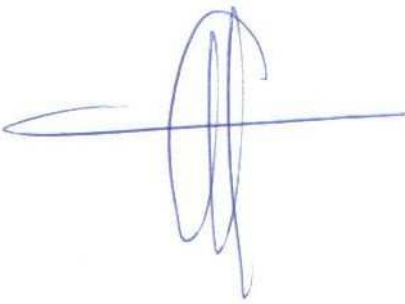

Selon la méthodologie de la norme NF EN 14348 (Juin 2005), le lot PR288-F12 du produit 1592 de la société CHRISTEYNS FRANCE, dans les conditions d'essai suivantes :

- en 30 secondes de temps de contact,
 - à la température d'essai de 20°C,
 - en présence d'albumine bovine à 0,3 g/l (conditions de propreté),
- présente une activité mycobactéricide (réduction supérieure ou égale à 4 Log décimaux) lorsqu'il est dilué à 80% (V/V) vis-à-vis de la souche *Mycobacterium avium* DSM 44157.

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

VII-SIGNATURES

Fait à DINARD,

Rédigé par	Validé par
<p>AF. GABILLET Responsable d'essai</p> 	<p>M.SESQUES Docteur en microbiologie Directeur technique p/o P. FOUTEL Responsable Qualité</p> 

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
Client
Project
Date of Delivery
Storage conditions
Active substances

PHAGORUB GEL SPS
LABORATOIRE PHAGOGENE, LOUZY, FRANCE
BT-PHA-04
16 February 2011
Ambient and darkness
Not known

Test Method and its validation

Method
Neutralizer

Filtration-neutralization technique
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
Product diluent used
Product test concentrations
Appearance product dilutions
Contact time
Test temperature
Interfering substance
Stability of mixture
Temperature of incubation
Identification of strain

5 April to 25 April 2011
Sterile hard water
20.0 % V/V; 40.0% V/V; 80.0% V/V
Colourless, clear product solution
 $t = 30s \pm 2 s$
 $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$
0.3 g/l bovine albumin
Precipitate absent throughout the test
 $37^{\circ}\text{C} \pm 1^{\circ}\text{C} + 5.0\% \text{CO}_2$
Mycobacterium terrae ATCC 15755

Conclusion

Following the EN 14348 (2005) protocol, **PHAGORUB GEL SPS**, possesses tuberculocidal activity (6.02 log₁₀ 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

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	$N = 1.77 \times 10^9$	$N_0 = 1.77 \times 10^8$	N_a (SEE TABLE 2)
Controls	$N_v = 5.20 \times 10^2$	$N_{v0} = 5.20 \times 10^1$	$A = 4.10 \times 10^1$ $B = 3.95 \times 10^1$ $C = 4.70 \times 10^1$

- 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	N_a	N_0/N_a	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

DISCLAIMER

BluTest (BT) has performed the Testing detailed in this report using reasonable skill and care and that BT has used reasonable endeavours to carry out the Testing [in accordance with an EN 14348 protocol]. All forecasts, recommendations and results contained in any report to the Company shall be submitted in good faith. However, other than as expressly set out in this report, no warranty is given (i) in relation to the Testing or the use(s) to which any results or deliverables produced in the course of the Testing are or may be put by the Company or their fitness or suitability for any particular purpose or under any special conditions notwithstanding that any such purpose or conditions may have been made known to BT or (ii) that the intended results or deliverables from the Testing can be achieved or (iii) that the Company can freely make use of the results or the deliverables without infringing any third party intellectual property rights and the Company will be deemed to have satisfied itself in this regard. BT shall have no liability (which is hereby excluded to the fullest extent permissible by law) in respect of any loss, liability or damage, including without limitation any indirect and/or consequential loss such as loss of profit or loss of business, market or goodwill, that the Company may suffer directly or indirectly as a result of or in connection with: (i) the performance of the Testing, except for direct loss arising from a breach of the foregoing warranties; (ii) the use of any materials, samples or other information provided by the Company for use in the Testing; and (iii) the Company's reliance upon or use of any results or deliverables provided as part of the Testing. The total liability of BT shall not exceed the sums paid to BT for the performance of the Testing.

LABORATOIRES : <input type="checkbox"/> PHAGOGENE <input checked="" type="checkbox"/> RIVADIS	Réf. : F-278/A	Page : 2/3
<u>Fiche</u> : 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 réalisée 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 toxicité de l'eau dure	A	(est supérieur ou égal à 0,05*Nv)
le témoin de toxicité 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 être inférieur à 150
le nombre de bactéries par boîte	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 leuoricide 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
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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

RAPPORT D'ESSAI

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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)

RAPPORT D'ESSAI

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

V- RESULTATS D'ESSAI

V_c : nombre de colonies comptées sur les boîtes,
N : nombre d'UFC / ml dans la suspension microbienne d'essai,
N₀ : nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de *N*,
N_v : nombre de cellules par ml de la suspension microbienne de validation,
N_{v0} : nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de *N_v*,
N_{vB} : 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 *N_v*,
N_a : 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 ($R=N_0/N_a$) 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)
<i>Candida albicans</i> DSM 1386 Lot 534	10 ⁻⁵ : Vc1 : 151 Vc2 : 155	Vc1 : 43 Vc2 : 38 (dilution 10 ⁻¹)	Vc1 : 48 Vc2 : 38 (dilution 10 ⁻³)	Vc1 : 29 Vc2 : 40	Vc1 : 36 Vc2 : 34	Vc1 : 34 Vc2 : 31
	10 ⁻⁶ : Vc1 : 17 Vc2 : 14 N= 1,53.10 ⁷ N ₀ = 1,53.10 ⁶ Log N ₀ =6,18	Nv= 405 Nv ₀ = 41	NvB= 4,30.10 ⁴	A= 35	B= 35	C= 33

L'essai est validé si :

N est compris entre 1,5.10⁷ et 5.10⁷ UFC/ml (7,17 ≤ lg *N* ≤ 7,70)

*N*₀ est compris entre 1,5.10⁶ et 5.10⁶ UFC/ml (6,17 ≤ lg *N* ≤ 6,70)

*N*_{v0} est compris entre 30 et 160 UFC/ml (*N*_v est compris entre 300 et 1600 UFC/ml)

*N*_{vB} est compris entre 3,0.10⁴ et 1,6.10⁵

A, *B* et *C* sont supérieurs ou égaux à 0,5 × *N*_{v0}

B (dilution-neutralisation) est égal ou supérieur à 0,0005 × *N*_{vB}

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
<i>Candida albicans</i> 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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N° 3588-1

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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.



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VII-SIGNATURES

Fait à DINARD, le 02/04/15

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

RAPPORT D'ESSAI
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TEST D'EFFICACITE FONGICIDE
SELON LA NORME NF EN 13624 (Novembre 2013)
1592

Applications = Friction hygiénique des mains
Friction chirurgicale des mains

(Méthode : dilution/neutralisation et dilution/neutralisation méthode modifiée)
Souche additionnelle : *Aspergillus brasiliensis* (ex niger) DSM 1988

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 : **20/07/15**
- Date de péremption : **20/07/18**
- Date de réception au laboratoire : **28/07/15**
- Aspect du produit : **Liquide limpide incolore**
- Conditions de stockage : **à température ambiante et à l'abri de la lumière**
- Diluant du produit recommandé par le fabricant : **non concerné**
- Matière(s) active(s) : **Non communiquées**

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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.

IV- CONDITIONS EXPERIMENTALES

- Période d'analyse : du 07/09/15 au 11/09/15
- Analyse réalisée par : M. TEULIER
- Diluant du produit utilisé au cours de l'essai : eau dure 30°f (et eau distillée pour méthode modifiée)
- Concentrations de produit testé (V/V) : 20% et 40%
: 97% (selon la méthode modifiée)
- Technique d'essai : dilution/neutralisation pour 20% et 40% ; dilution/neutralisation modifiée pour 97%
- Aspect des dilutions : opaques
- 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)
- Température d'incubation : 30°C (+/-1°C)
- Identification de la souche utilisée :
Aspergillus brasiliensis (ex niger) DSM 1988

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).

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V- RESULTATS D'ESSAI

V_c : nombre de colonies comptées sur les boîtes,

N: nombre d'UFC / ml dans la suspension microbienne d'essai,

N₀: nombre de cellules par ml dans le mélange d'essai au début du temps de contact, il représente un dixième de N (1/100 de N pour la méthode modifiée),

N_v : nombre de cellules par ml de la suspension microbienne de validation,

N_{v0} : nombre de cellules par ml dans les mélanges A, B et C au début du temps de contact. Il représente un dixième de N_v (1/100 de N_v pour la méthode modifiée),

N_{vB} : 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 N_v,

N_a : 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 ($R=N_0/N_a$) exprimé en logarithme

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Essai sur *Aspergillus brasiliensis* (ex niger) DSM 1988

Souche testée	Suspension microbienne d'essai	Essai de validation				
		Suspension microbienne (N _v)	Suspension microbienne (N _v ₀)	Conditions expérimentales (A)	Témoin du neutralisant ou de filtration (B)	Validation de la méthode (C)
<i>Aspergillus brasiliensis</i> DSM 1988 Lot 533	10 ⁻⁵ : Vc1 : 154 Vc2 : 151	Vc1 : 66 Vc2 : 58 (dilution 10 ⁻¹)	Vc1 : 57 Vc2 : 46 (dilution 10 ⁻³)	Vc1 : 54 Vc2 : 59	Vc1 : 65 Vc2 : 66	Pour 40% : Vc1 : 75 Vc2 : 57
	10 ⁻⁶ : Vc1 : 25 Vc2 : 27					
	N = 1,6.10 ⁷ N ₀ = 1,6.10 ⁶ Log N ₀ = 6,20	N _v = 620 N _{v0} = 62	N _{vB} = 5,20.10 ⁴	A = 57	B = 66	C = 66

L'essai est validé si :

N est compris entre 1,5.10⁷ et 5.10⁷ UFC/ml ($7,17 \leq \lg N \leq 7,70$)

*N*₀ est compris entre 1,5.10⁶ et 5.10⁶ UFC/ml ($6,17 \leq \lg N \leq 6,70$)

*N*_{v0} est compris entre 30 et 160 UFC/ml (*N*_v est compris entre 300 et 1600 UFC/ml)

*N*_{vB} est compris entre 3,0.10⁴ et 1,6.10⁵

A, *B* et *C* sont supérieurs ou égaux à 0,5 × *N*_{v0}

B (dilution-neutralisation) est égal ou supérieur à 0,0005 × *N*_{vB}

Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée	Concentrations testées % (V/V)						
		20%			40%		
		10 ⁰	10 ⁻¹	10 ⁻²	10 ⁰	10 ⁻¹	10 ⁻²
<i>Aspergillus brasiliensis</i> DSM 1988 Lot 533	Vc1	>330	>330	>330	>330	184	19
	Vc2	>330	>330	>330	>330	250	20
	Na	>3,3.10 ⁵			2,2.10 ⁴		
	lg Na	>5,52			4,34		
	Lg R	<0,68			1,86		

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Méthode modifiée sur *Aspergillus brasiliensis* (ex niger) DSM 1988

Souche testée	Suspension microbienne d'essai	Essai de validation				
		Suspension microbienne (N _v)	Suspension microbienne (N _{vB})	Conditions expérimentales (A)	Témoin du neutralisant ou de filtration (B)	Validation de la méthode (C)
<i>Aspergillus brasiliensis</i> DSM 1988 Lot 533	10 ⁻⁶ : Vc1 : 157 Vc2 : 138	Vc1 : 41 Vc2 : 48 (dilution 10 ⁻²)	Vc1 : 44 Vc2 : 49 (dilution 10 ⁻³)	Vc1 : 44 Vc2 : 39	Vc1 : 67 Vc2 : 56	Pour 97% : Vc1 : 47 Vc2 : 57
	10 ⁻⁷ : Vc1 : 19 Vc2 : 22	N _v = 4,5.10 ³ N _{v0} = 45	N _{vB} = 4,7.10 ⁴	A = 42	B = 62	
	N = 1,5.10 ⁸ N ₀ = 1,5.10 ⁶ log N ₀ = 6,18					

L'essai est validé si :
N est compris entre 1,5.10⁸ et 5.10⁸ UFC/ml (8,17 ≤ lg *N* ≤ 8,70)

*N*₀ est compris entre 1,5.10⁶ et 5.10⁶ UFC/ml (6,17 ≤ lg *N*₀ ≤ 6,70)

*N*_{v0} est compris entre 30 et 160 UFC/ml (*N*_v est compris entre 3000 et 16000 UFC/ml)

*N*_{vB} est compris entre 3,0.10⁴ et 1,6.10⁵
A, *B* et *C* sont supérieurs ou égaux à 0,5 × *N*_{v0}
B (dilution-neutralisation) est égal ou supérieur à 0,0005 × *N*_{vB}

Le quotient des dénombrements obtenus par moyenne pondérée est compris entre 5 et 15

Souche testée	Concentrations testées % (V/V)			
	97%			
	10 ⁰	10 ⁻¹	10 ⁻²	
<i>Aspergillus brasiliensis</i> DSM 1988 Lot 533	Vc1	12	1	0
	Vc2	13	0	0
	Na	< 140		
	lg Na	< 2,15		
	Lg R	> 4,03		

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VI- CONCLUSION

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),
- ne présente pas d'activité fongicide (réduction inférieure à 4 log décimaux), lorsqu'il est dilué à 20% et 40% (V/V), vis-à-vis de la souche *Aspergillus brasiliensis (ex niger)* DSM 1988.

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 fongicide (réduction supérieure ou égale à 4 log décimaux) selon la méthode modifiée de la norme, lorsqu'il est dilué à 97% (V/V), vis-à-vis de la souche *Aspergillus brasiliensis (ex niger)* DSM 1988.

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


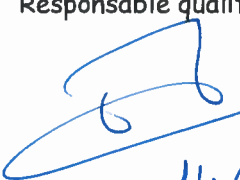
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VII-SIGNATURES

Fait à DINARD,

Rédigé par	Validé par
M. TEULIER Responsable d'essai  14/09/15	M.SESQUES Docteur en microbiologie Directeur technique p/o : P.FOUTEL Responsable qualité  14/09/15

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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 maladie 31
FR – 44120 Vertou



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).

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 421. 27819102, Telefax +49. 421. 2760283, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2015

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 cm² 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

- CO₂ 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)
- Polyesterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

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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.

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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* ($10\text{-}15 \times 10^3$ cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO₂-atmosphere. The cytopathic effect was read by using an inverted microscope after seven days. Calculation of the infective dose TCID₅₀/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

X_0 = log₁₀ 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 log₁₀ steps within the recommended exposure period. This corresponds to an inactivation of $\geq 99.99\%$.

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.

Titration of the virus control were performed after the longest exposure time (EN 5.5.7).

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

Inactivation tests were carried out in sealed test tubes in a water bath at $20\text{ °C} \pm 1.0\text{ °C}$. 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} .

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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 $\geq 4 \log_{10}$ reduction (maximal virus reduction $\geq 4.25 \pm 0.23$).
- 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 %) $\log_{10}\text{TCID}_{50}/\text{ml}$.
- 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 $6.75 \pm 0.33 \log_{10}\text{TCID}_{50}/\text{ml}$).
- 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.

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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 $\geq 4.25 \pm 0.23$ and $\geq 4.25 \pm 0.23$ at this time point (mean value $\geq 4.25 \pm 0.16$). 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

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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 (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.

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.

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The test results in this test report relate only to the items examined.

