Final test report C05ML260
submitted to

AS Chemi-Pharm
Põllu 132
Tallinn 10917
Estonia

Evaluation of the effectiveness of

Chemisept G/Chemisept G Color

Against

Bovine Viral Diarrhea Virus (BVDV) (surrogate of HCV)

in
a quantitative suspension test

Test method according to the guideline of BGA and DVV

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2005-12-30

5.6 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).

6. Results
In parallel with the inactivation tests, cytotoxicity of Chemisept G/G COLOR (80,0 %) and 0.7 % formaldehyde solution was measured. The formaldehyde solution was toxic for the *KOP-R cells* in the 1:1.000-dilutions. This corresponded to a $log_{10}CD_{50}$/mL of 4.50. (Table 1)
Examinations also showed that without treatment Chemisept G/G Color (80,0 %) had a $log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:100 and 1:10-dilutions),where-as no cytotoxitc effect after treatment with the colomns was measured resulting in a $log_{10}CD_{50}$/mL of ≤ 1,50 (Table 1).
These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated BVDV could be determined.
Virus titres without treatment with MicroSpin™ S-400 HR columns were 5,50 (assay without protein load), 5,88 (assay with BSA) and 5,75 $log_{10}CD_{50}$/mL (assay with FCS) (data not shown in table)

Results of inactivation tests are found in table 2. Formaldehyde (0.7 %) reduced the BVDV titre after 5 minutes by ≥ 0,25 log_{10}-steps. After 15, 30 and 60 minutes identical reduction factors werw ≥ 1,25 (Table 2).

Chemisept G/G Color was examined undeluted .Due the addition of virus suspension and interfering substance a test concsntration of 80,0 % resulted.Exposure time were 30, 60,120 seconds.

Testing Chemisept G/G Color undiluted, an effisncy was measured after exposure time 30 s (Table 2). At this time , no BVDV was detecable any longer. The reduction factors were ≥ 4.25 (assays without soil load) and ≥ 4.25 (assays with BSA) and ≥ 4,38 (assays with FCS). This

corresponds in all cases to an inactivation of ≥99,99%. According to the guidline of BGA/DVV, a disinfectant or a disinfectant solution at a particular concentrations is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four log_{10}-steps.

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 disinfectant Chemisept G/G Color for inactivation of BVDV (surrogate of hepatitis C virus) as follows:

undiluted **30 s**

Dr. J. Steinmann

Project number: C05ML260

Final report submitted to

CHEMI – PHARM AS
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Evaluation of the effectiveness of

CHEMISEPT G

against Adenovirus type 5

Test method according to guideline of BGA and DVV

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2006-03-12

4.4 Determination of cytotoxicity

For determination of cytotoxicity of the disinfectant, two parts by volume of PBS were mixed with eight parts by volume of the disinfectant, diluted with ice-cold DMEM and inoculated into cell culture. These tests were also performed with interfering substances.

4.5 Calculation of virucidal effectiveness

The virucidal effectiveness 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).

5. Results

In parallel with the inactivation tests, cytotoxicity of the 0.7% formaldehyde solution and of the hand disinfectant CHEMISEPT G (80.0%) was measured (Table 1). The formaldehyde solution was toxic for the A549 cells in the 1:1000 dilutions. This corresponded to a $\log_{10}CD_{50}$/mL of 4.50. Examinations showed that the hand disinfectant (80.0%) had a $\log_{10}CD_{50}$/mL of 3.50 (cytotoxicity in the 1:100 dilutions; table1).
These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated adenovirus is determined.

Results of inactivation tests are found in table 2 (raw data see appendix).

Formaldehyde (0.7%) reduced the adenovirus titre after 5 and 15 minutes by 1.00 and 2.12 $\log_{10}$ steps. After 30 and 60 minutes, reduction factors were ≥ 3.62 and ≥ 4.00 (Table 2).

