

**Project number: C05ML260**

**Final report submitted to**

**CHEMI – PHARM AS**

Põllu 132

10917 Tallinn

(<http://www.chemi-pharm.com>)

**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  $\geq 3.62$  and  $\geq 4.00$  (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  $\geq 5.00$  (assay without soil load),  $\geq 5.00$  (assay with BSA) and  $\geq 5.13$  (assay with FCS). These values correspond to an inactivation of  $\geq 99.999\%$  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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AS Chemi-Pharm  
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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**

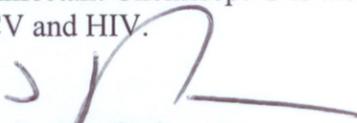
The following concentration and exposure time are necessary for inactivation of the two test viruses:

**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**

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Sitz der Gesellschaft: Bremen  
Geschäftsführerin: Dorothee Steinmann  
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Tervisekaitseinspeksioon • 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

Microbiologist

\* – Did not accredited by EAC



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Chemi-Pharm AS  
Pollu 132  
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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**

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Põllu 132

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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  $\geq 2.38$  and  $\geq 2.63$   $\log_{10}$  steps. A reduction factor of  $\geq 2.63$  was measured after 30 and 60 minutes contact time (table 2).

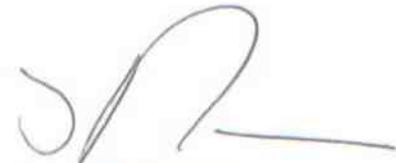
The hand disinfectant CHEMISEPT G was examined undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 0.5, 1.0, 2.0 and 5.0 minutes.

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  $\geq 4.63$  (assay without soil load),  $\geq 4.25$  (assay with BSA) and  $\geq 4.38$  (assay with FCS). At this exposure time, no HSV could be detected in all assays. These values correspond to an inactivation of  $\geq 99.99\%$  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**



Akrediteeritud L013

**TEST REPORT No 4301**

**Koopia**

**Name of the product:** Chemisept G

*Alsin*

**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 sterilized 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:**

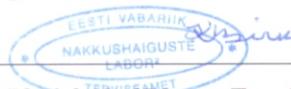
Concentration	Contact time	<i>Escherichia coli</i> ATCC 10538	<i>Staphylococcus aureus</i> ATCC 6538	<i>Streptococcus faecalis</i> ATCC 29212	<i>Pseudomonas aeruginosa</i> ATCC 15442	<i>Bacillus cereus</i> ATCC 11778	<i>Candida albicans</i> ATCC 10231
Without dilution	30 sec	0	0	0	0	0	0
Growth of tested 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





Akrediteeritud L013

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**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



\* – EAK poolt akrediteerimata

Project number: C05ML260

Final report submitted to

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(<http://www.chemi-pharm.com>)

**Evaluation of the  
effectiveness of**

**CHEMISEPT G**

**against**

**Polyomavirus SV 40**

Test method according to guideline of BGA and DWV

**Dr. Jochen Steinmann**

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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  $\geq 5.13$  (assay without protein load),  $\geq 4.88$  (assay with BSA) and  $\geq 5.00$  (assay with FCS), respectively. These RF correspond to an inactivation of  $\geq 99.999\%$  (assay with BSA  $\geq 99.99\%$ ) 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**

Project number: C05ML260

Final report submitted to

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10917 Tallinn

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**Evaluation of the  
effectiveness of**

**CHEMISEPT G**

**against  
Polyomavirus SV 40**

Test method according to guideline of BGA and DWV

**Dr. Jochen Steinmann**

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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  $\geq 5.13$  (assay without protein load),  $\geq 4.88$  (assay with BSA) and  $\geq 5.00$  (assay with FCS), respectively. These RF correspond to an inactivation of  $\geq 99.999\%$  (assay with BSA  $\geq 99.99\%$ ) 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**  
Põllu 132  
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(<http://www.chemi-pharm.com>)

**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  $\geq 4.38 \log_{10}$ -steps. This reduction corresponds to an inactivation of  $\geq 99.99\%$  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

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Põllu 132

10917 Tallinn

(<http://www.chemi-pharm.com>)

**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.

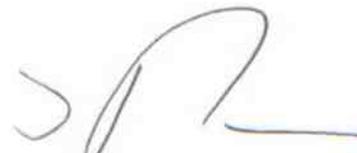
The hand disinfectant CHEMISEPT G exhibited a strong virucidal efficacy against the test virus. After an exposure time of 30 seconds no vaccinia virus was detectable any longer. The virus titres were reduced by  $\geq 4.25$  (assay without interfering substances),  $\geq 4.00$  (assay with BSA) and 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

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  
yeastocidal activity in the medical area.**