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

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Appendix:

Legend to the Tables

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

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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. 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.	n.d.
			1.5	n.d.	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.	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)

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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	0000 0000	n.d. n.d.	n.d. n.d.		
			1.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.	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.	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.	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	0000 0000		
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0040 0400	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 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	4444 4440	0040 0040	0000 0000	n.d. n.d.	n.d. n.d.	
			1.0	tttt tttt	4444 4444	4444 4444	0003 0040	0000 0000	0000 0000	0000 0000	0000 0000	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.	n.d.	n.d.
			60	n.d.	n.d.	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)

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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	4444	4444	4444	4444	4444	4444	0000	0000	n.d.	
				tttt	4444	4444	4444	4444	4444	4040	0000			
			60	1.0	n.d.	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.	n.d.
test product cytotoxicity	50.0%	clean conditions	n.a.	tttt	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	4404	4000	0000	4000	
				4444	4444	4444	4444	4444	4444	4000	4000	0000	0000	
virus control	n.a.	clean conditions	60	4444	4444	4444	4444	4444	4444	4444	4000	0000	0000	
				4444	4444	4444	4444	4444	4444	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)

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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	0444	0000	0000	n.d.	
				4444	4444	4444	4444	4444	4444	4004	0000		
			60	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.
test product cytotoxicity	25.0%	clean conditions	n.a.	0000	0000	0000	0000	0000	n.d.	n.d.	n.d.		
virus control	n.a.	clean conditions	0	4444	4444	4444	4444	4444	4444	4000	0000	4000	
				4444	4444	4444	4444	4444	4404	4000	4000	0000	
virus control	n.a.	clean conditions	60	4444	4444	4444	4444	4444	4444	4000	0000	0000	
				4444	4444	4444	4444	4444	4444	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.	n.d.
			1	n.d.	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.	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.	n.d. n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0000 0440	4400 0000	n.d. n.d.	n.d. n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	4004 4044	0004 0000	0000 0000	n.d. n.d.	n.d. n.d.
			60	tttt tttt	tttt tttt	4444 4444	4444 4444	0000 0000	0000 0000	0000 0000	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.	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)

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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 aftermin					> 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

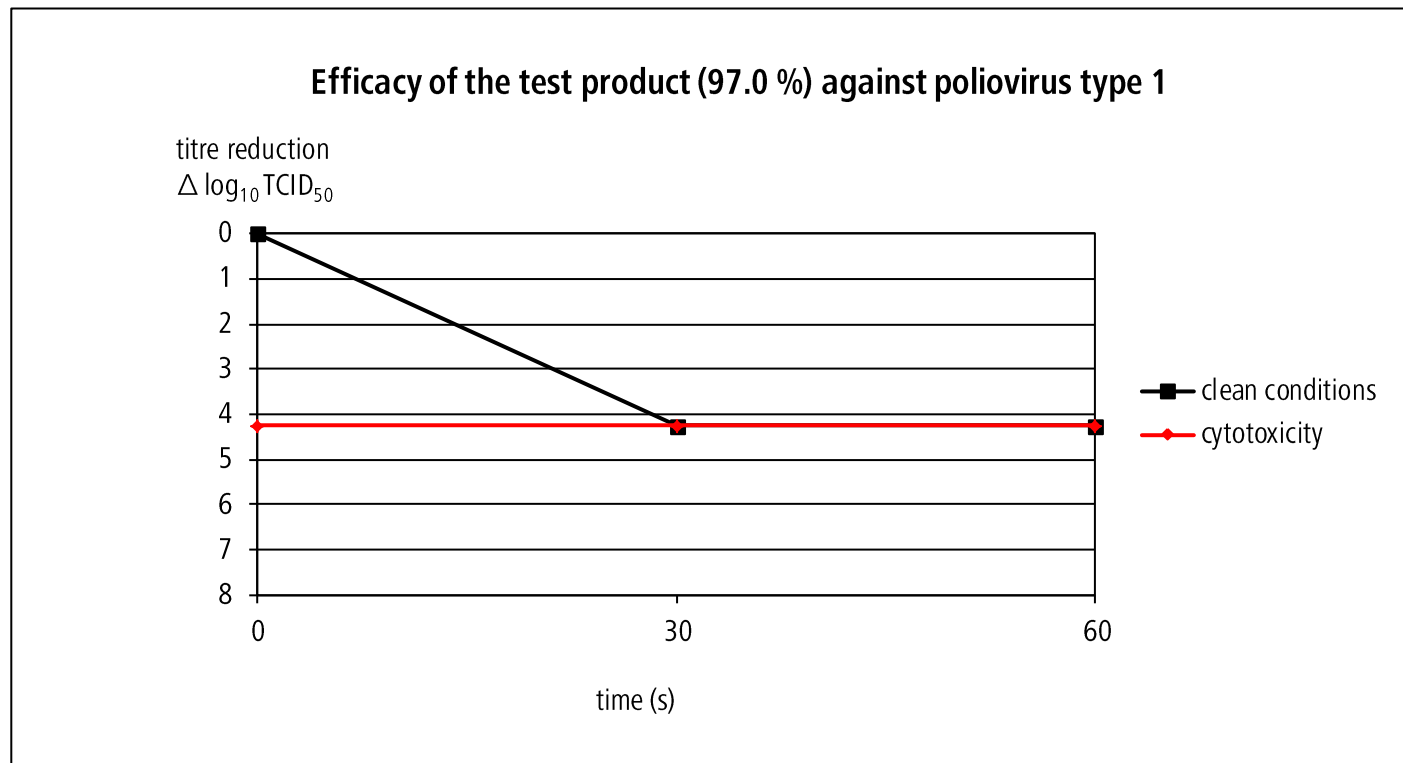
n.a. = not applicable n.d. = not done

Table 10b: Summary of results with 1592 and poliovirus type 1

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 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.

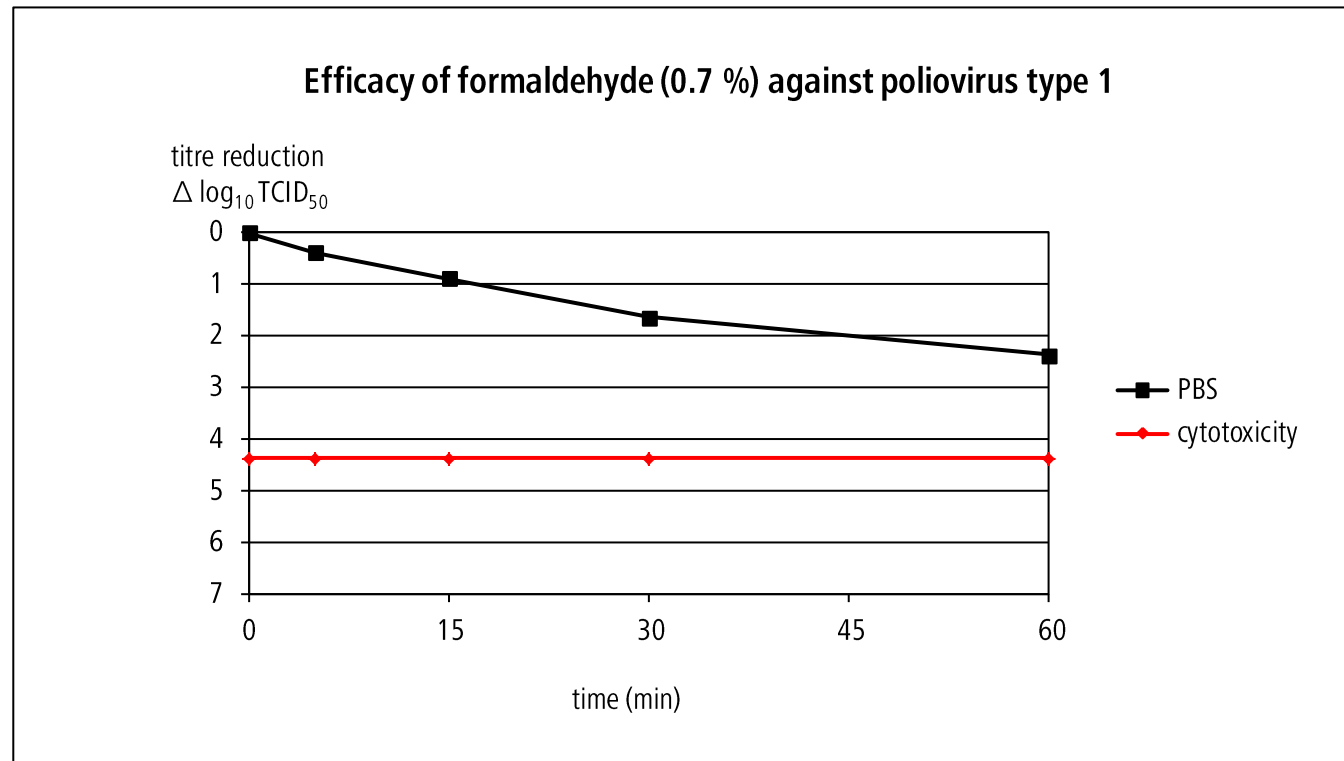
n.a. = not applicable n.d. = not done sens. = sensitivity

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



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



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10.07.2015

Test report C15L0111A

Evaluation of the effectiveness of
1592

Test virus: adenovirus type 5

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 maladie 31
FR – 44120 Vertou



1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 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) (kg)	72 % (w/w) ethanol
Appearance, odour	clear, colorless liquid, alcoholic
pH-values	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

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).

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3.2 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* (passage 121) originated from Vircell, S.L., Spain, 18320 Santa Fe (now BIOTRIN International GmbH, D-69126 Heidelberg).

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

- CO₂ 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).

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4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (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 (clean conditions EN 14476:2013)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water
Stability of product in the mix with virus and interfering substance (80.0 % solution)	no flocculation, minor precipitation
Virus strain	adenovirus type 5 strain adenoid 75 (ATCC VR-5)
Date of testing	09.03.2015– 10.07.2015
End of testing	10.07.2015

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 5.4.1 *A549 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.

Furthermore, the product was evaluated as 50.0 %, 25.0 % and 10.0 % solutions (demonstration of non-active range). These solutions were prepared with water immediately before the inactivation tests.

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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, 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 10³ cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO₂-atmosphere. The cytopathic effect was read by using an inverted microscope after ten days. Calculation of the infective dose TCID₅₀/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

X_0 = log₁₀ 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 log₁₀ 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 (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.

Titration of the virus control were performed after the longest exposure time (EN 5.5.7).

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

Inactivation tests were carried out in sealed test tubes in a water bath at $20\text{ °C} \pm 1.0\text{ °C}$. 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 was performed according to EN 5.5.4.2b.

Here, 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} .

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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 $\geq 4 \log_{10}$ reduction (maximal virus reduction $\geq 5.13 \pm 0.41$).
- b) The test product (undiluted) showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a $4 \log_{10}$ reduction of virus titre.
- c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see EN 5.7b) was $\geq 4.13 \pm 0.29$ (between 3.0 – 5.0) after 30 min and $\geq 4.13 \pm 0.29$ (between 3.5 – 5.5) after 60 min for adenovirus type 5.
- d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *A549 cells* showed no significant difference ($< 1 \log_{10}$; EN 5.7) of virus titre: 7.63 ± 0.25 (PBS) versus $7.63 \pm 0.49 \log_{10} \text{TCID}_{50}/\text{ml}$.
- e) The control of efficacy for suppression of disinfectant's activity showed no decrease ($< 0.5 \log_{10}$; EN 5.5.5.1) in virus titre (7.63 ± 0.49 versus $7.63 \pm 0.41 \log_{10} \text{TCID}_{50}/\text{ml}$).
- 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 adenovirus type 5 according to EN 14476:2013 is valid.

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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 (80.0 %) was able to inactivate adenovirus type 5 after 0.5 minutes in this quantitative suspension test. The reduction factor was $\geq 5.13 \pm 0.41$ (Table 3). This corresponded to an inactivation of ≥ 99.999 %.

Tested as 50.0 % solution the test product was not active within 30 seconds of exposure time (Table 4).

Examined 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 adenovirus type 5 after an exposure time of 30 seconds under clean conditions.

Therefore, the disinfectant 1592 can be declared as active against adenovirus type 5 as follows:

undiluted 30 seconds

Bremen, 10.07.2015

- Dr. Jochen Steinmann -
Scientific Director

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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 (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.

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.

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The test results in this test report relate only to the items examined.

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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 Pharmac; 162, 1931, 480-487

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Appendix:

Legend to the Tables

Table 1:	Raw data for 1592 (80.0 %) tested against adenovirus type 5
Table 2:	Raw data for 1592 (50.0 %) tested against adenovirus type 5
Table 3:	Raw data for 1592 (25.0 %) tested against adenovirus type 5
Table 4:	Raw data for 1592 (10.0 %) tested against adenovirus type 5
Table 5:	Raw data for formaldehyde solution (0.7 %) tested against adenovirus type 5
Table 6:	Raw data for control of efficacy for suppression of disinfectant activity
Table 7:	Raw data (adenovirus type 5) for cell sensitivity
Table 8 (a+b):	Summary of results with 1592 and adenovirus type 5

Legend to the Figures

Figure 1:	Virus-inactivating properties of 1592 (80.0 %)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

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Table 1: Raw data for 1592 (80.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3965)

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	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
			1	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
			tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			5	n.d.	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	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	3444	0030	0000	0000	
				4444	4444	4444	4444	4444	4444	0333	0000	0000	0000	
			60	4444	4444	4444	4444	4444	4444	3323	0004	0000	0000	
				4444	4444	4444	4444	4444	3440	2000	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)

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Table 2: Raw data for 1592 (50.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3965)

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	4444 4444	4444 4444	4330 3423	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	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.	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	3444 0333	0030 0000	0000 0000	0000 0000	
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3323 3440	0004 2000	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 (25.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3965)

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	4303	0300	0000	n.d.	
				4444	4444	4444	4444	4444	4232	3000	0000		
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
	n.a.	0000	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.			
test product cytotoxicity	25.0%	clean conditions	n.a.	0000	0000	0000	0000	0000	n.d.	n.d.	n.d.		
virus control	n.a.	clean conditions	0	4444	4444	4444	4444	4444	3444	0030	0000	0000	
				4444	4444	4444	4444	4444	0333	0000	0000	0000	
60	4444	4444	4444	4444	4444	4444	4444	4444	3323	0004	0000	0000	
				4444	4444	4444	4444	4444	3440	2000	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)

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Table 4: Raw data for 1592 (10.0 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3965)

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.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	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	1322 3333	0300 0000	0000 0000	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	3444 0333	0030 0000	0000 0000	0000 0000	
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3323 3440	0004 2000	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 formaldehyde solution (0.7 %) tested against adenovirus type 5 at 20 °C (quantal test; 8 wells) (3965)

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 3443	4440 0343	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	2230 2232	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 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	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	3344 0223	0000 0302	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 control of efficacy for suppression of disinfectant's activity (3965)

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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4300 3423	0000 2230	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)

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Table 7: Raw data (adenovirus type 5) for cell sensitivity (3965)

Product	Dilution	Dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4423 3334	0000 1000	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	4334 2300	0300 3001	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)

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Table 8a: Summary of results with 1592 and adenovirus type 5

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction after ... min
				0.5	1	2	30	60	
test product	80.0%	clean conditions	2.50	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	n.d.	0.5 (RF ≥ 5.13±0.41)
test product	50.0%	clean conditions	1.50	4.38±0.25	n.d.	n.d.	n.d.	n.d.	>0.5 (RF 3.25±0,48)
test product	25.0%	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.	7.63±0.25	n.d.	> 30

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

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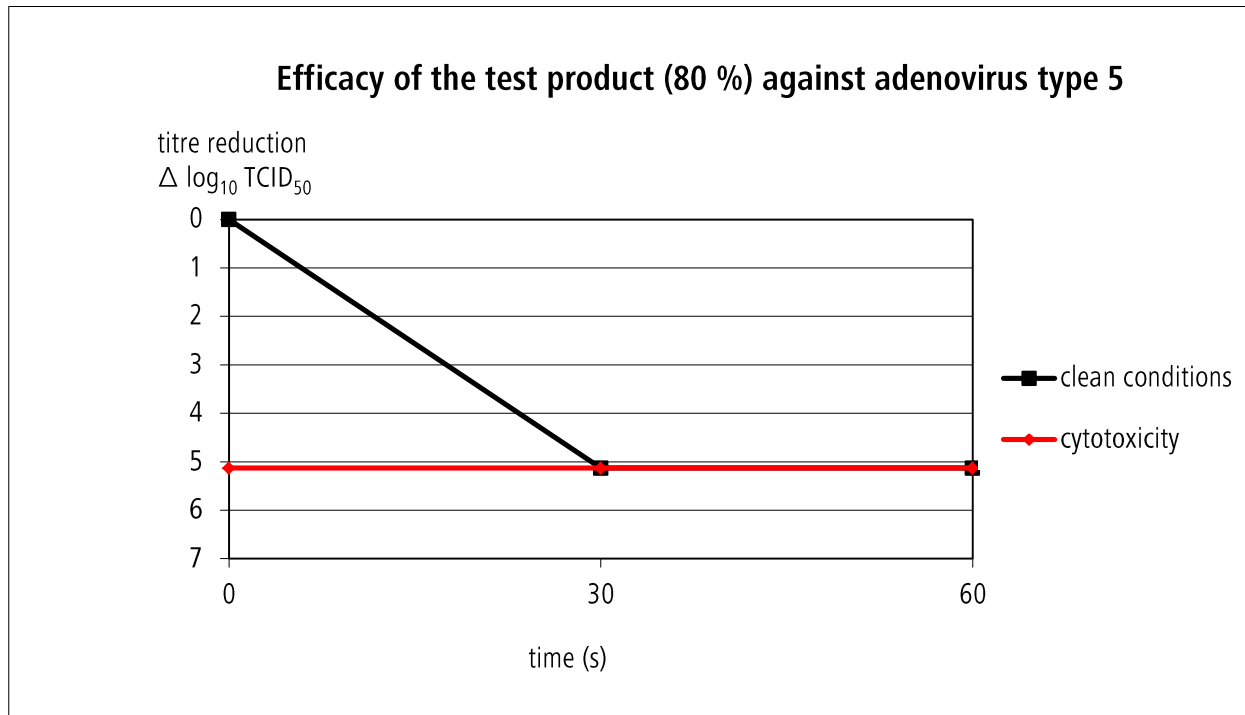
Table 8b: Summary of results with 1592 and adenovirus type 5

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7% (w/v)	PBS	3.50	n.d.	6.25±0.33	4.38±0.25	≤3.50±0.00	≤3.50±0.00	30 (RF ≥4.13±0.29)
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.41	n.a.
virus contr.	80.0%	clean conditions	n.a.	7.50±0.35	n.d.	n.d.	n.d.	7.63±0.41	n.a.
suppression control	80.0%	clean conditions	2.50	n.d.	n.d.	n.d.	n.d.	7.63±0.41	n.a.
sens.control PBS	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
sens. control test product	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.49	n.a.