The hand disinfectant CHEMISEPT G was undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 0.5, 1.0, 2.0 and 3.0 minutes.
Testing CHEMISEPT G undiluted, after an exposure time of 30 s a reduction of the virus titre was measured (table 2). The reduction factors were ≥ 5.00 (assay without soil load), ≥ 5.00 (assay with BSA) and ≥ 5.13 (assay with FCS). These values correspond to an inactivation of ≥ 99.999% meaning virucidal efficacy. According to the guideline of BGA/DVV, a disinfectant or a disinfectant solution at a particular concentration is having virucidal efficacy if within the recommended exposure period the titre is reduced at least by four $\log_{10}$.

Due to the lack of guidelines simulating practical conditions, results of this quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of adenovirus as follows:

undiluted	**30 s**

Dr. J. Steinmann

MIKROLAB

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Ihre Zeichen, Ihre Nachrichten vom | Unsere Zeichen, unsere Nachricht vom | Bremen ,den 2007-10-21

Virus-inactivating properties of the hand disinfectant Chemisept G of AS Chemi–Pharm

This summary is based on the following expert reports of MikroLab GmbH for the hand disinfectant Chemisept G of AS Chemi–Pharm:

vaccinia virus expert report 2006-02-18

BVDV expert report 2005-12-30

The following concentration and exposure time are necessary for inactivation of the two test viruses:

undiluted 30 s

in order to achieve a four $\log_{10}$ reduction (inactivation $\geq$ 99.99%) in a quantitative suspension test according to the guideline of the Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V. (DVV, German Association for the Control of Virus Diseases) and the Robert Koch-Institute (RKI).

After evaluation with vaccinia virus and Bovine Viral Diarrhea Virus (BVDV, surrogate of Hepatitis C Virus) the hand disinfectant Chemisept G can be declared as having "limited virucidal" properties according to a recommendation of an expert committee of RKI (Bundesgesundheitsbl 2004, 47: 62-66) and is thus able to inactivate all enveloped viruses.
Therefore, after successful experiments with the two above mentioned enveloped viruses the hand disinfectant Chemisept G is also effective against the so-called blood-borne viruses including HBV, HCV and HIV.

Dr. Jochen Steinmann

Tervisekaitseinspektsioon • Health Protection Inspectorate
Mikrobioloogia Kesklabor • Central Laboratory of Microbiology

Akrediteeritud L013

P.1/8

TEST REPORT 4580

Name of the product: CHEMISEPT G

Manufacturer: Chemi-Pharm Estonia Ltd

Sampled by: Chemi-Pharm Ltd., head of the laboratory M. Millner, +372 6778807

Place of sampling: Chemi-PharmLTD. Põllu 132 Tallinn 10917 Estonia

Date of sampling: 19.12.2005

Date and hour of receipt: 20.12.2005 at 08.35

Characteristics of the sample: No deviation the packaging and the labelling

Storage condition: room temperature and darkness

Used method of the examination: prEN 12791:2003* Chemical disinfectants and antiseptics – Surgical hand disinfectants – Test method and requirements (phase 2/step 2)

Neutralizers: According to prEN 12054:2001: polysorbate 80 (30 ml), saponin (30 g), histidine (1 g), cysteine (1 g) per litre diluent.

Results of the examination: see annexes 1-7

Conclusion: According to prEN 12791:2003, the product CHEMISEPT G is suitable for surgical hand disinfection in the following application: rub as many volumes of 3 ml onto the hands as is necessary to keep them wet for 3 min.

The product has also been tested for a sustained effect as there was the explicit claim for this feature. The test results demonstrated that reduction factors of the immediate effect and also of the 3 hours effect between Chemisept G and propan-1-ol 60 % (volume concentration). was not significantly different.

06.01.2006

TERVISEKAITSEINSPEKTSIOON MIKROBIOLOOGIA KESKLABOR TALLINN

Microbiologist

* – Did not accredited by EAC

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Chemi-Pharm AS
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Hamburg, 12 April 2006

Expert's report

Efficacy testing of the surgical skin disinfectant *CHEMISEPT G* according to EN 14348: 2005

The surgical skin disinfectant *CHEMISEPT G* was tested and evaluated in accordance with EN 14348: 2005. According to test report no. L 06/001 of Dr. Brill + Partner GmbH the test preparation proved to be tuberculocidal under low organic load.