**Testing date:** 06.04 – 10.04.2015

**Results:** look at appendix I



.....  
Raul Randsepp, MSc  
Head of Laboratory, microbiologist

**TEST RESULTS (yeasticial 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 <input type="checkbox"/>			$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	yes	$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	
Validation suspension $N_{TB}$			$V_{C1}$ 59+64=123		$V_{C2}$ 72+54=126; $\bar{x}=124,5$		$30 \leq \bar{x} N_{TB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>	

**Test suspension 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 <input type="checkbox"/>
	$10^{-7}$	<b>205</b>	<b>198</b>	
	$10^{-8}$	<b>25</b>	<b>12</b>	

### Experimental results

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

### Validation and controls

#### Dirty conditions

Validation suspension $N_{vo}$		Experimental conditions (A)		Neutralizer control (B)		Method validation (C)	
$V_{C1}$	$67+81=148$	$\bar{x} = 144,5$	$V_{C1}$ 78+91= 15 sec 169	$\bar{x} = 159,5$	$V_{C1}$ 69+63= 132	$\bar{x} = 132$	$V_{C1}$ 29+40=69 $\bar{x} = 74$
$V_{C2}$	$66+75=141$		$V_{C2}$ 85+65= 15 sec 150		$V_{C2}$		$V_{C2}$ 28+51=79
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	
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 <input type="checkbox"/>	

### Test suspension and test

Test suspension: $N$ and $N_0$	$N$		$V_{C1}$		$V_{C2}$	
	$10^{-7}$			<b>205</b>		<b>198</b>
	$10^{-8}$			<b>25</b>		<b>12</b>

$\bar{x} = 2,0 \times 10^9$ ;  $\log N = 9,30$   
 $N_0 = N/100$ ;  $\log N_0 = 7,30$   
 $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no

### Experimental results

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

### **Interpretation**

Using the **EN 13624:2013** method, there was tested product **CHEMISEPT G** at the temperature conditions  $20 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ , 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

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

### 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 temperature: +20° C; Incubation temperature: +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		$V_{C2}$ 69+58= 127		$V_{C2}$ 15 sek 69+58= 127	
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		
Validation suspension $N_{VB}$		$V_{C1}$ 66+48=114		$V_{C2}$ 50+55=105; $\bar{x}=109,5$		$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>		

#### Test suspension and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$	$\bar{x} = 1,87 \times 10^9$ ; $\log N = 9,27$ $N_0 = N/100$ ; $\log N_0 = 7,27$ $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no <input type="checkbox"/>
	$10^{-7}$	<b>164</b>	<b>203</b>	
	$10^{-8}$	<b>19</b>	<b>26</b>	

### 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''	clean
	10x	0	0					

### Validation and controls

#### Dirty conditions

Validation suspension $N_{10}$		Experimental conditions (A)		Neutralizer control (B)		Method validation (C)		
$V_{C1}$	67+52=121	$\bar{x}$ = 123,5	$V_{C1}$ 15 sec 54+62= 116	$\bar{x}$ = 121,5	$V_{C1}$ 97+112= 209	$\bar{x}$ = 209	$V_{C1}$ 73+59= 132	$\bar{x}$ = 139
$V_{C2}$	57+69=126		$V_{C2}$ 15 sec 68+59= 127		$V_{C2}$		$V_{C2}$ 70+76= 146	
$30 \leq \bar{x} N_{10} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>		
Validation suspension $N_{10}$		$V_{C1}$ 78+61=139		$V_{C2}$ 64+68=132; $\bar{x}$ =135,5		$30 \leq \bar{x} N_{10}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>		

### Test suspension and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$
	$10^{-7}$	164	203
	$10^{-8}$	19	26

$\bar{x} = 1,87 \times 10^9$ ;  $\log N = 9,27$   
 $N_0 = N/100$ ;  $\log N_0 = 7,27$   
 $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no

### 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 ( $R = N_0 / Na$ ;  $\text{LogR} = \text{Log}N_0 - \text{Log}Na$ )

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 temperature: +20° C; Incubation temperature: +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	
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>			$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>			$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		
Validation suspension $N_{VB}$			$V_{C1}$ 89+73=162			$V_{C2}$ 82+74=156; $\bar{x}=159$			$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>		

**Test suspension and test**

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$	$\bar{x} = 2.38 \times 10^9$ ; $\log N = 9,37$ $N_0 = N/100$ ; $\log N_0 = 7,37$ $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no <input type="checkbox"/>
	$10^{-7}$	<b>225</b>	<b>238</b>	
	$10^{-8}$	<b>32</b>	<b>28</b>	