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

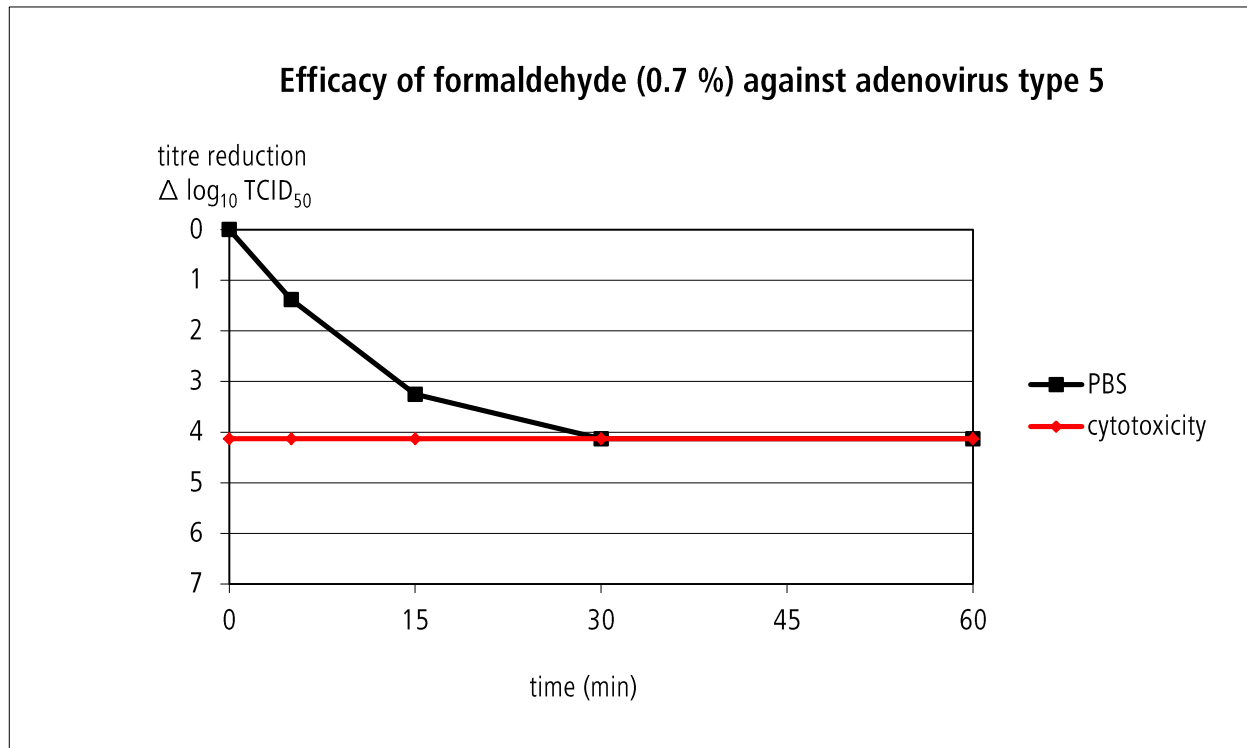
* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 421. 27819102, Telefax +49. 421. 2760283, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2015

Figure 1: Virus-inactivating properties of 1592 (80.0 %)



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



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ATTESTATION

Vertou le 14/03/2017

Conformité du Phagorub gel à la norme EN 14476 + A1 version 2015

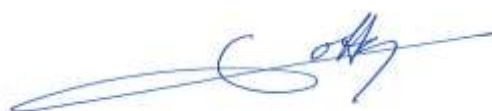
Le Produit Phagorub gel a été testé et est conforme à la norme de virucidie EN 14476 version 2013 et sur les virus nus de la norme (Polio, Adeno, Norovirus). Ceci confirme donc son activité virucide totale (virus nus et virus enveloppés).

Une version de cette norme est parue en 2015 → EN 14476 + A1.

Dans cette nouvelle version, la différence avec la version 2013 est juste l'ajout de possibilité de testé sur le virus de la vaccine, pour montrer une efficacité partielle de virucidie sur les virus enveloppés. Cette norme peut être désormais utilisée pour démontrer une efficacité partielle pour certains produits.

Comme le Phagorub gel a une efficacité sur les virus nus (beaucoup plus résistant aux désinfectants que les virus enveloppés), cette nouvelle version de la norme n'apporte rien pour ce produit. La norme EN 14476 version 2013 reste valide pour montrer l'efficacité virucide du Phagorub gel.

Pour diffusion aux utilisateurs.



Olivier COTTRON
Pharmacien Responsable

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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



passed 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

- CO₂ 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 10³ cells per well. Incubation was at 37°C in a CO₂-atmosphere (5.0 % CO₂ - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose (TCID₅₀) (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 $0.88 \pm 0.41 \log_{10}$ 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 $4.13 \pm 0.25 \log_{10}$ 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 $\geq 5.38 \pm 0.37$ and $\geq 5.13 \pm 0.25$ (assays without soil load) and $\geq 5.25 \pm 0.33$ and $\geq 5.50 \pm 0.44$ (assays with FCS), respectively. The following mean values were calculated: $\geq 5.26 \pm 0.25$ (assay without soil load) and $\geq 5.38 \pm 0.25$ (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. 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.

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The test results in this test report relate only to the items examined.



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 Pharmac; 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	n.a.	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	n.a.	n.a.	n.a.	15 s
controls		soil load	log₁₀TCID₅₀/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				
formaldehyde	0.7%	PBS	5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	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.	7.88±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.	7.75±0.33	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			log ₁₀ TCID ₅₀ /mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log ₁₀ reduction after
	conc.	soil load	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
test product	10.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	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.	> 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
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	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.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	8.00±0.44	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.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	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.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	tfff	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.		
			0.5	tfff	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.	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.	n.d.	n.d.	n.d.	n.d.	
			0.25	tfff	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			0.5	tfff	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
product cytotoxicity	80.0%	Aqua bidest.	n.a.	tfff	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.		
			n.a.	tfff	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
virus control	n.a.	Aqua bidest.	n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4444	4443	3004	0000		
	n.a.	10.0% FCS	n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4444	4213	0040	0000		

n.a. = not applicable t = cytotoxic 0 = no virus detectable
n.d. = not done 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	t t t t	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
			0.5	t t t t	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.	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.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	80.0%	10.0% FCS	0.25	t t t t	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
			0.5	t t t t	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.	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.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	10.0% FCS	n.a.	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
			n.a.	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
			n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4444	4344	0002	0000	0000
			n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4444	4343	0000	0000	0000

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

t = cytotoxic

0 = no virus detectable

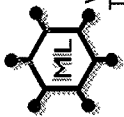
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)



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.	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.	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.	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.	n.d.	n.d.	n.d.	n.d.	
		0.25	4444	4444	4444	4444	4444	4444	4444	4444	4444	3424	0000	0000	0000	n.d.
		0.5	4444	4444	4444	4444	4444	4444	4444	4444	4444	4442	0024	0000	0000	n.d.
product cytotoxicity	10.0%	Aqua bidest.	1.0	n.d.	n.d.	n.d.	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.	n.d.	n.d.	n.d.	
			n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	10.0% FCS	n.a.	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
			n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4344	0002	0000	0000	0000
			n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4343	4300	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	4444	4444	4444	4444	4444	0310	0000	0000	n.d.
			15	tttt	tttt	4444	4444	4444	4444	1103	0000	0000	0000	n.d.
			30	tttt	tttt	4444	4444	2222	1012	0000	0000	0000	0000	n.d.
			60	tttt	tttt	2223	1012	0010	0000	0000	0000	0000	0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt	tttt	0000	0000	0000	0000	0000	0000	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	2443	0030	0000	n.d.
			4444	4444	4444	4444	4444	4433	3204	4000	
product	Aqua bidest.	1:10	4444	4444	4444	4444	4444	4433	0000	0000	n.d.
			4444	4444	4444	4444	4444	4442	0000	0000	
product	FCS	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product		1:1,000	4444	4444	4444	4444	4444	4343	0000	0000	n.d.
			4444	4444	4444	4444	4444	1412	0003	0000	
product		1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

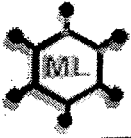
n.a. = not applicable

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)



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)

TEST REPORT

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





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

- CO₂ 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 $20^{\circ}C \pm 0.5^{\circ}C$. 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 $10-15 \times 10^3$ cells per well. Incubation was at $37^{\circ}C$ 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 μ l of the test virus suspension and 100 μ l Aqua bidest. and FCS, respectively were mixed with 800 μ l disinfectant at 20°C. At the end of the exposure time (15 s) 62.5 μ l 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 μ l / well). After 10 days of inoculation cultures were observed for cytopathic effects. The calculation of virus titre followed the formula of Lycke:

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

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 μ l Aqua bidest. were mixed with 800 μ l disinfectant (PBS as control). Then, an aliquot was diluted 1:1000 and 108 μ l 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 $\geq 1.25 \pm 0.48 \log_{10}$ steps. After 30 minutes a reduction factor of $\geq 1.25 \pm 0.48$ 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 $\geq 2.25 \pm 0.48$ (assay without soil load) and $\geq 2.38 \pm 0.37$ (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 log₁₀ 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 log₁₀ TCID₅₀ / 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) log₁₀ TCID₅₀.

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 log₁₀ = 0. The reduction factor is therefore log₁₀ 5.11 minus log₁₀ 0 = 5.11.

In the presence of FCS the result is log₁₀ = 0. The reduction factor is therefore log₁₀ 5.08 minus log₁₀ 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 log₁₀ 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. 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.

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9. Literature

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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			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	≤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.

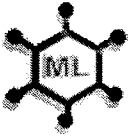
n.d. = not done n.a. = not applicable



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.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.	> 30 s	
controls			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.	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



20.11.2009

Test report no. P09ML928-1SI

Evaluation of the effectiveness of
Gel Mains Desinfectant

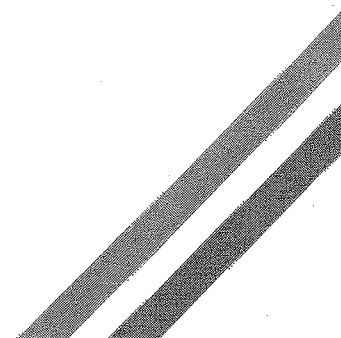
Test virus: Influenza A virus H1N1 (swine)

Method: following EN 14476:2007-02

TEST REPORT

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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

- CO₂ 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 cm² 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



performed monolayer of *MDCK cells* (placed in each well on the previous day; 100 µl aliquots with approx. 1.5×10^4 cells). Incubation took place at 37°C in a CO₂ incubator (5 % CO₂ content) for five days. Finally, cultures were observed for cytopathic effects with a reversed microscope and the infective dose TCID₅₀/ml was calculated with the method of Kärber (2) and Spearman (3) with the following formula:

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

meaning

X_0 = log₁₀ 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 log₁₀-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 $20^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

6.6 Inactivation assay following the method of Lycke¹

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 $\log_{10} \text{TCID}_{50} / \text{ml} = 6.83$. This value corresponded to the virus amount in the assays of $4.63 \log_{10} \text{TCID}_{50}$.

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.

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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 Pharmac; 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	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
Test product cytotoxicity	80.0%	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
				n.d.	n.d.	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	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
Formaldehyde cytotoxicity	0.7% (m/V)	PBS	15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Virus control	n.a.	PBS	30	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
Virus control	n.a.	PBS	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Virus control	n.a.	PBS	0	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
Virus control	n.a.	PBS	60	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	0000	
				4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	0000
				4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	4444	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 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	4444	4444	4444	4444	4444	0014	0000	0000	n.d.
				tttt	4444	4444	4444	4444	0001	0000	0000	n.d.	
				tttt	4444	4444	4444	4444	0000	0000	0000	n.d.	
Test product cytotoxicity	10.0%	PBS	1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Formaldehyde	0.7% (m/V)	PBS	2.0	tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
				tttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.
Formaldehyde cytotoxicity	0.7% (m/V)	PBS	5	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
Virus control	n.a.	PBS	15	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
Virus control	n.a.	PBS	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	PBS	60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	PBS	n.a.	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	n.d.
				tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	tttt	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.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	PBS	60	4444	4444	4444	4444	4444	4444	4004	0000	0000	n.d.
				4444	4444	4444	4444	4444	0000	0004	0000	0000	n.d.
				4444	4444	4444	4444	4444	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	4444	4344	0000	0000	0000	0000	0000	n.d.
		tttt	tttt	4444	1431	0040	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.	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.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	4144	4140	0000	0000	n.d.
			4444	4444	4444	4334	1411	0201	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	2000	0000	0000	n.d.
			4444	4444	4444	4444	4044	0000	0000	0000	n.d.
Test product	clean conditions	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.
			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Test product	dirty conditions	1:2,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			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
1 st assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4431 4444	0044 2204	0000 0000	0000 0000	n.d.
2 nd assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4444 4422	0030 0140	0000 0000	0000 0000	n.d.
3 rd assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	3444 4444	0004 0000	0000 0000	0000 0000	n.d.
4 th assay	PBS	4444 4444	4444 4444	4444 4444	4444 4444	4442 4444	0000 0000	0000 0000	0000 0000	n.d.
5 th 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
	plate 2/6	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
	plate 4/6	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
	plate 6/6	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.	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.	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.	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.	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.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.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.	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.	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.	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.	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

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EVIC france

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

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

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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	<u>Conduite d'étude</u> : J1 : traitement	22 juin 2009
29 juillet 2009	<u>Rapport final</u>	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 and 72h 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 ($t = 19 \pm 2^\circ\text{C}$) et humidité relative contrôlée ($\text{HR} = 50 \pm 20 \%$) 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 cm² environ) lui-même posé sur un carré d'adhésif Micropore® de 5 cm x 5 cm (soit 25 cm²).

Le pansement semi-occlusif a été appliqué sur la peau tondu 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 notation 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
<p>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 ($pH \leq 2$) ou fortement alcaline ($pH \geq 11,5$).</p> <ul style="list-style-type: none"> Ces destructions tissulaires apparaissent après un temps d'exposition ne dépassant pas 3 minutes ; ou un tel résultat est prévisible. Ces destructions tissulaires apparaissent après un temps d'exposition ne dépassant pas 4 heures ; ou un tel résultat est prévisible. 	<p>R 35 Provoque de graves brûlures</p> <p>R 34 Provoque des brûlures</p>
<p>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, <i>*jugée importante à partir des indices observés lors de l'essai :</i></p> <ul style="list-style-type: none"> 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 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 <p><i>*ou en cas de persistance à la fin de la période d'observation chez 2 animaux au moins</i></p>	<p>R 38 Irritant pour la peau</p>

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

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

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II	PERTINENCE DE L'ETUDE	3
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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-FOURAUULT (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 qualitative 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

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 %

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 %

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.

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) *[Signature]* 09/07/2010

Quality Assurance Personnel: Lucia CHIRITA *[Signature]* 09.07.2010

Head manager of the investigator centre: Alina NANU *[Signature]* 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

**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)

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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 mm²).

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 tight 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

TYPOLICAL 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

TYPOLICAL 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

TYPOLGICAL 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

TYPOLGICAL 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

TYPOLICAL 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
<p>No application of other products than the tested ones to the experimental area</p> <p>Deviation: none</p>	106	100 %
<p>No wearing of too tight or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patch</p> <p>Deviation: none</p>	106	100 %
<p>No bath (bathtub, swimming pool or sea), no hammam or sauna sessions during the study</p> <p>Deviation: none</p>	106	100 %
<p>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</p> <p>Deviation: none</p>	106	100 %
<p>No excessive sweating and no intensive sport liable to cause unsticking of the patch</p> <p>Deviation: none</p>	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
<p>No intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal</p> <p>Deviation: none</p>	106	100 %
<p>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</p> <p>Deviation: none</p>	106	100 %
<p>No vaccination during the study</p> <p>Deviation: none</p>	106	100 %
<p>At least 14 passages at the Institute (15 if a pre-inclusion visit was necessary)</p> <p>Deviation: none</p>	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: Oedema
 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

Aerobiocontamination study

Issue to: Health Care Division Phagogène

Product tested: Phagorub gel SPS (1592)

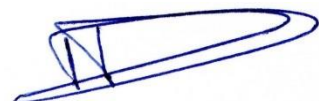
Tested packaging: 100ml capsule service, mounted pump 500ml, 1000ml airless

Test: Study of aerobiocontamination of a bottle of alcoholic gel

Tests: 2015/01/22 at 2015/03/08

Report issued the: 2015/09/07

Jérôme DUBOURGEOIS
Head of testing





CHRISTEYNS

1. Principle:

This study allows to assess the microbiological contamination of different alcoholic gel conditioning resistance when using them.

Microbiological cleanliness of the bottles is analyzed by an independent laboratory on samples in each of the bottles over a period of 6 months.

2. 2 Operating conditions:

Tested product samples:

- Phagorub gel SPS 100 ml batch 714789
- Phagorub gel SPS 500 ml batch 689227
- Phagorub gel SPS 1000 ml batch 731111

Frequency of sampling:

Phagorub gel SPS 100 ml:	1 per week
Phagorub gel SPS 500 ml:	1 ml per working day
Phagorub gel SPS 1000 ml:	2 samples per working day

3. Manipulations:

a. Sample

Depending on the above frequency, a dose (3ml) of product is taken from the bottle via its dispensing system. Samples are stored at room temperature in a room exposed to daylight.

b. Microbiological analysis

At the end of the 6 months of sampling, the bottles are sent to a microbiological analysis laboratory for the following tests:

- Enumeration of the mesophilic aerobic flora according to ISO 21149
- Enumeration of yeasts according to ISO 16212
- Enumeration of molds according to ISO 16212

4. Results

See analysis reports in appendices.

5. Conclusion

Phagorub Gel SPS can be used after opening without risk of aerobic contamination by its distribution system for a period of 6 months.

This study validates the 3 distribution systems: service capsule, long spout pump as well as the airless system.



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44120 VERTOU
Jérôme DUBOURGEOIS



COSMEBAC est certifié BPF
NF EN ISO 22716:2008
Certificat N°14-07-H014

Rapport d'analyses

1592 1L AIRLESS - N° DE LOT : 714789

N° dossier : 28831
N° étude : 340743
Commande client : 2015.07-113

Date de commande : 23/07/15
Date de réception : 27/07/15
Date de mise en analyse : 27/07/15
Edition du rapport : 03/08/15

Analyses Microbiologiques

Paramètres	Technique	Spécifications	Résultat	Unité	Conclusion
Dénombrement de la flore aérobie mésophile	NF EN ISO 21149	/	< 10	UFC/g	/
Dénombrement des levures	NF EN ISO 16212	/	< 10	UFC/g	/
Dénombrement des moisissures	NF EN ISO 16212	/	< 10	UFC/g	/

Remarques :

Rapport validé le : 03/08/15

Par : Antoine NOURY
Adjoint responsable laboratoire COSMEBAC

Les résultats ci-dessus ne concernent que l'échantillon soumis à COSMEBAC et seulement celui-ci.
Ce rapport d'analyses ne peut être reproduit, même partiellement, sans l'accord du laboratoire COSMEBAC.