A sufficient effect within the quantitative suspension test according to EN 14348: 2005 against *Mycobacterium terrae* was reached at the following concentration-time relationship:

100 % in 30 seconds.

Dr. Holger Brill

Project number: C05ML260

Final report submitted to

CHEMI – PHARM AS
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Evaluation of the effectiveness of

CHEMISEPT G

against Herpes Simplex Virus type 1

Test method according to guideline of BGA and DVV

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2006-02-23

4.5 Calculation of the virucidal activity

The virucidal effect 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).

5. Results

In parallel with the inactivation tests, cytotoxicity of the 0.7% formaldehyde solution and of the hand disinfectant CHEMISEPT G was measured. The formaldehyde solution was toxic for the BGM cells in the 1:1000 dilutions. This corresponds to a $\log_{10}CD_{50}$/mL of 4.50. Examinations showed that the hand disinfectant (80.0%) had a $\log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:10 dilutions).

These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated HSV type 1 is determined.

Results of inactivation tests are found in table 2 (raw data see appendix). There is no graphic presentation of the results since no kinetics of inactivation is visible.

Formaldehyde (0.7%) reduced the HSV titre after five and 15 minutes by ≥ 2.38 and ≥ 2.63 $\log_{10}$ steps. A reduction factor of ≥ 2.63 was measured after 30 and 60 minutes contact time (table 2).

The hand disinfectant CHEMISEPT G was examined undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 0.5, 1.0, 2.0 and 5.0 minutes.
Testing CHEMISEPT G undiluted (80.0%), after an exposure time of 30 seconds a reduction of the virus titre was measured (table 2). The reduction factors were ≥ 4.63 (assay without soil load), ≥ 4.25 (assay with BSA) and ≥ 4.38 (assay with FCS). At this exposure time, no HSV could be detected in all assays. These values correspond to an inactivation of ≥ 99.99% meaning virus-inactivating properties. According to the guideline of BGA/DVV (1,2) and EN 14476:2005 (6), a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating properties if within the recommended exposure period the titre is reduced at least by four $\log_{10}$.

Due to the lack of guidelines simulating practical conditions, the results of the quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for the inactivation of Herpes Simplex Virus type 1 as follows:

undiluted **30 s**

Dr. J. Steinmann

Tervisekaitseinspektsioon • Health Protection Inspectorate
Mikrobioloogia Kesklabor • Central Laboratory of Microbiology

Akrediteeritud L013

TEST REPORT No 4301 **Koopia**

Name of the product: Chemisept G

Manufactured: Chemi-Pharm Ltd.,

Sampled by: Chemi-Pharm Ltd., M. Milner

Place of sampling: Chemi-Pharm Ltd., Põllu 132, Tallinn10917

Active substance(s) and its/their concentration(s): not indicated

Aim of the examination: detection of disinfecting properties

Date and hour of receipt:23.12.2002 at 09.30

Storage condition: room temperature and darkness

Nature and characteristics of the sample: no deviation the packaging and the labelling

Period of analysis:27.12.-03.01.2005

Aim of the examination: detection of disinfecting properties

Test method: in-house method dilution-neutralization of Central Laboratory of Microbiology*

in-house method dilution-neutralization of Central Laboratory of Microbiology*

Dilutions of tested products were prepared with steriled drinking water from waterworks. Used neutralizer consists polysorbate 80 – 30 g/l, lecithin 3 g/l and L-histidine – 1 g/l.