### Experimental results

Concentration of the product. %	Dilution step	$V_{C1}$	$V_{C2}$	Na (= $\bar{x}$ *10)	log Na	logR	Contact time	Conditions
-	0	0	0	<140	<2,15	>5,22	15''	clean
10x	0	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 $\bar{x}$ = 143	$V_{C1}$	81+76=157 15 sek	$V_{C1}$	70+71=141	$V_{C1}$	66+69=135 30 sek
$V_{C2}$	81+68=149	$V_{C2}$	59+82=141 15 sek	$V_{C2}$		$V_{C2}$	75+70=145 30 sek
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	
Validation suspension $N_{TB}$		$V_{C1}$ 78+61=139		$V_{C2}$ 64+68=132; $\bar{x}$ =135,5		$30 \leq \bar{x} N_{TB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>	

### Test suspensioon and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$
$10^{-7}$			
		<b>225</b>	<b>238</b>
$10^{-8}$		<b>32</b>	<b>28</b>

$\bar{x} = 2.38 \times 10^9$ ;  $\log N = 9,37$   
 $N_0 = N/100$ ;  $\log N_0 = 7,37$   
 $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no

### Experimental results

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

### 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 ( $R = N_0 / Na$ ;  $\text{Log}R = \text{Log}N_0 - \text{Log}Na$ )

### 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 temperature: +20° C; Incubation temperature: +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		$V_{C2}$	83+68= 151		$V_{C2}$	83+68= 151	
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>					
Validation suspension $N_{VB}$		$V_{C1}$ 78+61=139		$V_{C2}$ 64+68=132; $\bar{x}=135,5$		$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>					

### Test suspension and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$
	$10^{-7}$	<b>271</b>	<b>325</b>
	$10^{-8}$	<b>25</b>	<b>35</b>

$\bar{x} = 2,98 \times 10^9$ ;  $\log N = 9,47$   
 $N_0 = N/100$ ;  $\log N_0 = 7,47$   
 $7,17 \leq \log N_0 \leq 7$ ; yes X; no

### 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	>5,32	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 $\bar{x}$ = 154,5	$V_{C1}$	101+97=198 15 sek	$V_{C1}$	131+103=234	$V_{C1}$	88+93=181
$V_{C2}$	81+671=15 1	$V_{C2}$	95+89=184 15 sek	$V_{C2}$	234	$V_{C2}$	79+94=173
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes <input checked="" type="checkbox"/> ; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	
Validation suspension $N_{VB}$		$V_{C1}$ 78+61=139		$V_{C2}$ 64+68=132; $\bar{x}$ =135,5		$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>	

### Test suspension and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$
	$10^{-7}$	271	325
	$10^{-8}$	25	35

$\bar{x} = 2,98 \times 10^9$ ;  $\log N = 9,47$   
 $N_0 = N/100$ ;  $\log N_0 = 7,47$   
 $7,17 \leq \log N_0 \leq 7$ ; yes X; no

### 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	>5,32	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 ( $R = N_0 / Na$ ;  $\text{Log}R = \text{Log}N_0 - \text{Log}Na$ )

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 temperature: +20° C; Incubation temperature: +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 $N_{10}$		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	
$30 \leq \bar{x} N_{10} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{10}$ ? yes X; no <input type="checkbox"/>				
Validation suspension $N_{VB}$		$V_{C1}$ 55+67=122		$V_{C2}$ 71+68=139; $\bar{x}=130,5$		$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>				

**Test suspension and test**

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$	$\bar{x} = 3,04 \times 10^9$ ; $\log N = 9,48$ $N_0 = N/100$ ; $\log N_0 = 7,48$ $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no <input type="checkbox"/>
	$10^{-7}$	<b>316</b>	<b>298</b>	
	$10^{-8}$	<b>31</b>	<b>24</b>	

### 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''	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	$\bar{x}$ = 152,5	$V_{C1}$ 15 sek 103+97= 200	$\bar{x}$ = 191,5	$V_{C1}$ 131+103= 234	$\bar{x}$ = 234	$V_{C1}$ 98+90= 188
$V_{C2}$	81+69=150		$V_{C2}$ 15 sek 89+94= 183		$V_{C2}$		$V_{C2}$ 88+101= 189
$30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/>		$\bar{x} A$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} B$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>		$\bar{x} C$ is $\geq 0,5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/>	
Validation suspension $N_{VB}$		$V_{C1}$ 78+61=139		$V_{C2}$ 64+68=132; $\bar{x}$ =135,5		$30 \leq \bar{x} N_{VB}/1000 \leq 160$ ? yes X; no <input type="checkbox"/>	

### Test suspension and test

Test suspension: $N$ and $N_0$	$N$	$V_{C1}$	$V_{C2}$
	$10^{-7}$	<b>316</b>	<b>298</b>
	$10^{-8}$	<b>31</b>	<b>24</b>