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NF EN ISO 22716:2008
Certificat N°14-07-H014

Rapport d'analyses

1592 500ml PM - N° DE LOT : 689227

N° dossier : 28831
N° étude : 340744
Commande client : 2015.07-113

Date de commande : 23/07/15
Date de réception : 27/07/15
Date de mise en analyse : 27/07/15
Edition du rapport : 03/08/15

Analyses Microbiologiques

Paramètres	Technique	Spécifications	Résultat	Unité	Conclusion
Dénombrement de la flore aérobie mésophile	NF EN ISO 21149	/	< 10	UFC/g	/
Dénombrement des levures	NF EN ISO 16212	/	< 10	UFC/g	/
Dénombrement des moisissures	NF EN ISO 16212	/	< 10	UFC/g	/

Remarques :

Rapport validé le : 03/08/15

Par : Antoine NOURY
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COSMEBAC est certifié BPF
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Certificat N°14-07-H014

Rapport d'analyses

1592 100ml - N° DE LOT : 731111

N° dossier : 28831
N° étude : 340745
Commande client : 2015.07-113

Date de commande : 23/07/15
Date de réception : 27/07/15
Date de mise en analyse : 27/07/15
Edition du rapport : 03/08/15

Analyses Microbiologiques

Paramètres	Technique	Spécifications	Résultat	Unité	Conclusion
Dénombrement de la flore aérobie mésophile	NF EN ISO 21149	/	< 10	UFC/g	/
Dénombrement des levures	NF EN ISO 16212	/	< 10	UFC/g	/
Dénombrement des moisissures	NF EN ISO 16212	/	< 10	UFC/g	/

Remarques :

Rapport validé le : 03/08/15

Par : Antoine NOURY
Adjoint responsable laboratoire COSMEBAC

Les résultats ci-dessus ne concernent que l'échantillon soumis à COSMEBAC et seulement celui-ci.
Ce rapport d'analyses ne peut être reproduit, même partiellement, sans l'accord du laboratoire COSMEBAC.



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

Christeys France
31 rue de la Maladrie
44120 VERTOU
France

CUSTOMER NUMBER
815

DATE
March 04, 2016

REPORT A 15263-2

PHAGO'RUB GEL SPS & PHAGO'RUB SOLUTION SPS

EQUIVALENCE TESTING (VAH)

Purpose

The equivalence of Phago'rub Gel SPS – batch 1592800806 – and of Phago'rub Solution SPS – batch 1590796750 (Christeys France, Vertou, France) should be evaluated by *in vitro* tests in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes" (2015) against the reference strain *Candida albicans*, as being determined as the most resistant test organism in our test report A 15263-1.

Test description

Product name:	Phago'rub Gel SPS	Phago'rub Solution SPS
Batch number:	1592800806	1590796750
Sample number:	P 155926	P 155927
pH-value (pH-meter):	100 %: 7.53 50 %: 6.40 25 %: 5.85 WSH: 7.19	100 %: 8.41 50 %: 7.99 10 %: 7.51 WSH: 7.13
pH-value (pH-strips):	100 %: 5-6	100 %: 5
Appearance	clear, colourless gel	clear colourless liquid
Appearance of dilutions:	clear to turbid solutions, flocculation with interfering substance	
Odour:	alcoholic	
Manufacturer:	Christeyns France, Vertou, France	
Storage conditions:	Room temperature	
Date of order:	October 13, 2015	
Date of delivery:	October 16, 2015	
Test date:	January 25, 2016 – February 11, 2016	
Basis:	Requirements and methods for VAH-certification of chemical disinfection processes (VAH, 2015)	
Test organisms:	<i>Candida albicans</i>	ATCC 10231
Test solution:	97 %, 80 %, 50 %, 10 %	
Active ingredients / 100 ml:	50 – 80 % Ethanol < 1 % propane-1,2,3-triol	
Neutralizer:	4 % Tween 80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS (Neutralizer XXIV)	
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes (dirty conditions)	
Test temperature:	20 ± 1 °C	
Incubation temperature:	30 ± 1 °C	

Test Method

Quantitative suspension test under dirty conditions

A suspension of test organisms including an interfering substance (0.3 % albumin + 0.3 % sheep erythrocytes in this case – dirty conditions) is added to a sample of Phago'rub Gel SPS or Phago'rub Solution SPS (diluted with hard water, if necessary). The mixture is maintained at $20 \pm 1^\circ\text{C}$ for the required contact time. At the end of the contact time, an aliquot is taken; the microbicidal activity in this portion is immediately neutralized. The number of surviving test organisms in each sample is determined by plating aliquots of the neutralized test suspensions and its dilutions. The reduction is calculated in relation to a sample containing water instead of the test product (water control, WSH control). The experimental conditions, the non-toxicity of the neutralizer and the dilution-neutralization method are validated according to the VAH methods:

- Co 1 = Water control (WSH)
- Co 2 = Method validation (Dilution-neutralization method)
- Co 3 = Non-toxicity of the neutralizer

The test was performed using *C. albicans* as test-organism. Detailed results are presented in tables 2 –3.

Results

In order to evaluate their equivalence, the yeasticidal activity of Phago'rub Gel SPS – batch 1592800806 – and of Phago'rub Solution SPS – batch 1590796750 – was assayed in quantitative suspension tests in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes" (VAH, 2015). Detailed results are given in tables 2-3 and summarized in table 1:

Tab. 1: Reduction (log RF) obtained in the VAH (2015) quantitative suspension tests against *C. albicans* for the selected product concentrations at contact times of 15 s, 30 s and 60 s under dirty conditions.

Test condition	Equivalence testing - Reduction (log RF) obtained against <i>C. albicans</i> within 15 s, 30 s and 60 s in the quantitative suspension test with					
	Phago'rub Gel SPS batch 1592800806			Phago'rub Solution SPS batch 1590796750		
	15 s	30 s	60 s	15 s	30 s	60 s
97 %	≥ 5.53	≥ 5.51	≥ 5.46	≥ 5.37	≥ 5.45	≥ 5.49
80 %	≥ 5.53	≥ 5.51	≥ 5.46	≥ 5.37	≥ 5.45	≥ 5.49
50 %	1.21	3.19	≥ 5.46	3.30	5.15	≥ 5.49
10 %	0.08	0.10	0.01	0.08	0.05	0.00

According to the "VAH Requirements and methods for VAH-certification of chemical disinfection processes", the products Phago'rub Gel SPS and Phago'rub Solution SPS, when applied at a product concentration of at least 80 %, do both possess yeasticidal activity in 15 s at 20 °C under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes) for reference strain *C. albicans* (tables 2 - 3).

Accordingly, and thereby following the "Geschäftsordnung der Desinfektionsmittelkommission des VAH – Stand 15.12.2005", both formulations can be considered equivalent in their microbicidal activity.

Results are considered validated according to VAH requirements (2015).

Greifswald, March 04, 2016



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



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

Table 2: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago'rub Gel SPS Batch-Number: 1592800806
 Test organism: *C. albicans* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 48 h Neutralizer: XXIV
 Test suspension: 4.50 *10⁷ cfu/0.1 ml (7.65 log) Incubation temperature: 30 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	0	0.00	≥ 5.53	0	0.00	≥ 5.51	0	0.00	≥ 5.46
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ¹	2			0			0		
	0.1 ml 10 ²	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 5.53	0	0.00	≥ 5.51	0	0.00	≥ 5.46
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
50 %	1 ml 10 ⁰	> 330			214	2.33	3.19	0	0.00	≥ 5.46
	0.1 ml 10 ⁰	> 330			20	2.30		0		
	0.1 ml 10 ¹	256	4.41	1.21	1			0		
	0.1 ml 10 ²	17	4.23		0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	282	5.45	0.08	255	5.41	0.10	283	5.45	0.01
WSH (co 1)	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	311	5.53		295	5.51		270	5.46	
	0.1 ml 10 ²	37			35			31		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	323	3.23E+04	4.62
	0.1 ml 10 ¹	51	5.10E+04	
control 2 (co 2) - 80 %	0.1 ml 10 ⁰	332		
	0.1 ml 10 ¹	33	3.30E+04	4.52
control 2 (co 2) - 50 %	0.1 ml 10 ⁰	> 330		
	0.1 ml 10 ¹	29	2.90E+03	4.46
control 3 (co 3)	0.1 ml 10 ⁰	325	3.25E+04	4.62
	0.1 ml 10 ¹	51	5.10E+04	
Validation suspension:			4.50E+04	4.65
97 % - 60 s			controls o.k.?	Yes

Table 3: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: February 11, 2016 Order number: A 15263
 Substance: Phago'rub Solution SPS Batch-Number: 1590796750
 Test organism: *C. albicans* Sample number: P 155927
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 48 h Neutralizer: XXIV
 Test suspension : 3.30 *10⁷ cfu/0.1 ml (7.52 log) Incubation temperature: 30 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	0	0.00	≥ 5.37	0	0.00	≥ 5.45	0	0.00	≥ 5.49
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 5.37	0	0.00	≥ 5.45	0	0.00	≥ 5.49
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
50 %	1 ml 10 ⁰	100	2.00	3.30	2	0.30	≥ 5.15	0	0.00	≥ 5.49
	0.1 ml 10 ⁰	14	2.15		0			0		
	0.1 ml 10 ⁻¹	1			0			0		
	0.1 ml 10 ⁻²	0			0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	194	5.29	0.08	252	5.40	0.05	310	5.49	0.00
WSH (co 1)	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	218	5.37		289	5.45		224	5.49	
	0.1 ml 10 ⁻³	25			28			39		

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 [co 2] - 97 %	0.1 ml 10 ⁰	> 330		
	0.1 ml 10 ⁻¹	36	3.60E+04	4.56
control 3 [co 3]	0.1 ml 10 ⁰	> 330		
	0.1 ml 10 ⁻¹	40	4.00E+04	4.60
Validation suspension:			3.30E+04	4.52
		97 % - 60 s	controls o.k.?	Yes

Legend:

MW	=	average value
x	=	average value
RF	=	reduction factor
> 330	=	not countable
n.d.	=	not determined
Co 1	=	Control 01
Co 2	=	Control 02
Co 3	=	Control 03

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Christeyns France
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44120 VERTOU
France

DATE / DATUM
March 08, 2016 / 08.03.2016

GUTACHTERLICHE STELLUNGNAHME / EXPERT'S REPORT
- PHAGO'RUB GEL SPS -

Im März 2015 und im Januar und Februar 2016 wurden durch die Hygiene Nord GmbH mit dem Prüfmuster Phago'rub Gel SPS, einem Mittel zur Händedesinfektion der Firma Christeyns France, Vertou, Frankreich, folgende Untersuchungen auf Basis der EN 1500 (2013) und der „ANFORDERUNGEN UND METHODEN zur VAH-Zertifizierung chemischer Desinfektionsverfahren“ (2015) durchgeführt:

The efficacy of Phago'rub Gel SPS as a hygienic handrub (manufactured by Christeyns France, Vertou, France) was evaluated by the testing laboratory Hygiene Nord GmbH, Greifswald, Germany. The following tests were performed in the month of March, 2015 and during the months of January and February 2016 in accordance with the EN 1500 (2013) and the "REQUIREMENTS AND TEST METHODS for the VAH-certification of chemical disinfectants" (2015):

1. Bestimmung der bakteriostatischen und levurostatischen Wirksamkeit sowie geeigneter Neutralisationsmittel. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 15263-1 der Hygiene Nord GmbH vom 04.03.2016 enthalten.

Determination of a suitable neutralizer and analysis of the bacteriostatic and fungistatic activity. Results are presented in the Hygiene Nord GmbH test report A 15263-1 (March 04, 2016).

2. Im quantitativen Suspensionsversuch wurde die bakterizide und levurozide Wirksamkeit des Prüfmusters Phago´rub Gel SPS unter hoher organischer Belastung bestimmt (0,3 % Albumin + 0,3 % Schaferythrozyten) untersucht. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 15263-1 der Hygiene Nord GmbH vom 04.03.2016 enthalten.

The bactericidal and yeasticidal activity of Phago´rub Gel SPS was evaluated by quantitative suspension tests under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes). Results are presented in the Hygiene Nord GmbH test report A 15263-1 (March 04, 2016).

3. Durch praxisnahe Versuche zur hygienischen Händedesinfektion wurde die Wirksamkeit des Prüfmusters Phago´rub Gel SPS unter Anwendungsbedingungen untersucht. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 15086 der Hygiene Nord GmbH vom 08.03.2016 enthalten.

The activity of Phago´rub Gel SPS as a hygienic handrub was evaluated under conditions simulating practical conditions. Results are presented in the Hygiene Nord GmbH test report A 15086 (March 08, 2016).

ZUSAMMENFASSUNG / SUMMARY

Nach Bewertung der Ergebnisse kann festgestellt werden, dass das Prüfprodukt Phago´rub Gel SPS den „ANFORDERUNGEN UND METHODEN zur VAH-Zertifizierung chemischer Desinfektionsverfahren“ (2015) genügt, da folgende Wirkungen beobachtet wurden:

Upon evaluation of the test results it can be concluded that Phago´rub Gel SPS complies with the "REQUIREMENTS AND TEST METHODS for the VAH-certification of chemical disinfectants" (2015):

- Bakterizide und levurozide Wirksamkeit in den *in vitro* - Tests:

Im quantitativen Suspensionstest unter hoher Belastung wurden die Prüfspezies *P. aeruginosa*, *E. coli*, *P. mirabilis*, *E. hirae*, *S. aureus* und *C. albicans* bei der Konzentration-Zeit-Relation 80 % / 15 s in einem ausreichenden Maße inaktiviert ($\log \text{RF} \geq 5$ bzw. $\log \text{RF} \geq 4$).

Bactericidal and yeasticidal activity in the in vitro tests:

*In the quantitative suspension test under dirty conditions, the product possesses bactericidal ($\log \text{RF} \geq 5$) and yeasticidal ($\log \text{RF} \geq 4$) efficacy against the test organisms *P. aeruginosa*, *E. coli*, *P. mirabilis*, *E. hirae*, *S. aureus* and *C. albicans* within the contact time of 15 s at a product concentration of 80 %.*

- Bestätigung der Wirksamkeit unter praxisnahen Bedingungen: Die Anforderungen an ein Produkt zur hygienischen Händedesinfektion wurden bei Feuchthalten der Hände mit einem Produktvolumen von 3 ml innerhalb einer Einwirkzeit von 30 s erfüllt.

- Confirmation of that efficacy under conditions simulating practical conditions (hygienic handrub). The product fulfilled the requirements if the hands were kept moist with 3 ml of the product for a contact time of 30 s.

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c/o Hygiene Nord GmbH
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Für die Aufnahme in die Desinfektionsmittelliste des VAH können daher folgende Anwendungsempfehlung für Phago´rub Gel SPS gegeben werden:

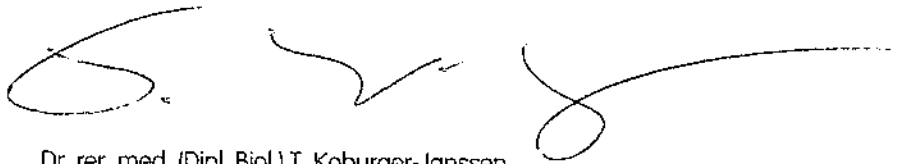
It can therefore be recommended to include Phago´rub Gel SPS in the VAH List of Disinfectants as follows:

Phago´rub Gel SPS:

Hygienische Händedesinfektion / Hygienic handrub:

100 % (3 ml) / 30 s

Greifswald, 08.03.2016 / March 08, 2016



Dr. rer. med. (Dipl. Biol.) T. Koburger-Janssen



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 04, 2016

REPORT A 15263-1
PHAGO'RUB GEL SPS
BACTERICIDAL AND YEASTICIDAL EFFICACY
(VAH 2015)

Purpose

The bactericidal and yeastocidal efficacy of the hygienic handrub Phago'rub Gel SPS (Christeyns France, Vertou, France) should to be evaluated by in vitro-tests in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes" (2015).

Test description

Manufacturer:	Christeyns France, Vertou, France		
Product name:	Phago'rub Gel SPS Hygienic hand antiseptic		
Batch number	1592800806		
Sample number:	P 155926		
Date of manufacture:	September 14, 2015		
Best before:	September 14, 2018		
Storage conditions:	Room temperature		
Date of order:	October 13, 2015		
Date of delivery:	October 16, 2015		
Test date:	January 18, 2016 – February 11, 2016		
Basis:	Requirements and methods for VAH-certification of chemical disinfection processes (VAH, 2015)		
Test organisms:	<i>Staphylococcus aureus</i>	ATCC 6538	
	<i>Enterococcus hirae</i>	ATCC 10541	
	<i>Pseudomonas aeruginosa</i>	ATCC 15442	
	<i>Escherichia coli</i> K12	NCTC 10538	
	<i>Proteus mirabilis</i>	ATCC 14153	
	<i>Candida albicans</i>	ATCC 10231	
Test solution:	97 %, 80 %, 50 %, 25 %, 12.5 %, 10 %, 1 %		
Active ingredients in 100 g:	72 g Ethanol		
Odour:	alcoholic		
Appearance :	clear, colourless gel		
Appearance of dilutions:	clear to turbid solutions, flocculation with interfering substance		
pH-values (pH-meter):	100 %: 7.53	50 %: 6.40	25 %: 5.85
	WSH: 7.19		
pH-values (pH-stripes):	100 %: 5-6	10 %: 7	
Neutralizer:	4 % Tween80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS (Neutralizer XXIV)		
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes (dirty conditions)		
Test temperature:	20 ± 1 °C		
Incubation temperature:	36 ± 1 °C (<i>C. albicans</i> : 30 ± 1°C)		

Test Method

MIC / Selection of neutralizer

The test was performed in accordance with the "Requirements and methods for VAH-certification of chemical disinfection processes". Accordingly, the following test organisms were used:

<i>S. aureus</i>	ATCC 6538	<i>P. aeruginosa</i>	ATCC 15442
<i>E. coli</i> K12	NCTC 10538	<i>E. hirae</i>	ATCC 10541
<i>P. mirabilis</i>	ATCC 14153	<i>C. albicans</i>	ATCC 10231

The test was performed using the following growth medium:

- Tryptone Soya Broth (TSB)

The product is diluted with hard water; tests are performed at room temperature (20 ± 1 °C). The test organisms are incubated at 36 ± 1 °C (*C. albicans*: 30 ± 1 °C). Detailed results presented in table 1.