Conditions of the examination: 20±1°C for application and 37±1°C for incubation

Test results:

Concent-ration	Contact time	*Escheri-chia coli* ATCC 10538	*Staphylo-coccus aureus* ATCC 6538	*Streptcoc-cus faecalis* ATCC 29212	*Pseudomo-nas aeruginosa* ATCC 15442	*Bacillus cereus* ATCC 11778	*Candida albicans* ATCC 10231
Without dilution	30 sec	0	0	0	0	0	0
Growth of tested strains		+	+	+	+	+	+

\+ the growth of the organisms exists

0 the growth of the organisms is absent

The test results applies for the tested sample only

* – EAK poolt akrediteerimata

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Tervisekaitseinspektsioon • Health Protection Inspectorate
Mikrobioloogia Kesklabor • Central Laboratory of Microbiology

Akrediteeritud L013

Conclusion: According to the in-house dilution-neutralization method of Central Laboratory of Microbiology, the tested product „ Chemisept G " had the bactericidal effects without dilution during contact time 30 sek to all referenced strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC27853 and *Candida albicans* ATCC 10231

Microbiologist K. Birk

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Project number: C05ML260

Final report submitted to

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Evaluation of the effectiveness of

CHEMISEPT G

against Polyomavirus SV 40

Test method according to guideline of BGA and DVV

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2006-03-09

6. Results

In parallel with the inactivation tests, cytotoxicity of CHEMISEPT G (80.0%) and formaldehyde (0.7%) was measured. The formaldehyde solution was toxic for the CV-1 cells in the 1:1000 dilutions. This corresponds to a $\log_{10}CD_{50}$/mL of 4.50 (Table 1).
Examinations showed that the hand disinfectant CHEMISEPT G had a $\log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:10 dilution; Table 1).
These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated SV 40 is determined.

Results of inactivation tests are found in table 2. Formaldehyde (0.7%) reduced the SV 40 titre after 5 and 15 minutes by 0.13 $\log_{10}$ steps. After 30 and 60 minutes RF were 0.63 and 1.25 (Table 2).

The hand disinfectant CHEMISEPT G was examined undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times of the inactivation experiments were 3, 5, 15 and 20 minutes.

Testing CHEMISEPT G undiluted against SV 40, an efficacy was measured after an exposure time of five minutes in all assays without and with interfering substances (Table 2). The RF were ≥ 5.13 (assay without protein load), ≥ 4.88 (assay with BSA) and ≥ 5.00 (assay with FCS), respectively. These RF correspond to an inactivation of ≥ 99.999% (assay with BSA ≥ 99.99%) indicating virus-inactivating properties of the test product.
Due to the lack of guidelines simulating practical conditions, results of this quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of SV 40 as follows:

undiluted **5 min**

Dr. J. Steinmann

Final report submitted to

CHEMI – PHARM AS
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Evaluation of the effectiveness of

CHEMISEPT G

against

human rotavirus strain Wa

Test method according to guideline of BGA and DVV

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2006-01-15

6. Results

In parallel with inactivation tests, cytotoxicity of CHEMISEPT G (80.0%) was measured. Examinations showed that the hand disinfectant tested undiluted exhibited a cytotoxic effect at the dilution of 1:10. This means a $\log_{10}CD_{50}$/mL value (analogous to the $\log_{10}TCID_{50}$ value) of 2.50 (Table 1).

These tests to measure the cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated rotavirus is determined.

Results of inactivation tests are found in table 2. CHEMISEPT G was tested undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 30, 60 and 120 seconds.

The hand disinfectant CHEMISEPT G tested undiluted exhibited strong virus-inactivating properties against the test virus. After an exposure time of 30 s no rotavirus virus could be detected any longer. The virus titre reduction was ≥ 4.38 $\log_{10}$-steps. This reduction corresponds to an inactivation of ≥ 99.99% and demonstrates a rotavirus efficacy. According to the guideline of BGA and DVV and also to EN 14476:2005 (5), a disinfectant is considered as having virucidal efficacy if within the recommended exposure time the titre is reduced by four $\log_{10}$-steps.