$\bar{x} = 3,04 \times 10^9$ ;  $\log N = 9,48$   
 $N_0 = N/100$ ;  $\log N_0 = 7,48$   
 $7,17 \leq \log N_0 \leq 7,7$ ; yes X; no

### Experimental results

Concentration of the product. %	Dilution step	$V_{C1}$	$V_{C2}$	Na ( $=\bar{x} \cdot 10$ )	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=  $N_0/N_a$ ; LogR=Log $N_0$  - Log  $N_a$ )

### Interpretation

Using EN 13727 methodologies, there was tested ready-to-use product for hand rubbing - **CHEMISEPT G**, in temperature at  $20\text{ °C} \pm 1\text{ °C}$ , with the contact times: 15 seconds and the conditions: clean and dirty. The dilution neutralization method was 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 testorganisms 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.



Raul Raudsepp

Head of laboratory, microbiologist

Test report No. 03/2015

DISINFECTION EFFICIENCY TEST FOR PRE-SURGICAL SKIN  
TREATMENT COMPOSITES

**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:** 16.03.2015  
**Date of registration:** 16.03.2015  
**Test material conditions:** no specific features, sample in the manufacturers tare  
**Storage conditions:** in room temperature, not in the direct sunlight  
**Active substance – conc.:** ethyl alcohol – 75 wt%; quaternary ammonium compounds 0,1 wt%  
**Appearance of the product:** transparent liquid  
**Contact time:** 30, 60, 90 and 120 sec  
**Test concentration:** ready to use  
**Reference solution:** propan-1-ol 60% (vol/vol)  
**Testing method:** sampling microbes onto the contact slides and afterward incubation of  
**Testing date:** 17.03.2015  
**Results:** look appendix 1



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**Skin disinfection procedure with the reference solution and product under test**

**Experimental results**

Product: **CHEMISEPT G**

Reference solution: propan-1-ol 60% (vol/vol)

Disinfection procedure: sprinkled 2 ml on 10 cm<sup>2</sup> area;

Tested using bacterial/fungal specific slides, after: 30 sec, 60 sec, 90 sec and 120 sec

Test group: 5 volunteers

Test Date: 17.03.2015

No.	Skin type	Non-treated area; cfu/ml				Test area; cfu/ml				Reference area; cfu/ml			
		30''	60''	90''	120''	30''	60''	90''	120''	30''	60''	90''	120''
1	Sebaceous rich	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>2</sup>	0	0	0	0	0	0	0	0
	Sebaceous poor	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>3</sup>	0	0	0	0	0	0	0	0
2	Sebaceous rich	10 <sup>5</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>4</sup>	0	0	0	0	0	0	0	0
	Sebaceous poor	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>3</sup>	0	0	0	0	10 <sup>2</sup>	0	10 <sup>2</sup>	0
3	Sebaceous rich	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>2</sup>	0	0	0	0	0	0	0	0
	Sebaceous poor	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>4</sup>	0	0	0	0	0	0	0	0
4	Sebaceous rich	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>3</sup>	0	0	0	0	0	0	0	0
	Sebaceous poor	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>3</sup>	0	0	0	0	0	0	0	0
5	Sebaceous rich	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>3</sup>	0	0	0	0	10 <sup>2</sup>	0	0	0
	Sebaceous poor	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>3</sup>	0	10 <sup>2</sup>	0	0	10 <sup>2</sup>	0	0	10 <sup>2</sup>
Mean Lg S.r,		3,2	3,0	2,8	2,8	0	0	0	0	0,8	0	0	0
Mean Lg S.p.		4,0	3,8	3,2	3,2	0	0,4	0	0	0,4	0	0,4	0,4
Reduction Factor for Sebaceous rich area						3,2	3,0	2,8	2,8	2,4	3,0	2,8	2,8
Reduction Factor for Sebaceous poor area						4,0	3,4	3,2	3,2	3,6	3,8	2,8	2,8

**Interpretation:**

Using the current method, there was tested product **CHEMISEPT G** (Batch No. 69090215), in ready to use conditions, with the contact times: 30, 60, 90 and 120 sec. The sampling method<sup>1</sup> was been used for the testing of product effect onto the skin microflora as in the sebaceous rich as poor areas.

**Summary:**

By the test results conclusion can be made, that tested product **CHEMISEPT G has both bactericidal and fungicidal effect** onto skin microflora in case of the different surfaces (sebaceous rich/poor) disinfection, at the contact times 30, 60, 90 and 120 sec. In comparison with the reference substance (propan-1-ol 60 vol%) the tested product exhibits slight more noticeable influence.

**References:**

<sup>1)</sup> M.S. Favero, *et al.*; Microbiological Sampling of Surfaces; Journal of Applied Bacteriology, Vol. 31, Issue 3, pp 336–343, 1968.



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