Quantitative suspension test under dirty conditions

A test suspension of bacteria including an interfering substance (0.3 % albumin + 0.3 % sheep erythrocytes – dirty conditions) is added to a sample of Phago'rub Gel SPS (diluted with hard water, if necessary). The mixture is maintained at 20 ± 1 °C for the required contact time. At the end of the contact time, an aliquot is taken; the microbicidal activity in this portion is immediately neutralized. The number of surviving test organisms in each sample is determined by plating aliquots of the neutralized test suspensions and its dilutions. The reduction is calculated in relation to a sample containing water instead of the test product (water control, WSH control).

The experimental conditions, the non-toxicity of the neutralizer and the dilution-neutralization method are validated according to the DGHM standard methods:

- Co 1 = Water control (WSH)
- Co 2 = Method validation (Dilution-neutralization method)
- Co 3 = Non-toxicity of the neutralizer

The test was performed using *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa*, *E. coli*, *Proteus mirabilis* and *Candida albicans* as test-organisms.

Results are presented in tables 2 – 7.

Results


Selection of neutralizer / Minimal inhibitory concentration (MIC)

In accordance with the test results presented in table 1, 4 % Tween 80 + 3 % Saponin + 0.4 % Lecithine + 0.25 % SDS (Neutralizer XXIV) was selected as a suitable neutralizer for the test bacteria and *C. albicans*. A product concentration of 25 % was determined as the minimal inhibitory concentration at the methodically determined contact time of 48 h (Tab. 1).

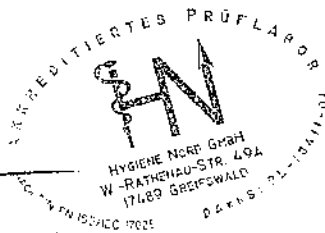
Quantitative suspension test

According to the "VAH Requirements and methods for VAH-certification of chemical disinfection processes", the batch 1592800806 of the product Phago'rub Gel SPS, applied at a concentration of at least 80 % possesses bactericidal and yeasticidal efficacy at 20 °C in 15 s under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes) for reference strains *S. aureus*, *E. hirae*, *aeruginosa*, *E. coli*, *P. mirabilis* and *C. albicans*, with *C. albicans* being the limiting test organism (Tab. 2 – 7).

Greifswald, March 04, 2016


Dr. rer. med. (Dipl. Biol.) T. Koberger-Janssen

- General Manager -




Prof. Dr. med. A. Kramer

- MD for Hygiene and Environmental Medicine -

Table 1: Identification of a suitable neutralizer (according to VAH methods, 2015)

Date: January 22, 2016
 Substance: Phago'rub Gel SPS
 Test organism: see table 1
 Incubation temperature: 36 ± 1 °C (*C. albicans*: 30 ± 1°C)
 Incubation time: 48 h

Order number: A 15263
 Sample number: P 155926
 Lot number: 1592800806
 Test suspension: see table 1

concentration [%]	<i>S. aureus</i> 2.50*10 ⁸ cfu /ml (8.40 log)	<i>E. hirae</i> 1.80*10 ⁸ cfu/ml (8.26 log)	<i>E. coli</i> 1.70*10 ⁸ cfu/ml (8.23 log)	<i>P. mirabilis</i> 2.00*10 ⁸ cfu/ml (8.30 log)	<i>P. aeruginosa</i> 2.70*10 ⁸ cfu/ml (8.43 log)	<i>C. albicans</i> 4.40*10 ⁷ cfu/ml (7.64 log)
	0 II XXIV VI	0 II XXIV VI	0 II XXIV VI	0 II XXIV VI	0 II XXIV VI	0 II XXIV VI
50	-	-	-	-	-	-
25	-	-	-	-	-	-
12.5	-	(+)	(+)	(+)	-	(+)
1	+	+	+	+	+	+
control*	+	+	+	+	+	+

0 = WSH (water of standardized hardness)
 II = 3 % Tween 80 + 3 % Saponin + 0.1 % Histidine + 0.1 % Cysteine
 XXIV = 4 % Tween80 + 3 % Saponin + 0.4 % Lecithin + 0.25 % SDS
 VI = 3 % Tween80 + 3 % Saponin + 0.1 % Histidine + 0.1 % Cysteine + 0.3 % Lecithin

*control = WSH
 - = no growth
 + = growth
 (+) = minimal growth

Table 2: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago*rub Gel SPS Batch-Number: 1592800806
 Test organism: *S. aureus* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 24 h – 48 h Neutralizer: XXIV
 Test suspension : 1.50 *10⁸ cfu/0.1 ml (8.18 log) Incubation temperature: 36 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	5	0.70	5.02	0	0.00	≥ 5.70	0	0.00	≥ 5.64
	0.1 ml 10 ⁰	2			0			0		
	0.1 ml 10 ¹	1			0			0		
	0.1 ml 10 ²	1			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 5.72	0	0.00	≥ 5.70	0	0.00	≥ 5.64
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
50 %	1 ml 10 ⁰	0	0.00	≥ 5.72	0	0.00	≥ 5.70	0	0.00	≥ 5.64
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	134	5.13	0.59	> 330	> 5.52	< 0.18	> 330	> 5.52	< 0.18
WSH (co 1)	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	52	5.72		50	5.70		44	5.64	
	0.1 ml 10 ⁴	4			3			0		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	156	1.56E+04	4.19
	0.1 ml 10 ¹	11		
control 2 (co 2) - 80 %	0.1 ml 10 ⁰	125	1.25E+04	4.21
	0.1 ml 10 ¹	20	2.00E+04	
control 2 (co 2) - 50 %	0.1 ml 10 ⁰	136	1.36E+04	4.13
	0.1 ml 10 ¹	13		
control 3 (co 3)	0.1 ml 10 ⁰	159	1.59E+04	4.19
	0.1 ml 10 ¹	15	1.50E+04	
Validation suspension:			1.40E+04	4.15
97 % - 60 s			controls o.k.?	Yes

Table 3: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago'rub Gel SPS Batch-Number: 1592800806
 Test organism: *E. hirae* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 24 h – 48 h Neutralizer: XXIV
 Test suspension : 6.70 *10⁸ cfu/0.1 ml (8.83 log) Incubation temperature: 36 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	0	0.00	≥ 6.12	0	0.00	≥ 6.09	0	0.00	≥ 6.05
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	8			1			0		
	0.1 ml 10 ³	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 6.12	0	0.00	≥ 6.09	0	0.00	≥ 6.05
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			1			0		
	0.1 ml 10 ³	0			0			0		
50 %	1 ml 10 ⁰	0	0.00	≥ 6.12	8	0.90	5.19	4	0.60	5.45
	0.1 ml 10 ¹	0			0			1		
	0.1 ml 10 ²	0			0			0		
	0.1 ml 10 ³	0			2			1		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	> 330			> 330			> 330		
	0.1 ml 10 ³	≥ 330	> 5.52	< 0.60	≥ 330	> 5.52	< 0.57	≥ 330	> 5.52	< 0.53
WSH (co 1)	0.1 ml 10 ²	> 330			> 330			> 330		
	0.1 ml 10 ³	> 330			> 330			> 330		
	0.1 ml 10 ⁴	131	6.12		123	6.09		112	6.05	
	0.1 ml 10 ⁵	12			13			11		

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ²	129	1.29E+04	4.14
	0.1 ml 10 ³	15	1.50E+04	
control 2 (co 2) - 80 %	0.1 ml 10 ²	177	1.77E+04	4.26
	0.1 ml 10 ³	19	1.90E+04	
control 2 (co 2) - 50 %	0.1 ml 10 ²	226	2.26E+04	4.32
	0.1 ml 10 ³	19	1.90E+04	
control 3 (co 3)	0.1 ml 10 ²	162	1.62E+04	4.19
	0.1 ml 10 ³	15	1.50E+04	
Validation suspension:			6.70E+04	4.83
		97 % - 60 s	controls o.k.?	Yes

Table 4.1: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago[®]rub Gel SPS Batch-Number: 1592800806
 Test organism: *P. aeruginosa* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 24 h – 48 h Neutralizer: XXIV
 Test suspension : 3.50 *10⁸ cfu/0.1 ml (8.54 log) Incubation temperature: 36 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	8	0.90	5.60	0	0.00	≥ 6.49	0	0.00	≥ 6.53
	0.1 ml 10 ⁻¹	1			0			0		
	0.1 ml 10 ⁻²	5			0			0		
	0.1 ml 10 ⁻³	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 6.50	224	2.35	4.19 *	0	0.00	≥ 6.53
	0.1 ml 10 ⁻¹	0			18	2.26		0		
	0.1 ml 10 ⁻²	0			0			0		
	0.1 ml 10 ⁻³	0			0			0		
50 %	1 ml 10 ⁰	0	0.00	≥ 6.50	0	0.00	≥ 6.49	0	0.00	≥ 6.53
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
	0.1 ml 10 ⁻³	0			0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	> 330			> 330			> 330		
	0.1 ml 10 ⁻³	> 330	> 5.52	< 0.99	> 330	> 5.52	< 0.97	> 330	> 5.52	< 1.01
WSH (co 1)	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	> 330			> 330			> 330		
	0.1 ml 10 ⁻³	259	6.50		262	6.49		243	6.53	
	0.1 ml 10 ⁻⁴	38			36			43		

* see table 4.2 for verification

Controls and Validation:

		number of colonies (cfu/plate)	cfu /ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	215	2.15E+04	4.27
	0.1 ml 10 ⁻¹	16	1.60E+04	
control 2 (co 2) - 80 %	0.1 ml 10 ⁰	182	1.82E+04	4.33
	0.1 ml 10 ⁻¹	25	2.50E+04	
control 2 (co 2) - 50 %	0.1 ml 10 ⁰	174	1.74E+04	4.27
	0.1 ml 10 ⁻¹	20	2.00E+04	
control 3 (co 3)	0.1 ml 10 ⁰	148	1.48E+04	4.32
	0.1 ml 10 ⁻¹	27	2.70E+04	
Validation suspension:			3.90E+04	4.59
		97 % - 60 s	controls o.k.?	Yes

Table 4.2: Results of the quantitative suspension test (according to VAH methods, 2015)

Date:	February 10, 2016	Order number:	A 15263
Substance:	Phago'rub Gel SPS	Batch-Number:	1592800806
Test organism:	<i>P. aeruginosa</i>	Sample number:	P 155926
Interfering substance:	0.3 % albumin + 0.3 % sheep erythrocytes		
Incubation time:	24 h – 48 h	Neutralizer:	XXIV
Test suspension :	2.10 *10 ⁸ cfu/1 ml (8.32 log)	Incubation temperature:	36 ± 1 °C
Method:	Dilution-neutralization-method	Reaction temperature:	20 ± 1 °C

concentration	dilution	time: 30 s		
		cfu / plate	cfu / plate	cfu / plate
80 %	1 ml 10 ⁰	0	0.00	≥ 6.22
	0.1 ml 10 ¹	0		
	0.1 ml 10 ¹	0		
	0.1 ml 10 ²	0		
50 %	1 ml 10 ⁰	0	0.00	≥ 6.22
	0.1 ml 10 ⁰	0		
	0.1 ml 10 ¹	0		
	0.1 ml 10 ²	0		
WSH (co 1)	0.1 ml 10 ⁰	> 330		
	0.1 ml 10 ¹	> 330		
	0.1 ml 10 ²	171	6.22	
	0.1 ml 10 ³	16		

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	132	1.32E+04	4.13
	0.1 ml 10 ¹	14	1.40E+04	
control 3 (co 3)	0.1 ml 10 ⁰	152	1.52E+04	4.21
	0.1 ml 10 ¹	17	1.70E+04	
Validation suspension:			2.10E+04	4.32
		97 % - 30 s	controls o.k.?	Yes

Table 5: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago'rub Gel SPS Batch-Number: 1592800806
 Test organism: *P. mirabilis* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 24 h – 48 h Neutralizer: XXIV
 Test suspension : 7.50 *10⁸ cfu/0.1 ml (8.88 log) Incubation temperature: 36 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	0	0.00	≥ 6.46	0	0.00	≥ 6.44	0	0.00	≥ 6.51
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 6.46	0	0.00	≥ 6.44	0	0.00	≥ 6.51
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
50 %	1 ml 10 ⁰	0	0.00	≥ 6.46	0	0.00	≥ 6.44	0	0.00	≥ 6.51
	0.1 ml 10 ⁰	0			0			0		
	0.1 ml 10 ⁻¹	0			0			0		
	0.1 ml 10 ⁻²	0			0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	≥ 330	> 5.52	< 0.94	≥ 330	> 5.52	< 0.92	≥ 330	> 5.52	< 0.99
WSH (co 1)	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ⁻¹	> 330			> 330			> 330		
	0.1 ml 10 ⁻²	259	6.46		260	6.44		295	6.51	
	0.1 ml 10 ⁻³	32			29			35		

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	270	2.70E+04	4.47
	0.1 ml 10 ⁻¹	32	3.20E+04	
control 2 (co 2) - 80 %	0.1 ml 10 ⁰	241	2.41E+04	4.43
	0.1 ml 10 ⁻¹	30	3.00E+04	
control 2 (co 2) - 50 %	0.1 ml 10 ⁰	234	2.34E+04	4.39
	0.1 ml 10 ⁻¹	26	2.60E+04	
control 3 (co 3)	0.1 ml 10 ⁰	269	2.69E+04	4.49
	0.1 ml 10 ⁻¹	35	3.50E+04	
Validation suspension:			7.50E+04	4.88
		97 % - 60 s	controls o.k.?	Yes

Table 6: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago'rub Gel SPS Batch-Number: 1592800806
 Test organism: *E. coli* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 24 h – 48 h Neutralizer: XXIV
 Test suspension : 3.90 *10⁸ cfu/0.1 ml (8.59 log) Incubation temperature: 36 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁶	0	0.00	≥ 6.58	4	0.60	5.94	0	0.00	≥ 6.52
	0.1 ml 10 ⁶	0			0			0		
	0.1 ml 10 ¹	1			0			0		
	0.1 ml 10 ²	0			0			0		
80 %	1 ml 10 ⁶	0	0.00	≥ 6.58	0	0.00	≥ 6.54	0	0.00	≥ 6.52
	0.1 ml 10 ⁶	0			0			0		
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
50 %	1 ml 10 ⁶	0	0.00	≥ 6.58	0	0.00	≥ 6.54	0	0.00	≥ 6.52
	0.1 ml 10 ⁶	0			0			0		
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
10 %	1 ml 10 ⁶	> 330			> 330			> 330		
	0.1 ml 10 ⁶	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	> 330	> 5.52	< 1.06	> 330	> 5.52	< 1.03	> 330	> 5.52	< 1.00
WSH (co 1)	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	> 330			> 330			> 330		
	0.1 ml 10 ³	> 330			> 330			> 330		
	0.1 ml 10 ⁴	38	6.58		35	6.54		33	6.52	

Controls and Validation:

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁶	> 330		
	0.1 ml 10 ¹	39	3.90E+04	4.59
control 2 (co 2) - 80 %	0.1 ml 10 ⁶	> 330		
	0.1 ml 10 ¹	37	3.70E+04	4.57
control 2 (co 2) - 50 %	0.1 ml 10 ⁶	> 330		
	0.1 ml 10 ¹	42	4.20E+04	4.62
control 3 (co 3)	0.1 ml 10 ²	> 330		
	0.1 ml 10 ¹	38	3.80E+04	4.58
Validation suspension:			3.90E+04	4.59
		97 % - 60 s	controls o.k.?	Yes

Table 7: Results of the quantitative suspension test (according to VAH methods, 2015)

Date: January 26, 2016 Order number: A 15263
 Substance: Phago'rub Gel SPS Batch-Number: 1592800806
 Test organism: *C. albicans* Sample number: P 155926
 Interfering substance: 0.3 % albumin + 0.3 % sheep erythrocytes
 Incubation time: 48 h Neutralizer: XXIV
 Test suspension : 4.50 *10⁷ cfu/0.1 ml (7.65 log) Incubation temperature: 30 ± 1 °C
 Method: Dilution-neutralization-method Reaction temperature: 20 ± 1 °C

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	cfu / plate	cfu / plate	cfu / plate	log ₁₀ (x)	RF	cfu / plate	log ₁₀ (x)	RF
97 %	1 ml 10 ⁰	0	0.00	≥ 5.53	0	0.00	≥ 5.51	0	0.00	≥ 5.46
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	2			0			0		
	0.1 ml 10 ³	0			0			0		
80 %	1 ml 10 ⁰	0	0.00	≥ 5.53	0	0.00	≥ 5.51	0	0.00	≥ 5.46
	0.1 ml 10 ¹	0			0			0		
	0.1 ml 10 ²	0			0			0		
	0.1 ml 10 ³	0			0			0		
50 %	1 ml 10 ⁰	> 330			214	2.33	3.19	0	0.00	≥ 5.46
	0.1 ml 10 ¹	> 330			20	2.30		0		
	0.1 ml 10 ²	256	4.41	1.21	1			0		
	0.1 ml 10 ³	17	4.23		0			0		
10 %	1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	> 330			> 330			> 330		
	0.1 ml 10 ³	282	5.45	0.08	255	5.41	0.10	283	5.45	0.01
WSH (co 1)	0.1 ml 10 ⁰	> 330			> 330			> 330		
	0.1 ml 10 ¹	> 330			> 330			> 330		
	0.1 ml 10 ²	311	5.53		295	5.51		270	5.46	
	0.1 ml 10 ³	37			35			31		

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log ₁₀ (x)
control 2 (co 2) - 97 %	0.1 ml 10 ⁰	323	3.23E+04	4.62
	0.1 ml 10 ¹	51	5.10E+04	
control 2 (co 2) - 80 %	0.1 ml 10 ⁰	332		
	0.1 ml 10 ¹	33	3.30E+04	4.52
control 2 (co 2) - 50 %	0.1 ml 10 ⁰	> 330		
	0.1 ml 10 ¹	29	2.90E+03	4.46
control 3 (co 3)	0.1 ml 10 ⁰	325	3.25E+04	4.62
	0.1 ml 10 ¹	51	5.10E+04	
Validation suspension:			4.50E+04	4.65
		97 % - 60 s	controls o.k.?	Yes

Legend:

MW	=	average value
x	=	average value
RF	=	reduction factor
> 330	=	not countable
n.d.	=	not determined
Co 1	=	Control 01
Co 2	=	Control 02
Co 3	=	Control 03



Christeyns France
31 rue de la Maladrie
44120 VERTOU
France

CUSTOMER NUMBER
815

DATE
March 08, 2016

REPORT A 15086
PHAGO'RUB GEL SPS
HYGIENIC HANDRUB (EN 1500)

Purpose

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

Test Description

Manufacturer:	Christeyns France, Vertou, France			
Product:	Phago'rub Gel SPS Hygienic hand antiseptic			
Sample number:	P 151168			
Batch number:	PR195-F14			
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:	<i>Escherichia coli</i> K 12 NCTC 10538			
Test solutions:	100 %			
Active ingredients in 100 g:	72 g Ethanol			
Odour:	alcoholic			
Appearance:	colourless clear gel			
Appearance of dilution:	10 %: colourless clear liquid, white flocculation			
pH – value (pH-meter)	100 %: 9.02	80 %: 8.42	10 %: 7.26	WSH: 7.20
pH – value (pH-stripes)	100 %: 5 -6	10 %: 6-7		
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 Phago'rub Gel 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 Phago'rub Gel 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: $4.80 \cdot 10^8$ 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 Phago'rub Gel 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

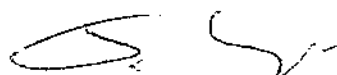
The overall mean values of the test product formulation Phago'rub Gel SPS at a contact time of 30 s are:

prevalue	5.92
postvalue	1.62
reduction factor (RF)	4.30

The mean log reduction (RF) for the test product (Phago'rub Gel 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 = 0.49).