Due to the lack of guidelines simulating practical conditions, results of the quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of rotavirus as follows:

undiluted 30 s

Bremen, 2006-01-15

- Dr. J. Steinmann -

Project number: C05ML260

Final report submitted to

CHEMI – PHARM AS
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Evaluation of the effectiveness of

CHEMISEPT G

against Vaccinia virus strain Elstree

Test method according to guideline of BGA and DVV

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2006-02-18

6. Results

In parallel with the inactivation tests, cytotoxicity of the 0.7% formaldehyde solution and of the hand disinfectant was measured (Table 1). 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. Examinations showed that the hand disinfectant (80.0%) also had a $\log_{10}CD_{50}$/mL of 3.50 (cytotoxicity in the 1:100 dilutions).

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

Formaldehyde (0.7%) reduced the vaccinia virus titre after 5 and 15 minutes by 1.12 and 1.50 $\log_{10}$ steps. Reduction factors of 1.75 and 2.75 were measured after 30 and 60 minutes contact time (table 2).

Results of inactivation tests are found in table 2. CHEMISEPT G was tested undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. The exposure times were 0.5, 1.0, 2.0 and 5.0 minutes.

The hand disinfectant CHEMISEPT G exhibited a strong virucidal efficacy against the test virus. After an exposure time of 30 seconds no vaccinia virus was detectable any longer. The virus titres were reduced by ≥ 4.25 (assay without interfering substances), ≥ 4.00 (assay with BSA) and ≥ 4.25 $\log_{10}$-steps (assay with FCS). This corresponds in all cases to an inactivation of ≥ 99.99%. According to the guideline of BGA/DVV, 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.

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 CHEMISEPT G for inactivation of vaccinia virus as follows:

undiluted **30 s**

Bremen, 2006-02-18

- Dr. J. Steinmann -

Nordic Tersus
Laboratory

Test report No. 12/2015

EVALUATION OF YEASTICIDAL AND FUNGICIDAL ACTIVITIES (EN 13624)

Name of the product:	**CHEMISEPT G**
Batch number:	LOT 69090215
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA Maris Millner, +372-51-77-090
Date of delivery:	06.04.2015
Date of registration:	06.04.2015
Test material conditions:	no specific features, sample in the manufacturers tare
Storage conditions:	in room temperature, dark
Appearance of the product:	amount 1000 ml
Contact time:	15 sec, clean and dirty conditions
Interfering substance:	3,0 g/l bovine albumin and 3,0 ml/l sheep blood erythrocytes
Test neutralizer:	polysorbate 80, 30 g/l; saponine, 30 g/l; lecithin, 3 g/l
Test organisms:	*Candida albicans* ATCC 10231
Testing method:	**EVS-EN 13624:2013** **Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.**
Testing date:	06.04 – 10.04.2015
Results:	look at appendix 1

ACCREDITED LABORATORY No. ... REPUBLIC OF ESTONIA

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Raul Raudsepp, MSc

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EESTI AKREDITEERIMISKESKUS
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Appendix 1

TEST RESULTS (yeasticidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Candida albicans* ATCC 10231;
Test temperature: +20° C; Incubation temperature: +30° C
Solvents: water;
Interfering substance: 3,0 g/l bovine albumin and 3,0 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015

Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	67+81=148	$\bar{x}$ = 144,5	V_{C1} 15 sek	45+65= 110	$\bar{x}$ = 124, 5	V_{C1}	71+63= 134	$\bar{x}$ = 134	V_{C1} 30 sek	45+16= 61	$\bar{x}$ = 78
V_{C2}	66+75=141		V_{C2} 15 sek	62+77= 139		V_{C2}			V_{C2} 30 sek	42+53= 95	
$30 \leq \bar{x}\ N_{vo} \leq 160$? yes X; no □			$\bar{x}$ A is $\geq 0{,}5\ \bar{x}\ N_{vo}$? yes X; no □			$\bar{x}$ **B** is $\geq 0{,}5\ \bar{x}\ N_{vo}$? yesX; no □			$\bar{x}$ **C** is $\geq 0{,}5\ \bar{x}\ N_{vo}$? yes X; no □		
Validation suspension N_{VB}			V_{C1} 59+64=123			V_{C2} 72+54=126; $\bar{x}$=124,5			$30 \leq \bar{x}\ N_{VB}/1000 \leq 160$? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	$\bar{x} = 2{,}0 \times 10^9$; $\log N = 9{,}30$ $N_0 = N/100$; $\log N_0 = 7{,}30$ $7{,}17 \leq \log N_0 \leq 7{,}7$; yes X; no □
	10^{-7}	**205**	**198**	
	10^{-8}	**25**	**12**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	34	17	510	2,71	4,61	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	67+81=148	x̄ = 144,5	V_{C1} 15 sec	78+91= 169	x̄ = 159,5	V_{C1}	69+63= 132	x̄ = 132	V_{C1}	29+40= 69	x̄ = 74
V_{C2}	66+75=141		V_{C2} 15 sec	85+65= 150		V_{C2}			V_{C2}	28+51= 79	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ **B** is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ **C** is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 59+64=123			V_{C2} 72+54=126; x̄=124,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	x̄ = 2,0 x 10^9; log$N$ = 9,30 $N_0 = N/100$; logN_0 = 7,30 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**205**	**198**	
	10^{-8}	**25**	**12**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	17	28	225	2,35	5,17	15''	dirty
	10x	0	0					
	10x	0	0					