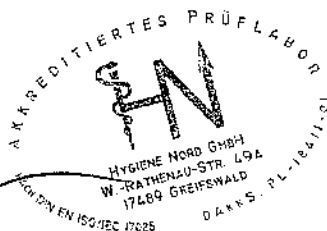
The test product formulation Phago'rub Gel SPS does therefore correspond to the requirements of the EN 1500 (2013) for the hygienic handrub when hands are kept moist with 3 ml of the product for a contact time of 30 s.

Greifswald, March 08, 2016



Dr rer. med. (Dipl. Biol.) T. Koburger-Janssen

- 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 15086
Product:	Phago´rub Gel SPS	Sample number:	P 151168
Test organism:	<i>E. coli</i>	Batch number:	PR195-F14
Interfering substance:	none	Neutralizer:	XXIV
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h – 48 h
Test suspension (N):	4.80*10 ⁸ cfu / ml	Test temperature	20 ± 1 °C
Test suspension (N _v):	1.25*10 ⁵ cfu / ml		
Validation Suspension (N _v):	1.58*10 ⁹ cfu / ml		

Neutralization – control and validation:

Validation suspension (N _{v0})				Neutralizer control (control B)				Method validation (Control C) 80 %						
cfu / Plate 1 & 2		V _c	\bar{x} (cfu / ml)	cfu / Plate 1 & 2		V _c	\bar{x} (cfu / ml)	cfu / Plate 1 & 2		V _c	\bar{x} (cfu / ml)			
V _{d1}	86	75	161	158	V _{d1}	69	76	145	141.5	V _{d1}	76	54	130	132.5
V _{d2}	72	83	155		V _{d2}	70	68	138		V _{d2}	72	63	135	
30 ≤ \bar{x} of N _{v0} ≤ 160?				\bar{x} of B is ≥ 0.0005* \bar{x} of N _{v0} ?				\bar{x} of C is ≥ 0.5* \bar{x} of N _{v0} ?						
<input checked="" type="checkbox"/> Yes* <input type="checkbox"/> No				<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No				<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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)	cfu / Plate 1 & 2		V _c	\bar{x} (cfu / ml)	cfu / Plate 1 & 2		V _c	\bar{x} (cfu / ml)			
V _{d1}	95	80	175	175	V _{d1}	95	80	175	175	V _{d1}	95	80	175	175
V _{d2}	83	92	175		V _{d2}	83	92	175		V _{d2}	83	92	175	
\bar{x} of C is ≥ 0.5* \bar{x} of N _{v0} ?														
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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	abs (0.00 - 0.38) = 0.39
RP	PP		RP	PP		
4.52	4.52	0.00	4.47	4.09	0.38	
result < 2.00 ?						
<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No						

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

test date: March 26, 2015
 reference: 60 % propan-2-ol
 test product: Phago[®]rub Gel SPS
 neutralizer: XXIV

order number: A 15086
 batch number: 093192228
 batch number: PR195-F14
 sample number: P 151168

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 ⁶	0.1 ml 10 ⁵	0.1 ml 10 ⁴	0.1 ml 10 ³	0.1 ml 10 ⁴	1 ml 10 ⁶	0.1 ml 10 ⁵	0.1 ml 10 ⁴
1	right	152	26	12	4	0	162	25	26	1	0
	left	187	28	78	4	0	151	19	> 330	33	4
2	right	28	6	16	1	0	41	4	28	1	0
	left	55	9	10	0	0	57	5	14	1	0
3	right	84	22	130	17	0	97	7	60	6	0
	left	23	5	38	6	1	60	8	4	0	0
4	right	16	1	216	24	4	8	0	180	7	0
	left	20	1	20	1	0	21	2	30	2	0
5	right	76	14	2	0	0	68	11	0	0	0
	left	58	8	6	1	0	65	6	2	0	0
6	right	174	43	0	0	0	> 330	42	0	0	0
	left	146	15	0	0	0	194	25	0	0	0
7	right	115	12	0	0	0	118	14	0	0	0
	left	105	23	0	0	0	89	10	0	0	0
8	right	97	5	74	6	0	139	20	26	3	0
	left	58	5	0	0	0	131	16	2	0	0
9	right	54	8	224	23	2	93	12	> 330	150	25
	left	72	6	26	1	0	85	11	> 330	171	33
10	right	12	1	> 330	62	4	50	4	> 330	168	33
	left	9	0	> 330	59	8	36	4	> 330	102	15
11	right	171	32	2	0	0	> 330	34	> 330	50	2
	left	127	21	22	1	0	> 330	32	> 330	36	0
12	right	65	10	8	0	0	82	16	100	11	0
	left	70	4	16	1	0	95	11	46	4	0
13	right	132	27	0	0	0	76	12	26	2	0
	left	120	22	12	0	0	63	10	12	0	0
14	right	64	10	26	6	0	92	12	10	1	0
	left	69	11	62	5	1	70	12	12	1	0
15	right	35	2	26	2	0	155	24	> 330	106	14
	left	51	4	8	1	0	155	24	30	4	0
16	right	50	8	10	2	0	49	12	26	5	0
	left	44	8	42	3	0	38	8	16	5	0
17	right	105	25	> 330	38	2	110	18	> 330	91	14
	left	133	8	> 330	129	21	144	16	> 330	> 330	38
18	right	> 330	40	92	11	0	170	31	> 330	36	0
	left	141	19	24	2	0	139	27	40	5	0
19	right	59	8	6	0	0	64	9	14	0	0
	left	45	5	2	0	0	57	7	2	0	0
20	right	20	1	140	18	1	32	6	76	11	1
	left	3	0	108	10	1	16	1	> 330	48	8

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

test product: Phago'rub Gel SPS
reference: 60 % propan-2-ol

sample number: P 151168
order number: A 15086

subject	hand	reference product					test product						
		prevalue log		postvalue log		reduction-factors	prevalue log		postvalue log		reduction-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	6.23	6.21	1.41	1.97	4.24		
	L	6.29		1.89			6.19		2.52				
2	R	5.45	5.59	1.20	1.10	4.49	5.61	5.68	1.45	1.30	4.39		
	L	5.74		1.00			5.76		1.15				
3	R	5.98	5.67	2.13	1.85	3.82	5.99	5.88	1.78	1.19	4.69		
	L	5.36		1.58			5.78		0.60				
4	R	5.20	5.25	2.34	1.82	3.43	4.90	5.11	2.26	1.87	3.25		
	L	5.30		1.30			5.32		1.48				
5	R	5.91	5.84	0.30	0.54	5.30	5.83	5.82	0.00	0.15	5.67		
	L	5.76		0.78			5.81		0.30				
6	R	6.30	6.23	0.00	0.00	6.23	6.62	6.46	0.00	0.00	6.46		
	L	6.17		0.00			6.30		0.00				
7	R	6.06	6.06	0.00	0.00	6.06	6.08	6.01	0.00	0.00	6.01		
	L	6.07		0.00			5.95		0.00				
8	R	5.99	5.88	1.87	0.93	4.94	6.16	6.14	1.41	0.86	5.28		
	L	5.76		0.00			6.13		0.30				
9	R	5.73	5.79	2.35	1.88	3.91	5.97	5.95	3.20	3.23	2.71		
	L	5.86		1.41			5.93		3.27				
10	R	5.08	5.02	2.79	2.78	2.24	5.70	5.63	3.26	3.14	2.48		
	L	4.95		2.77			5.56		3.03				
11	R	6.27	6.20	0.30	0.82	5.38	6.53	6.52	2.70	2.63	3.89		
	L	6.13		1.34			6.51		2.56				
12	R	5.81	5.83	0.90	1.05	4.78	5.95	5.96	2.00	1.83	4.13		
	L	5.85		1.20			5.98		1.66				
13	R	6.16	6.14	0.00	0.54	5.60	5.88	5.84	1.41	1.25	4.59		
	L	6.11		1.08			5.80		1.08				
14	R	5.81	5.82	1.41	1.60	4.22	5.96	5.90	1.00	1.04	4.86		
	L	5.84		1.79			5.85		1.08				
15	R	5.54	5.63	1.41	1.16	4.47	6.21	6.21	3.04	2.26	3.95		
	L	5.71		0.90			6.21		1.48				
16	R	5.70	5.67	1.00	1.31	4.36	5.69	5.63	1.41	1.31	4.33		
	L	5.64		1.62			5.58		1.20				
17	R	6.07	6.10	2.58	2.86	3.24	6.07	6.11	2.98	3.28	2.83		
	L	6.12		3.13			6.16		3.58				
18	R	6.60	6.38	1.96	1.67	4.71	6.26	6.22	2.56	2.08	4.14		
	L	6.16		1.38			6.18		1.60				
19	R	5.77	5.71	0.78	0.54	5.17	5.81	5.78	1.15	0.72	5.06		
	L	5.65		0.30			5.76		0.30				
20	R	5.30	4.89	2.16	2.10	2.79	5.51	5.35	1.88	2.28	3.07		
	L	4.48		2.03			5.20		2.68				
arithm. mean		5.80		1.30		4.49		5.92		1.62		4.30	
absolute SD		0.40		0.80		1.04		0.34		1.02		1.09	
relative SD		6.88		61.09		23.14		5.80		62.78		25.30	

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: Phago'rub Gel SPS

order number: A 15086
 sample number: P 151168

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: 0.11

Subject	RP-PP	Mean pairwise differences $(d_{i+} + d_{i-})/2$ (no duplicates, only values \geq median)										
		11	9	13	12	18	1	15	17	4	19	2
11	1.49	1.49										
9	1.20	1.35	1.20									
13	1.01	1.25	1.11	1.01								
12	0.65	1.07	0.93	0.83	0.65							
18	0.57	1.03	0.89	0.79	0.61	0.57						
1	0.52	1.01	0.86	0.77	0.59	0.55	0.52					
15	0.52	1.01	0.86	0.77	0.59	0.55	0.52	0.52				
17	0.41	0.95	0.81	0.71	0.53	0.49 ⁵³	0.47	0.47	0.41			
4	0.18	0.84	0.69	0.60	0.42	0.38	0.35	0.35	0.30	0.18		
19	0.11	0.80	0.66	0.56	0.38	0.34	0.32	0.32	0.26	0.15	0.11	
2	0.10	0.80	0.65	0.56	0.38	0.34	0.31	0.31	0.26	0.14	0.11	
7	0.05	0.77	0.63	0.53	0.35	0.31	0.29	0.29	0.23	0.12		
16	0.03	0.76	0.62	0.52	0.34	0.30	0.28	0.28	0.22	0.11		
6	-0.23	0.63	0.49	0.39	0.21	0.17	0.15	0.15				
10	-0.24	0.63	0.48	0.39	0.21	0.17	0.14	0.14				
20	-0.28	0.61	0.46	0.37	0.19	0.15	0.12	0.12				
8	-0.34	0.58	0.43	0.34	0.16	0.12						
5	-0.37	0.56	0.42	0.32	0.14							
14	-0.64	0.43	0.28	0.19								
3	-0.87	0.31	0.17									

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 0.49, 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
> 300	=	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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Unser Zeichen
Dr.Pi/cs

Datum
8. März 2016

Gutachten

zum Präparat: Phago´rub gel SPS
als Mittel zur: hygienischen Händedesinfektion

Die Prüfung des Präparats erfolgte gemäß

DIN EN 1500 (2013-07) „Chemische Desinfektionsmittel und Antiseptika, Hygienische Händedesinfektion, Prüfverfahren und Anforderungen Phase 2, Stufe 2“.

Begutachtungskriterium waren die „Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren, Stand 2. April 2015“.

Der dem Gutachten zugrunde liegende Prüfbericht mit der Bezeichnung „PL 15-66 Prüfbericht Feuchthalten der Hände über 30 s 160308“ datiert vom 8. März 2016.

Bestimmung der bakteriostatischen und levurostatischen Wirkung sowie geeigneter Neutralisationsmittel (Tab. 1 im Prüfbericht vom 08.03.2016):

Ermittelte Hemmtiter:

Prüforganismus	Konzentration des Prüfprodukts (%)	
	ohne Neutralisationsmittel	mit optimalem Neutralisationsmittel
<i>S. aureus</i>	10	25
<i>E. hirae</i>	10	25
<i>P. aeruginosa</i>	10	10
<i>E. coli</i>	10	10
<i>P. mirabilis</i>	25	25
<i>C. albicans</i>	10	10

Als optimales Neutralisationsmittel erwies sich die Kombination aus:

3,0 % Polysorbat 80, 3,0 % Saponin, 0,1 % L-Histidin, 0,1 % Cystein

Diese Kombination wurde zur Neutralisation in allen weiteren Untersuchungen angewendet.

Bestimmung der bakteriziden und levuroziden Wirkung im qualitativen Suspensionsversuch (Tab. 2 im Prüfbericht vom 08.03.2016)

Testkeim	Konzentration des Prüfprodukts (%) bei Einwirkzeit in		
	15 s	30 s	60 s
<i>P. aeruginosa</i>	100	100	100
<i>E. coli</i>	100	50	50
<i>P. mirabilis</i>	100	50	50

Aufgrund der nachgewiesenen mikrobioziden Wirksamkeiten werden die Prüforganismen *S. aureus*, *E. hirae*, *P. aeruginosa* und *C. albicans* für die quantitativen Suspensionsversuche ausgewählt.

Bestimmung der bakteriziden und levuroziden Wirkung im quantitativen Suspensionsversuch (Tab. 3 – 6 im Prüfbericht vom 08.03.2016)

In folgenden Konzentrations-/Zeitrelationen wurde eine ausreichende Reduktion der Testkeime unter hoher Belastung (0,3 % Albumin + 0,3 % Schaferythrozyten) erreicht:

Testkeim	Wirksame Konzentration (%) bei Einwirkzeit in		
	15 s	30 s	60 s
<i>S. aureus</i>	80	80	80
<i>E. hirae</i>	80	50	50
<i>P. aeruginosa</i>	50	50	50
<i>C. albicans</i>	80	50	50
Alle Testkeime	80	80	80

Prüfung der Gleichwertigkeit im quantitativen Suspensionsversuch unter hoher Belastung (0,3 % Albumin + 0,3 % Schaferythrozyten) (Tab. 3a im Prüfbericht vom 08.03.2016)

Testkeim <i>S. aureus</i>	Reduktionsfaktoren nach Einwirkzeit in		
	15 s	30 s	60 s
80 % Phago´rub gel SPS	6,28	6,38	6,26
80 % Phago´rub Solution SPS	6,28	6,38	6,26
50 % Phago´rub gel SPS	1,73	3,69	4,45
50 % Phago´rub Solution SPS	2,35	4,00	4,83
25 % Phago´rub gel SPS	<0,80	<0,90	<0,78
25 % Phago´rub Solution SPS	<0,80	<0,90	<0,78

**Versuche zur hygienischen Händedesinfektion nach DIN EN 1500 (2013-07)
(Tab. 7 - 11 im Prüfbericht vom 08.03.2016)**

In der Prüfung auf Eignung zur hygienischen Händedesinfektion wurde mit dem Referenz-Desinfektionsverfahren (Propan-2-ol, 60 % V/V) ein mittlerer Reduktionsfaktor von 4,45 erreicht. Die mit dem Prüfprodukt **Phago´rub gel SPS** erzielte Reduktion nach 30 s betrug 4,31.

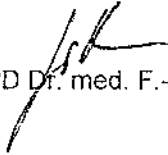
Der Test auf Nicht-Unterlegenheit ($p = 0,025$, einseitig) zeigt, dass das Prüfprodukt **Phago´rub gel SPS** dem Referenz-Desinfektionsverfahren (Propan-2-ol, 60 % V/V) nicht unterlegen ist.

Zusammenfassung und Bewertung

Die Anforderungen gemäß DIN EN 1500 (2013-07) „Chemische Desinfektionsmittel und Antiseptika, Hygienische Händedesinfektion, Prüfverfahren und Anforderungen (Phase 2, Stufe 2)“ werden von **Phago´rub gel SPS** als Mittel zur **hygienischen Händedesinfektion** bei einer Anwendung von

Feuchthalten der Hände – 30 s

erfüllt.


PD Dr. med. F.-A. Pitten

Unser Zeichen:
Dr.Pi/cs

Datum:
8. März 2016

Prüfbericht

zum Präparat: Phago´rub gel SPS / Phago´rub Solution SPS
als Mittel zur: hygienischen Händedesinfektion
Prüfverfahren: Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren,
Stand 2. April 2015
DIN EN 1500 (2013-07) „Chemische Desinfektionsmittel und Antiseptika - Hygienische
Händedesinfektion - Prüfverfahren und Anforderungen, Phase 2, Stufe 2“

Auftraggeber: Christeyns GmbH, Offenburg
IKI-Labor-Nr.: PL 15/66
Produkt: Phago´rub gel SPS / Phago´rub Solution SPS
Chargen-Nr.: Phago´rub gel SPS: 1592 805636 /
Phago´rub Solution SPS: LOT 23011
Lieferdatum: 05.10.2016
Zeitraum der Prüfung: 13.10.2015 bis 25.02.2016
Lagerbedingungen: dunkel, Raumtemperatur
Wirksubstanzen und deren Konzentration: 72 % w/w Ethanol
Aussehen: klare, farblose Flüssigkeit / klares, farbloses Gel
Geruch: produktspezifisch / produktspezifisch
pH-Wert des Konzentrats: 8,20 / 8,30
Verwendete Testkeime: *Staphylococcus aureus* ATCC 6538
Enterococcus hirae ATCC 10541
Escherichia coli K12 NCTC 10538
Proteus mirabilis ATCC 14153
Pseudomonas aeruginosa ATCC 15442
Candida albicans ATCC 10231

Tabelle 1

Bestimmung der bakteriostatischen und levurostatischen Wirkung sowie geeigneter Neutralisationsmittel (Standardmethode 7)

Prüfprodukt: Phago´rub Solution SPS
Prüfdatum: 13.10.2015
Prüftemperatur: 20 ± 1°C

Keimgehalt der Prüfsuspensionen (KbE/ml):

<i>S. aureus</i>	1,7 x 10 ⁸	<i>E. hirae</i>	1,7 x 10 ⁸
<i>E. coli</i>	2,8 x 10 ⁸	<i>P. mirabilis</i>	1,6 x 10 ⁸
<i>P. aeruginosa</i>	1,6 x 10 ⁸	<i>C. albicans</i>	1,5 x 10 ⁷

Hemmkonzentrationen nach 48 h Inkubation bei 36 ± 1°C (*C. albicans* bei 30 ± 1°C)

Neutralisations- mittel und Testkeime	Präparateendkonzentrationen (%)				
	50	25	10	5	WSH
ohne					
<i>S. aureus</i>	-	-	-	+	+
<i>E. hirae</i>	-	-	-	+	+
<i>E. coli</i>	-	-	-	+	+
<i>P. mirabilis</i>	-	-	+	+	+
<i>P. aeruginosa</i>	-	-	-	+	+
<i>C. albicans</i>	-	-	-	+	+
3,0 % Polysorbat 80 + 0,3 % Lezithin + 0,1 % Cystein					
<i>S. aureus</i>	-	-	+	+	+
<i>E. hirae</i>	-	-	+	+	+
<i>E. coli</i>	-	-	-	+	+
<i>P. mirabilis</i>	-	-	+	+	+
<i>P. aeruginosa</i>	-	-	-	+	+
<i>C. albicans</i>	-	-	-	+	+
3,0 % Polysorbat 80 + 3,0 % Saponin + 0,1 % L-Histidin + 0,1 % Cystein					
<i>S. aureus</i>	-	-	+	+	+
<i>E. hirae</i>	-	-	+	+	+
<i>E. coli</i>	-	-	-	+	+
<i>P. mirabilis</i>	-	-	+	+	+
<i>P. aeruginosa</i>	-	-	-	+	+
<i>C. albicans</i>	-	-	-	+	+
3,0 % Polysorbat 80 + 0,3 % Lezithin + 0,1 % L-Histidin + 0,5 % Natrium-Thiosulfat					
<i>S. aureus</i>	-	-	-	+	+
<i>E. hirae</i>	-	-	+	+	+
<i>E. coli</i>	-	-	-	+	+
<i>P. mirabilis</i>	-	-	+	+	+
<i>P. aeruginosa</i>	-	-	-	+	+
<i>C. albicans</i>	-	-	-	+	+

+ = Wachstum der Mikroorganismen
- = kein Wachstum der Mikroorganismen

Tabelle 2

Bestimmung der bakteriziden und levuroziden Wirksamkeit im qualitativen Suspensionsversuch (Standardmethode 8)

Prüfprodukt: Phago´rub Solution SPS
 Prüfdatum: 28.10.2016
 Testtemperatur: 20 ± 1°C
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l, VE-Wasser ad 1000 ml

Incubation of the subcultures at 36 ± 1°C (*C. albicans* at 30 ± 1°C)

Versuch ohne Belastung

Testkeime und Präparateendkonzentrationen (%)	Einwirkzeit		
	15 s	30 s	60 s
<i>P. aeruginosa</i> 1,8 x 10 ⁸ KbE/ml			
100	-	-	-
50	+	+	+
25	+	+	+
10	+	+	+
5	+	+	+
WSH	+	+	+
<i>E. coli</i> 1,51 x 10 ⁸ KbE/ml			
100	-	-	-
50	+	-	-
25	+	+	+
10	+	+	+
5	+	+	+
WSH	+	+	+
<i>P. mirabilis</i> 1,5 x 10 ⁸ KbE/ml			
100	-	-	-
50	+	-	-
25	+	+	+
10	+	+	+
5	+	+	+
WSH	+	+	+

+ = Wachstum der Mikroorganismen
 - = kein Wachstum der Mikroorganismen

Tabelle 3

Bestimmung der bakteriziden und levuroziden Wirksamkeit im quantitativen Suspensionsversuch (Standardmethode 9)

Prüfprodukt: Phago'rub Solution SPS
 Prüfdatum: 12.11.2015
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l, VE-Wasser ad 1000 ml
 Testsuspension: *S. aureus* ATCC 6538 1,7 x 10⁸ KbE/ml
 Testtemperatur: 20 ± 1°C
 Belastung: 0,3 % Albumin + 0,3 % Schafbluterythrozyten

Konzentration (%)	Reduktionsfaktoren (lg) nach Einwirkzeit in									
	15 s			30 s			60 s			
	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	
Kontrolle 1	10 ⁻⁴	228			168			145		
	10 ⁻⁵	18	6,26		14	6,15		15	6,18	
Kontrolle 2	10 ⁻¹							154	3,19	
	10 ⁻²							12		
Kontrolle 3	10 ⁻¹							164		
	10 ⁻²							14	3,15	
80	10 ⁰	0	0	6,26	0	0	6,15	0	0	6,18
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
50	10 ⁰	>330			>330			21	1,32	4,85
	10 ⁻¹	>330			>330			1		
	10 ⁻²	>330			34	3,53	2,62	0		
	10 ⁻³	52	4,72	1,54	2			0		
25	10 ⁰	>330			>330			>330		
	10 ⁻¹	>330			>330			>330		
	10 ⁻²	>330			>330			>330		
	10 ⁻³	>330	>5,48	<0,78	>330	>5,48	<0,67	>330	>5,48	<0,66

Tabelle 3a

Prüfung auf GLEICHWERTIGKEIT im quantitativen Suspensionsversuch (Standardmethode 9)

Prüfprodukt: Phago`rub gel SPS / Phago`rub Solution SPS
 Prüfdatum: 25.02.2016
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l,
 VE-Wasser ad 1000 ml
 Testsuspension: *S. aureus* ATCC 6538 2,3 x 10⁸ KbE/ml
 Testtemperatur: 20 ± 1°C
 Belastung: 0,3 % Albumin + 0,3 % Schafbluterythrozyten

Konzentration Produkt	Reduktionsfaktoren (lg) nach Einwirkzeit in									
	15 s			30 s			60 s			
	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	
Kontrolle 1	10 ⁻⁴ 10 ⁻⁵	162 19			138 24			157 18		
Kontrolle 2 Phago`rub gel SPS	10 ⁻¹ 10 ⁻²						181 18			
Kontrolle 2 Phago`rub Solution SPS	10 ⁻¹ 10 ⁻²						198 20			
Kontrolle 3	10 ⁻¹ 10 ⁻²						180 18			
80 % Phago`rub gel SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	0 0 0 0	0	6,28	0 0 0 0	0	6,38	0 0 0 0	0	6,26
80 % Phago`rub Solution SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	0 0 0 0	0	6,28	0 0 0 0	0	6,38	0 0 0 0	0	6,26
50 % Phago`rub gel SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	>330 >330 >330 35			>330 49 4 0	2,69	3,69	65 4 0 0	1,81	4,45
50 % Phago`rub Solution SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	>330 >330 85 10	3,93	2,35	>330 24 2 0	2,38	4,00	27 1 0 0	1,43	4,83
25 % Phago`rub gel SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	>330 >330 >330 >330			>330 >330 >330 >330			>330 >330 >330 >330		
25 % Phago`rub Solution SPS	10 ⁰ 10 ⁻¹ 10 ⁻² 10 ⁻³	>330 >330 >330 >330	>5,48	<0,80	>330 >330 >330 >330	>5,48	<0,90	>330 >330 >330 >330	>5,48	<0,78

Tabelle 4

Bestimmung der bakteriziden und levuroziden Wirksamkeit im quantitativen Suspensionsversuch (Standardmethode 9)

Prüfprodukt: Phago´rub Solution SPS
 Prüfdatum: 12.11.2015
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l, VE-Wasser ad 1000 ml
 Testsuspension: *E. hirae* ATCC 10541 1,7 x 10⁸ KbE/ml
 Testtemperatur: 20 ± 1°C
 Belastung: 0,3 % Albumin + 0,3 % Schafbluterythrozyten

Konzentration (%)	Reduktionsfaktoren (lg) nach Einwirkzeit in									
	15 s			30 s			60 s			
	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	
Kontrolle 1	10 ⁻⁴ 10 ⁻⁵	146 12	6,16		113 16	6,20		112 10	6,05	
Kontrolle 2	10 ⁻¹ 10 ⁻²							278 19	3,28	
Kontrolle 3	10 ⁻¹ 10 ⁻²							289 20	3,30	
80	10 ⁰	0	0	6,16	0	0	6,20	0	0	6,05
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
50	10 ⁰	0	0	6,16	0	0	6,20	0	0	6,05
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
25	10 ⁰	>330			>330			>330		
	10 ⁻¹	>330			>330			>330		
	10 ⁻²	>330			>330			>330		
	10 ⁻³	>330	>5,48	<0,68	>330	>5,48	<0,72	>330	>5,48	<0,57

Tabelle 5

Bestimmung der bakteriziden und levuroziden Wirksamkeit im quantitativen Suspensionsversuch (Standardmethode 9)

Prüfprodukt: Phago´rub Solution SPS
 Prüfdatum: 12.11.2015
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l, VE-Wasser ad 1000 ml
 Testsuspension: *P. aeruginosa* ATCC 15442 2,4 x 10⁸ KbE/ml
 Testtemperatur: 20 ± 1°C
 Belastung: 0,3 % Albumin + 0,3 % Schafbluterythrozyten

Konzentration (%)	Reduktionsfaktoren (lg) nach Einwirkzeit in									
	15 s			30 s			60 s			
	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	
Kontrolle 1	10 ⁻⁴ 10 ⁻⁵	164 14	6,15		114 9	6,06		94 10	5,97	
Kontrolle 2	10 ⁻¹ 10 ⁻²							180 16	3,20	
Kontrolle 3	10 ⁻¹ 10 ⁻²							160 14	3,15	
80	10 ⁰	0	0	6,15	0	0	6,06	0	0	5,97
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
50	10 ⁰	0	0	6,15	0	0	6,06	0	0	5,97
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
25	10 ⁰	>330			>330			>330		
	10 ⁻¹	>330			>330			>330		
	10 ⁻²	>330			>330			>330		
	10 ⁻³	130	5,11	1,04	104	5,02	1,04	98	4,99	0,98

Tabelle 6

Bestimmung der bakteriziden und levuroziden Wirksamkeit im quantitativen Suspensionsversuch (Standardmethode 9)

Prüfprodukt: Phago-rub Solution SPS
 Prüfdatum: 12.11.2015
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l, VE-Wasser ad 1000 ml
 Testsuspension: *C. albicans* ATCC 10231 2,4 x 10⁷ KbE/ml
 Testtemperatur: 20 ± 1°C
 Belastung: 0,3 % Albumin + 0,3 % Schafbluterythrozyten

Konzentration (%)	Reduktionsfaktoren (lg) nach Einwirkzeit in									
	15 s			30 s			60 s			
	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	KbE/ml	lg KbE	RF	
Kontrolle 1	10 ⁻⁴ 10 ⁻⁵	22 2	5,34		23 2	5,36		21 2	5,32	
Kontrolle 2	10 ⁻¹ 10 ⁻²							173 14	3,15	
Kontrolle 3	10 ⁻¹ 10 ⁻²							170 19	3,28	
80	10 ⁰	0	0	5,34	0	0	5,36	0	0	5,32
	10 ⁻¹	0			0			0		
	10 ⁻²	0			0			0		
	10 ⁻³	0			0			0		
50	10 ⁰	43	1,63	3,71	8	0,90	4,46	0	0	5,32
	10 ⁻¹	2			1			0		
	10 ⁻²	1			0			0		
	10 ⁻³	0			0			0		
25	10 ⁰	>330			>330			>330		
	10 ⁻¹	>330			>330			>330		
	10 ⁻²	>330			>330			>330		
	10 ⁻³	170	5,23	0,11	190	5,28	0,08	200	5,30	0,02

Tabelle 7

Hygienische Händedesinfektion nach DIN EN 1500
Referenz-Händedesinfektion – Versuchsergebnisse

Produkt: Propan-2-ol 60 % vol.
 Anwendung: 3 ml/30 s einreiben, einmal wiederholen
 Prüfdatum: 10.02.2016 und 11.02.2016
 Prüfkeim: *E. coli* K 12 NCTC 10538
 Mittlere Keimzahl in der Kontaminationsflüssigkeit: $4,6 \times 10^8$ KbE/ml und $3,5 \times 10^8$ KbE/ml

Proband		Anzahl KbE je Platte aus Verdünnung 10^x					
Nummer	Hand links oder rechts	Vorwerte			Nachwerte		
		10^{-3}	10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	l	>330	<u>164*</u>	<u>15*</u>	<u>9</u>	0	0
	r	>330	<u>172*</u>	<u>29*</u>	<u>14</u>	2	0
2	l	>330	<u>86*</u>	<u>17*</u>	<u>3</u>	0	0
	r	>330	<u>93</u>	<u>10</u>	<u>0</u>	<u>6</u>	<u>0</u>
3	l	>330	>330	<u>40</u>	<u>50</u>	<u>3</u>	<u>3</u>
	r	>330	>330	<u>43</u>	<u>48</u>	<u>10</u>	<u>1</u>
4	l	>330	<u>66</u>	<u>11</u>	>330	<u>31</u>	<u>4</u>
	r	>330	<u>114</u>	<u>10</u>	>330	<u>32</u>	<u>3</u>
5	l	0	0	0	<u>10</u>	<u>4</u>	<u>0</u>
	r	0	0	0	<u>5</u>	<u>0</u>	<u>0</u>
6	l	>330	<u>248*</u>	<u>25*</u>	<u>7</u>	<u>1</u>	<u>0</u>
	r	>330	>330	<u>40</u>	<u>21</u>	<u>1</u>	<u>0</u>
7	l	>330	<u>28</u>	<u>2</u>	>330	<u>131</u>	<u>12</u>
	r	>330	<u>97*</u>	<u>14*</u>	>330	<u>144*</u>	<u>18*</u>
8	l	>330	<u>149*</u>	<u>14*</u>	>330	>330	<u>96</u>
	r	>330	<u>84</u>	<u>13</u>	>330	>330	<u>84</u>
9	l	>330	<u>148*</u>	<u>18*</u>	>330	<u>99</u>	<u>10</u>
	r	>330	<u>104*</u>	<u>19*</u>	<u>37</u>	<u>2</u>	<u>0</u>
10	l	>330	<u>133*</u>	<u>14*</u>	>330	<u>143*</u>	<u>21*</u>
	r	>330	<u>134*</u>	<u>14*</u>	>330	>330	<u>63</u>
11	l	>330	<u>214*</u>	<u>40*</u>	<u>15</u>	<u>0</u>	<u>0</u>
	r	>330	<u>134*</u>	<u>17*</u>	<u>7</u>	<u>0</u>	<u>0</u>
12	l	>330	<u>74</u>	<u>6</u>	<u>22</u>	<u>1</u>	<u>0</u>
	r	>330	<u>67</u>	<u>7</u>	<u>90*</u>	<u>14*</u>	<u>0</u>
13	l	>330	<u>68</u>	<u>10</u>	<u>230*</u>	<u>18*</u>	<u>2</u>
	r	>330	<u>110</u>	<u>9</u>	>330	<u>26</u>	<u>4</u>
14	l	>330	<u>138*</u>	<u>14*</u>	<u>2</u>	<u>0</u>	<u>0</u>
	r	>330	<u>137*</u>	<u>19*</u>	<u>0</u>	<u>0</u>	<u>0</u>
15	l	>330	<u>51</u>	<u>8</u>	<u>22</u>	<u>0</u>	<u>0</u>
	r	<u>125*</u>	<u>17*</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
16	l	>330	<u>61</u>	<u>3</u>	<u>151</u>	<u>10</u>	<u>0</u>
	r	>330	<u>41</u>	<u>6</u>	<u>8</u>	<u>0</u>	<u>0</u>
17	l	>330	<u>100</u>	<u>6</u>	<u>1</u>	<u>0</u>	<u>0</u>
	r	>330	<u>136*</u>	<u>15*</u>	<u>5</u>	<u>0</u>	<u>0</u>
18	l	>330	>330	<u>34</u>	<u>17</u>	<u>4</u>	<u>0</u>
	r	>330	>330	<u>29</u>	<u>7</u>	<u>1</u>	<u>0</u>
19	l	>330	>330	<u>30</u>	<u>3</u>	<u>2</u>	<u>0</u>
	r	>330	<u>254*</u>	<u>25*</u>	<u>2</u>	<u>0</u>	<u>0</u>
20	l	<u>194*</u>	<u>15*</u>	<u>2</u>	<u>37</u>	<u>4</u>	<u>0</u>
	r	<u>182</u>	<u>12</u>	<u>1</u>	<u>123*</u>	<u>22*</u>	<u>0</u>
21	l	<u>93</u>	<u>11</u>	<u>2</u>	<u>28</u>	<u>0</u>	<u>0</u>
	r	<u>100</u>	<u>8</u>	<u>1</u>	<u>24</u>	<u>1</u>	<u>0</u>
22	l	>330	>330	<u>41</u>	<u>1</u>	<u>0</u>	<u>0</u>
	r	>330	<u>150</u>	<u>12</u>	<u>92</u>	<u>10</u>	<u>3</u>

unterstrichen = Keimzahl wurde für die weitere Berechnung verwendet
 na = nicht auswertbar
 * zeigt die angrenzenden Verdünnungen an, die zur Berechnung verwendet wurden