Interpretation

Using the **EN 13624:2013** method, there was tested product **CHEMISEPT G** at the temperature conditions 20 °C ± 1 °C, with the contact time 15 seconds and the clean and dirty conditions. The dilution neutralization method has been used for the testing of product effect onto the microorganism *Candida albicans* ATCC 10231. In clean and dirty conditions (test regime) tested product was been active against the test organism in the selected contact times.

Conclusion

By the test results it can be made conclusion, that tested product **CHEMISEPT G has yeasticidal effect** at the contact time 15 seconds.

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Raul Raudsepp, MSc

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. 13/2015

EVALUATION OF BACTERICIDAL ACTIVITY (EN 13727)

Name of the product:	**CHEMISEPT G**
Batch number:	LOT 69090215
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA Maris Millner, +372-51-77-090
Date of delivery:	16.04.2015
Date of registration:	16.04.2015
Test material conditions:	no specific features, sample in the manufacturers tare
Storage conditions:	in room temperature, dark
Appearance of the product:	amount 1000 ml
Contact time:	15 sec, clean and dirty conditions
Interfering substance:	3,0 g/l bovine albumin and 3,0 ml/l sheep blood erythrocytes
Test neutralizer:	polysorbate 80, 30 g/l; saponine, 30 g/l; lecithin, 3 g/l
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442; *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli K12* NCTC 10538.
Testing method:	**EVS-EN 13727:2012+A1:2013** **Quantitative suspension test for the valuation of bactericidal activity in the medical area**
Testing date:	06.04 – 10.04.2015
Results:	look at appendix 1-4

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Raul Raudsepp, MSc

Head of Laboratory, microbiologist

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EESTI AKREDITEERIMISKESKUS
AK
ESTONIAN ACCREDITATION CENTRE

Appendix 1

TEST RESULTS (bactericidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Pseudomonas aeruginosa* ATCC 15442;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	67+54=121	$\bar{x}$ = 123,5	V_{C1} 15 sek	48+56= 104	$\bar{x}$ = 111	V_{C1}	97+115= 212	$\bar{x}$ = 212	V_{C1} 15 sek	55+67= 122	$\bar{x}$ = 124,5
V_{C2}	57+69=126		V_{C2} 15 sek	60+58= 118		V_{C2}			V_{C2} 15 sek	69+58= 127	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ **B** is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ **C** is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 66+48=114			V_{C2} 50+55=105; $\bar{x}$=109,5			30 ≤ $\bar{x}$ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	$\bar{x}$ = 1,87 x 10^9; log$N$ = 9,27 N_0 = N/100; logN_0 = 7,27 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**164**	**203**	
	10^{-8}	**19**	**26**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,12	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	67+52=121	x̄ = 123,5	V_{C1} 15 sec	54+62= 116	x̄ = 121,5	V_{C1}	97+112= 209	x̄ = 209	V_{C1}	73+59= 132	x̄ = 139
V_{C2}	57+69=126		V_{C2} 15 sec	68+59= 127		V_{C2}			V_{C2}	70+76= 146	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ **B** is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ **C** is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	x̄ = 1,87 x 10^9; log$N$ = 9,27 $N_0 = N/100$; logN_0 = 7,27 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**164**	**203**	
	10^{-8}	**19**	**26**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,12	15''	dirty
	10x	0	0					