Tabelle 8

Hygienische Händedesinfektion nach DIN EN 1500
Versuchsergebnisse

Produkt: Phago'rub gel SPS
 Anwendung: Feuchthalten der Hände über 30 s
 Prüfdatum: 10.02.2016 und 11.02.2016
 Prüfkeim: *E. coli* K 12 NCTC 10538
 Mittlere Keimzahl in der Kontaminationsflüssigkeit: $4,6 \times 10^8$ KbE/ml und $3,5 \times 10^9$ KbE/ml

Proband		Anzahl KbE je Platte aus Verdünnung 10^x					
Nummer	Hand links oder rechts	Vorwerte			Nachwerte		
		10^3	10^4	10^5	10^0	10^{-1}	10^{-2}
1	l	>330	<u>165*</u>	<u>19*</u>	<u>57</u>	6	0
	r	>330	<u>164*</u>	<u>17*</u>	<u>29</u>	0	0
2	l	>330	<u>214*</u>	<u>23*</u>	6	0	0
	r	>330	>330	<u>40</u>	<u>39</u>	3	0
3	l	>330	>330	<u>31</u>	>330	<u>40</u>	3
	r	>330	>330	<u>30</u>	>330	<u>40</u>	5
4	l	>330	<u>226*</u>	<u>27*</u>	<u>214*</u>	<u>18*</u>	3
	r	>330	<u>247*</u>	<u>28*</u>	>330	<u>124</u>	<u>13</u>
5	l	170	17	1	235	24	0
	r	112	15	0	59	6	0
6	l	>330	<u>248*</u>	<u>22*</u>	<u>25</u>	5	0
	r	>330	>330	<u>35</u>	<u>53</u>	3	0
7	l	>330	<u>56</u>	6	>330	<u>101</u>	8
	r	>330	<u>63</u>	7	>330	<u>117</u>	9
8	l	>330	<u>224*</u>	<u>36*</u>	>330	>330	<u>132</u>
	r	>330	<u>189*</u>	<u>14*</u>	>330	>330	<u>64</u>
9	l	>330	<u>172*</u>	<u>17*</u>	<u>102</u>	11	2
	r	>330	>330	<u>38</u>	>330	<u>89</u>	7
10	l	>330	<u>246*</u>	<u>35*</u>	>330	<u>86</u>	12
	r	>330	<u>204*</u>	<u>30*</u>	>330	>330	<u>62</u>
11	l	>330	<u>294*</u>	<u>28*</u>	<u>59</u>	6	0
	r	>330	<u>221*</u>	<u>33*</u>	>330	<u>28</u>	6
12	l	>330	<u>124*</u>	<u>15*</u>	<u>2</u>	0	0
	r	>330	<u>146*</u>	<u>27*</u>	<u>144*</u>	<u>25*</u>	2
13	l	>330	<u>160</u>	9	>330	<u>83</u>	8
	r	>330	<u>112</u>	12	65	5	1
14	l	>330	<u>176*</u>	<u>17*</u>	<u>6</u>	0	0
	r	>330	<u>143*</u>	<u>14*</u>	<u>7</u>	0	0
15	l	>330	<u>91</u>	9	<u>7</u>	0	0
	r	172*	<u>24*</u>	2	0	0	0
16	l	>330	<u>140*</u>	<u>14*</u>	<u>192</u>	10	2
	r	>330	<u>72</u>	4	<u>74</u>	6	0
17	l	>330	<u>126*</u>	<u>15*</u>	0	0	0
	r	>330	<u>95</u>	12	0	0	0
18	l	>330	<u>103</u>	10	<u>30</u>	5	1
	r	>330	<u>68</u>	11	<u>18</u>	1	0
19	l	>330	<u>130</u>	12	0	0	0
	r	>330	<u>64</u>	9	4	2	0
20	l	<u>154*</u>	<u>16*</u>	0	<u>1</u>	0	0
	r	<u>188*</u>	<u>18*</u>	1	<u>7</u>	0	0
21	l	>330	<u>31</u>	2	<u>52</u>	10	0
	r	<u>136*</u>	<u>15*</u>	2	<u>21</u>	2	1
22	l	>330	>330	<u>40</u>	<u>116</u>	10	2
	r	>330	>330	<u>29</u>	>330	<u>216*</u>	<u>25*</u>

unterstrichen = Keimzahl wurde für die weitere Berechnung verwendet
 na = nicht auswertbar
 * zeigt die angrenzenden Verdünnungen an, die zur Berechnung verwendet wurden

Tabelle 9

Hygienische Händedesinfektion nach DIN EN 1500

 Liste der berechneten lg-Werte (Mittelwerte aus linker und rechter Hand) und
 lg-Reduktionsfaktoren aus den Versuchsergebnissen

Proband	Chronologische Abfolge	Referenz Händedesinfektion (RP) (Propan-2-ol, Volumenkonzentration 60 %)			Händedesinfektion mit dem zu prüfenden Produkt (PP)		
		lg Vorwerte	lg Nachwerte	lg Reduktion	lg Vorwerte	lg Nachwerte	lg Reduktion
1	PP→RP	6,24	1,05	5,19	6,22	1,61	4,61
2	RP→PP	5,97	0,24	5,73	6,47	1,18	5,29
3	PP→RP	6,62	1,69	4,93	6,48	2,60	3,88
4	RP→PP	5,94	2,50	3,44	6,38	2,71	3,67
5	PP→RP	nb	nb	nb	nb	nb	nb
6	RP→PP	6,50	1,08	5,42	6,47	1,56	4,91
7	PP→RP	5,73	3,14	2,59	5,77	3,04	2,73
8	RP→PP	6,05	3,95	2,10	6,32	3,96	2,36
9	PP→RP	6,11	2,28	3,83	6,41	2,48	3,93
10	RP→PP	6,13	3,49	2,64	6,37	3,36	3,01
11	PP→RP	6,25	1,01	5,24	6,41	2,11	4,30
12	RP→PP	5,85	1,66	4,19	6,15	1,24	4,91
13	PP→RP	5,94	2,38	3,56	6,13	2,37	3,76
14	RP→PP	6,15	0,15	6,00	6,20	0,81	5,39
15	PP→RP	5,41	0,67	4,74	5,60	0,42	5,18
16	RP→PP	5,70	1,54	4,16	6,00	2,08	3,92
17	PP→RP	6,07	0,35	5,72	6,04	0,00	6,04
18	RP→PP	6,50	1,04	5,46	5,92	1,37	4,55
19	PP→RP	6,44	0,39	6,05	5,96	0,30	5,66
20	RP→PP	5,27	1,84	3,43	5,23	0,42	4,81
21	PP→RP	4,98	1,41	3,57	5,31	1,52	3,79
22	RP→PP	6,39	0,98	5,41	6,53	2,70	3,83

RP → PP = Abfolge: zuerst RP, dann PP
 PP → RP = Abfolge: zuerst PP, dann RP
 nb = nicht berechnet

Tabelle 9 (Fortsetzung)

Proband	Chronologische Abfolge	Referenz Händedesinfektion (RP) (Propan-2-ol, Volumenkonzentration 60 %)			Händedesinfektion mit dem zu prüfenden Produkt (PP)		
		Ig Vorwerte	Ig Nachwerte	Ig Reduktion	Ig Vorwerte	Ig Nachwerte	Ig Reduktion
X	gesamt	6,01	1,56	4,45	6,11	1,80	4,31
S		0,42	1,07	1,20	0,38	1,08	0,96
NN		21	21	21	21	21	21
X	RP → PP	6,04	1,68	4,36	6,19	1,94	4,24
S		0,36	1,22	1,34	0,37	1,11	0,97
NN		11	11	11	11	11	11
X	PP → RP	5,98	1,44	4,54	6,03	1,65	4,39
S		0,49	0,93	1,10	0,38	1,07	1,00
NN		10	10	10	10	10	10
RP → PP = Abfolge: zuerst RP, dann PP				X = Mittelwert			
PP → RP = Abfolge: zuerst PP, dann RP				s = Standardabweichung			
NN = Anzahl der Werte (Probanden)							

Differenz der Mittelwerte der Reduktionsfaktoren (RP → PP): $4,36 - 4,24 = 0,12$

Differenz der Mittelwerte der Reduktionsfaktoren (PP → RP): $4,54 - 4,39 = 0,15$

Absolute Differenz der Differenzen: $\text{abs}\{(0,12)-(0,15)\} = 0,03$

Überprüfung der Annahmekriterien

- ein vollständiger Satz von Ergebnissen von 21 Probanden steht zur Verfügung (demzufolge mehr als die Mindestzahl von 18);
- Mittelwert der Ig-Vorwerte für RP = 6,01 und für PP = 6,11 (demzufolge beide größer als 5,00);
- bei RP dürfen nicht mehr als 3 einzelne Ig-Reduktionen kleiner als 3,00 auftreten, dabei ist RP = 3 (demzufolge ist der Wert nicht größer als 3);
- bei der Gruppe mit der Abfolge RP → PP beträgt die Differenz von log R: $4,36 - 4,24 = 0,12$,
bei der Gruppe mit der Abfolge PP → RP beträgt die Differenz von log R: $4,54 - 4,39 = 0,15$,
die absolute Differenz der mittleren Differenzen beträgt: $\text{abs}\{(0,12)-(0,15)\} = 0,03$ (demzufolge weniger als 2,00);
- Die grundlegenden Grenzwerte (s. Tabelle nächste Seite) wurden eingehalten;
Die Ergebnisse aus den Tabellen 7 und 8 wurden zur Berechnung der gewichteten mittleren Keimzahlen verwendet, alle Quotienten gewichteter mittlerer Keimzahlen liegen zwischen 5 und 15.

Alle Annahmekriterien sind erfüllt.

Tabelle 9 (Fortsetzung)

Grundlegende Grenzwerte

Prüfprodukt: Phago´rub Solution SPS
 Prüfdatum: 18.02.2016
 Neutralisationsmittel: Polysorbat 80 30 g/l, Saponin 30 g/l, L-Histidin 1 g/l, Cystein 1 g/l,
 VE-Wasser ad 1000 ml
 Testorganismus: *E. coli* K 12 NCTC 10538
 Inkubation: 36 ± 1°C, 48 h

	Verd.	V _{C1}	V _{C2}	Ergebnis	Grenzwert	Grenzwert eingehalten	
						ja	nein
Testsuspension (N)	10 ⁻⁶ 10 ⁻⁷	256 34	220 18	N = 2,4 x 10 ⁸	1,5 x 10 ⁸ ≤ N ≤ 5,0 x 10 ⁸	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Validierungssuspension (N _V)	10 ⁻¹	72	56	N _V = 6,4 x 10 ²	3,0 x 10 ² ≤ N ≤ 1,6 x 10 ³	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Validierungssuspension (N _{VO})				N _{VO} = N _V /10 = 64	30 ≤ N _{VO} ≤ 160	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Validierungssuspension (N _{VB})	10 ⁻³	50	68	N _{VB} = 5,9 x 10 ⁵	3,0 x 10 ⁴ ≤ N ≤ 1,6 x 10 ⁵	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Kontrolle der Neutralisation (B) / Neutralisationszeit: 10 s		102	84	B = 93	B ≥ 0,0005 x N _{VB}	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Verfahrensvalidierung (C) Testkonzentration: 80 % Einwirkzeit: 30 s Belastung: 20 % CSS Neutralisationszeit: 10 s		80	76	C = 78	C ≥ 0,5 x N _{VO}	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Tabelle 10

Hygienische Händedesinfektion nach DIN EN 1500

Berechnung der einzelnen Differenzen von lg R von RP - PP

Proband	lg-Reduktion (R)		
	Referenzverfahren (RP)	Produkt-Prüfverfahren (PP)	Differenz RP - PP
1	5,19	4,61	0,58
2	5,73	5,29	0,44
3	4,93	3,88	1,05
4	3,44	3,67	-0,23
5	nb	nb	nb
6	5,42	4,91	0,51
7	2,59	2,73	-0,14
8	2,10	2,36	-0,26
9	3,83	3,93	-0,10
10	2,64	3,01	-0,37
11	5,24	4,30	0,94
12	4,19	4,91	-0,72
13	3,56	3,76	-0,20
14	6,00	5,39	0,61
15	4,74	5,18	-0,44
16	4,16	3,92	0,24
17	5,72	6,04	-0,32
18	5,46	4,55	0,91
19	6,05	5,66	0,39
20	3,43	4,81	-1,38
21	3,57	3,79	-0,22
22	5,41	3,83	1,58

Tabelle 11

Hygienische Händedesinfektion nach DIN EN 1500

Sortierung der einzelnen Differenzen und Berechnungen der oberen 97,5 %-Vertrauensgrenzen nach Hodges-Lehmann

Sortierte Differenzen		Mittlere paarweise Differenzen $(d_i + d_{ii})/2$												
		1,58	1,05	0,94	0,91	0,61	0,58	0,51	0,44	0,39	0,24	-0,1		
1	1,58	1,58 ¹												
2	1,05	1,32 ²	1,05 ⁸											
3	0,94	1,26 ³	0,99 ¹¹	0,94 ¹³										
4	0,91	1,25 ⁴	0,98 ¹²	0,93 ¹⁴	0,91 ¹⁶									
5	0,61	1,10 ⁵	0,83 ¹⁷	0,77 ²⁰	0,76 ²²	0,61 ⁴¹								
6	0,58	1,08 ⁶	0,82 ¹⁸	0,76 ²¹	0,75 ²³	0,60 ⁴²	0,58 ⁴⁵							
7	0,51	1,05 ⁷	0,78 ¹⁹	0,73 ²⁶	0,71 ²⁹	0,56 ⁴⁷	0,55 ⁴⁸	0,51 ⁵¹						
8	0,44	1,01 ⁹	0,74 ²⁵	0,69 ³¹	0,68 ³⁴	0,52 ⁴⁹	0,51 ⁵⁰	0,48 ⁵⁵	0,44 ⁵⁸					
9	0,39	0,99 ¹⁰	0,72 ²⁸	0,67 ³⁵	0,65 ³⁸	0,50 ⁵²	0,49 ⁵³	0,45 ⁵⁷	0,42	0,39				
10	0,24	0,91 ¹⁵	0,65 ³⁷	0,59 ⁴³	0,58 ⁴⁴	0,43	0,41	0,38	0,34	0,32	0,24			
11	-0,10	0,74 ²⁴	0,48 ⁵⁴	0,42	0,41	0,26	0,24	0,21	0,17	0,15	0,07	-0,10		
12	-0,14	0,72 ²⁷	0,46 ⁵⁶	0,40	0,39	0,24	0,22	0,19	0,15	0,13	0,05	-0,12		
13	-0,20	0,69 ³⁰	0,43	0,37	0,36	0,21	0,19	0,16	0,12	0,10	0,02	-0,15		
14	-0,22	0,68 ³²	0,42	0,36	0,35	0,20	0,18	0,15	0,11	0,09	0,01	-0,16		
15	-0,23	0,68 ³³	0,41	0,36	0,34	0,19	0,18	0,14	0,11	0,08	0,01	-0,17		
16	-0,26	0,66 ³⁵	0,39	0,34	0,33	0,17	0,16	0,13	0,09	0,06	-0,01	-0,18		
17	-0,32	0,63 ³⁹	0,37	0,31	0,30	0,15	0,13	0,10	0,06	0,04	-0,04	-0,21		
18	-0,37	0,61 ⁴⁰	0,34	0,28	0,27	0,12	0,11	0,07	0,03	0,01	-0,07	-0,24		
19	-0,44	0,57 ⁴⁶	0,31	0,25	0,24	0,09	0,07	0,04	0,00	-0,02	-0,10	-0,27		
20	-0,72	0,43 ⁵⁹	0,16	0,11	0,09	-0,06	-0,07	-0,11	-0,14	-0,17	-0,24	-0,41		
21	-1,38	0,43 ⁶⁰	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	-0,36	

Der Medianwert ist der 11. Wert: -0,10.

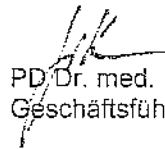
Die kleinen Exponenten geben die Ränge an.

Test auf Nicht-Unterlegenheit


Die mittleren paarweisen Differenzen, die den Medianwert (hier: -0,10) nicht überschreiten, wurden berechnet. Der kritische Wert für Vorzeichenrangtests für gepaarte Stichproben nach Wilcoxon ist 59, bei einem Eingangswert von $n = 21$ und einem einseitigen Signifikanzniveau von 0,025. Folglich ist $c = 59 + 1 = 60$. Die paarweisen Differenzen wurden in absteigender Reihenfolge sortiert. Der 60. Wert ist 0,43. Folglich ist die einseitige obere 97,5 %-Vertrauensgrenze für die Differenz der lg R zwischen RP und PP 0,43, was geringer ist als die vereinbarte Grenze für Unterlegenheit von 0,6. Deshalb wird die Hypothese der Unterlegenheit von PP verworfen und es kann die Schlussfolgerung gezogen werden, dass die zu prüfende Zubereitung PP RP nicht unterlegen ist.

Produkt: Phago´rub gel SPS / Phago´rub Solution SPS

Hinweis: Die Prüfergebnisse beziehen sich ausschließlich auf den genannten Prüfgegenstand. Auszugsweise Wiedergabe dieses Berichtes nur mit schriftlicher Genehmigung des Instituts für Krankenhaushygiene und Infektionskontrolle, Gießen.



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