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

N_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (bactericidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	63+74=137	$\bar{x}$ = 143	V_{C1}	65+70= 135	$\bar{x}$ = 125,5	V_{C1}	70+71=141	$\bar{x}$ = 141	V_{C1}	51+67= 118	$\bar{x}$ = 111,5
V_{C2}	81+68=149		V_{C2}	48+68= 116		V_{C2}			V_{C2}	49+56= 105	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ **B** is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ **C** is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 89+73=162			V_{C2} 82+74=156; $\bar{x}$=159			30 ≤ $\bar{x}$ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: *N and* N_0	*N*	V_{C1}	V_{C2}	$\bar{x}$ = 2.38 x 10^9; log*N* = 9,37 N_0 = *N*/100; logN_0 = 7,37 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**225**	**238**	
	10^{-8}	**32**	**28**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,22	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	63+72=137	x̄ = 143	V_{C1} 15 sek	81+76= 157	x̄ =	V_{C1}	70+71= 141	x̄ = 141	V_{C1} 30 sek	66+69= 135	x̄ = 140
V_{C2}	81+68=149		V_{C2} 15 sek	59+82= 141		V_{C2}			V_{C2} 30 sek	75+70= 145	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ **B** is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ **C** is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	x̄ = 2.38 x 10^9; log$N$ = 9,37 N_0 = N/100; logN_0 = 7,37 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**225**	**238**	
	10^{-8}	**32**	**28**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,22	15''	dirty
	10x	0	0					

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

N_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 3

TEST RESULTS (bactericidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	72+86=158	$\bar{x}$ = 154,5	V_{C1}	80+8= 167	$\bar{x}$ = 168	V_{C1}	131+103= 234	$\bar{x}$ = 234	V_{C1}	76+84= 160	$\bar{x}$ = 155,5
V_{C2}	81+70=151		V_{C2}	79+90= 169		V_{C2}			V_{C2}	83+68= 151	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ **B** is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ **C** is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; $\bar{x}$=135,5			30 ≤ $\bar{x}$ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: *N and* N_0	N	V_{C1}	V_{C2}	$\bar{x}$ = 2.98 x 10^9; log$N$ = 9,47 N_0 = N/100; logN_0 = 7,47 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**271**	**325**	
	10^{-8}	**25**	**35**	

Experimental results

Concentration of the product.%	**Dilution step**	V_{C1}	V_{C2}	**Na** (=x̄*10)	**log Na**	**logR**	**Contact time**	**Conditions**
RTU	-	0	0	<140	<2,15	>5,32	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	72+86=158	x̄ = 154,5	V_{C1} 15 sek	101+97=198	x̄ = 191	V_{C1}	131+103=234	x̄ = 234	V_{C1}	88+93=181	x̄ = 177
V_{C2}	81+671=151		V_{C2} 15 sek	95+89=184		V_{C2}			V_{C2}	79+94=173	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ **B** is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ **C** is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	x̄ = 2.98 x 10^9; log$N$ = 9,47 N_0 = N/100; logN_0 = 7,47 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**271**	**325**	
	10^{-8}	**25**	**35**	

Experimental results

Concentration of the product.%	**Dilution step**	V_{C1}	V_{C2}	**Na** (=x̄*10)	**log Na**	**logR**	**Contact time**	**Conditions**
RTU	-	0	0	<140	<2,15	>5,32	15''	dirty
	10x	0	0					

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

N_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor ($R = N_0 / Na$; $LogR = Log N_0 - Log\ Na$)

Appendix 4

TEST RESULTS (bactericidal suspension test)

Product: **CHEMISEPT G**
Dilution neutralization method; Spread plate;
Neutralizers: Polysorbate 80, 30 g/l; Saponine, 30 g/l; lecithin, 3 g/l
Test organism: *Escherichia coli* K12 NCTC 10538;
Test temperatuure: +20° C; Incubation temperatuure: +37° C
Solvents: water;
Interfering substance: BSA 3,0 g/l and sheep blood erythrocytes 3,0 ml/l
Nordic Tersus Laboratory LLC.; Date of test: 06.04 – 10.04.2015
Responsible person: Raul Raudsepp

Validation and controls

Clean conditions

Validation suspension N_{vo}			**Experimental conditions (A)**			**Neutralizer control (B)**			**Method validation (C)**		
V_{C1}	67+80=147	$\bar{x}$ = 133,5	V_{C1} 15 sek	95+99= 194	$\bar{x}$ = 179,5	V_{C1}	98+108= 206	$\bar{x}$ = 206	V_{C1}	86+90= 176	$\bar{x}$ = 169,5
V_{C2}	62+58=120		V_{C2} 15 sek	79+86= 165		V_{C2}			V_{C2}	78+85= 163	
30 ≤ $\bar{x}$ N_{vo} ≤160 ? yes X; no □			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □			$\bar{x}$ **B** is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ **C** is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 55+67=122			V_{C2} 71+68=139; $\bar{x}$=130,5			30 ≤ $\bar{x}$ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: *N and* N_0	*N*	V_{C1}	V_{C2}	$\bar{x}$ = 3,04 x 10^9; log*N* = 9,48
	10^{-7}	**316**	**298**	N_0 = *N*/100; logN_0 = 7,48
	10^{-8}	**31**	**24**	7,17≤ logN_0≤7,7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,33	15''	clean
	10x	0	0					

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Neutralizer control (B)			Method validation (C)		
V_{C1}	72+83=155	x̄ = 152,5	V_{C1} 15 sek	103+97= 200	x̄ = 191,5	V_{C1}	131+103= 234	x̄ = 234	V_{C1}	98+90= 188	x̄ = 188,5
V_{C2}	81+69=150		V_{C2} 15 sek	89+94= 183		V_{C2}			V_{C2}	88+101= 189	
30 ≤ x̄ N_{vo} ≤160 ? yes X; no □			x̄ A is ≥ 0,5 x̄ N_{vo}? yes X; no □			x̄ **B** is ≥ 0,5 x̄ N_{vo}? yesX; no □			x̄ **C** is ≥ 0,5 x̄ N_{vo}? yes X; no □		
Validation suspension N_{VB}			V_{C1} 78+61=139			V_{C2} 64+68=132; x̄=135,5			30 ≤ x̄ $N_{VB}/1000$ ≤160 ? yes X; no □		

Test suspensioon and test

Test suspension: N *and* N_0	N	V_{C1}	V_{C2}	x̄ = 3,04 x 10^9; log$N$ = 9,48 $N_0 = N/100$; logN_0 = 7,48 7,17≤ logN_0≤7,7; yes X; no □
	10^{-7}	**316**	**298**	
	10^{-8}	**31**	**24**	

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
RTU	-	0	0	<140	<2,15	>5,33	15''	dirty
	10x	0	0					

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

N_{VB} = cfu/ml on the neutralizer control

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Interpretation

Using **EN 13727** methodics, there was tested ready-to-use product for hand rubbing - **CHEMISEPT G**, in temperature at 20 °C ± 1 °C, with the contact times: 15 seconds and the conditions: clean and dirty. The dilution neutralization method was been used for the testing of product effect onto the microorganisms: *Pseudomonas aeruginosa* ATCC 15442; *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli* K12 NCTC 10538. In clean and dirty conditions tested product was active against all the testorganims at the all tested contact times.

Conclusions

By the test results it can be made conclusion, that tested product **CHEMISEPT G has bactericidal effect** in case of hand rubbing at the conditions tested, as treated by the product the surviving microorganisms count was decreasing at least five grades.

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Raul Raudsepp

Head of laboratory, microbiologist