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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10917 Tallinn
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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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AS Chemi–Pharm
Põllu 132

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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 K. Birk

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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.**

Brill

Dr. Holger Brill

**Project number: C05ML260**

**Final report submitted to**

**CHEMI – PHARM AS**
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 ≥ 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**

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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

EESTI VABARIIK NAKKUSHAIGUSTE TERVISEAMET

* – EAK poolt akrediteerimata

**Project number: C05ML260**

**Final report submitted to**

**CHEMI – PHARM AS**
Põllu 132
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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**
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 ≥ 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**
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 ≥ 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<br>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**<br>**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 L263 NORDIC TERSUS LABORATORY OÜ REPUBLIC OF ESTONIA

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Raul Raudsepp, MSc

Head of Laboratory, microbiologist

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EESTI AKREDITEERIMISKESKUS ESTONIAN ACCREDITATION CENTRE
Akrediteeritud L263

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* $N_0$ | $N$ | $V_{C1}$ | $V_{C2}$ | $\bar{x} = 2{,}0 \times 10^9$; $\log N = 9{,}30$<br>$N_0 = N/100$; $\log N_0 = 7{,}30$<br>$7{,}17 \leq \log N_0 \leq 7{,}7$; 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<br>$N_0 = N/100$; log$N_0$ = 7,30<br>7,17≤ log$N_0$≤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.

ACCREDITED LABORATORY, No L268, ISO/IEC 17025, NORDIC TERSUS LABORATORY OÜ, REPUBLIC OF ESTONIA

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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<br>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**<br>**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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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<br>$N_0$ = $N$/100; log$N_0$ = 7,27<br>7,17≤ log$N_0$≤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<br>$N_0 = N/100$; log$N_0$ = 7,27<br>7,17≤ log$N_0$≤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=Log$N_0$ - 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<br>$N_0$ = *N*/100; log$N_0$ = 7,37<br>7,17≤ log$N_0$≤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<br>$N_0$ = $N$/100; log$N_0$ = 7,37<br>7,17≤ log$N_0$≤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=Log$N_0$ - 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<br>$N_0$ = $N$/100; log$N_0$ = 7,47<br>7,17≤ log$N_0$≤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<br>$N_0$ = $N$/100; log$N_0$ = 7,47<br>7,17≤ log$N_0$≤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; log$N_0$ = 7,48 |
| | $10^{-8}$ | **31** | **24** | 7,17≤ log$N_0$≤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<br>$N_0 = N/100$; log$N_0$ = 7,48<br>7,17≤ log$N_0$≤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=Log$N_0$ - 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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REPUBLIC OF ESTONIA

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Raul Raudsepp

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. 04/2015

# DISINFECTION EFFICIENCY TEST FOR PRE-SURGICAL SKIN TREATMENT COMPOSITES

| | |
|---|---|
| **Name of the product:** | **CHEMISEPT G COLOR** |
| **Batch number:** | LOT 71020215 |
| **Manufacturer:** | Chemi-Pharm Ltd. |
| **Client, representative:** | Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA<br>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:** | coloured 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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Appendix 1

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

## Experimental results

Product: **CHEMISEPT G COLOR**
Reference solution: propan-1-ol 60% (vol/vol)
Disinfection procedure: sprinkled 2 ml on 10 $cm^2$ 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 | $10^3$ | $10^3$ | $10^2$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sebaceous poor | $10^4$ | $10^3$ | $10^4$ | $10^4$ | $10^2$ | 0 | 0 | 0 | 0 | 0 | 0 | $10^2$ |
| 2 | Sebaceous rich | $10^2$ | $10^2$ | $10^3$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sebaceous poor | $10^4$ | $10^3$ | $10^3$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | Sebaceous rich | $10^3$ | $10^3$ | $10^3$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sebaceous poor | $10^3$ | $10^4$ | $10^4$ | $10^4$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | Sebaceous rich | $10^2$ | $10^2$ | $10^4$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sebaceous poor | $10^4$ | $10^4$ | $10^4$ | $10^4$ | 0 | 0 | 0 | 0 | $10^2$ | 0 | 0 | $10^2$ |
| 5 | Sebaceous rich | $10^3$ | $10^2$ | $10^3$ | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Sebaceous poor | $10^4$ | $10^4$ | $10^4$ | $10^4$ | 0 | 0 | $10^2$ | 0 | 0 | $10^2$ | 0 | 0 |
| Mean Lg S.r, | | 2,6 | 2,4 | 3,0 | 3,0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean Lg S.p. | | 3,8 | 3,6 | 3,8 | 3,8 | 0,4 | 0 | 0,4 | 0 | 0,4 | 0,4 | 0 | 0,8 |
| Reduction Factor for Sebaceous rich area | | | | | | 2,6 | 2,4 | 3,0 | 3,0 | 2,6 | 2,4 | 3,0 | 3,0 |
| Reduction Factor for Sebaceous poor area | | | | | | 3,4 | 3,6 | 3,4 | 3,8 | 3,4 | 3,2 | 3,8 | 3.0 |

**Interpretation:**

Using the current method, there was tested product **CHEMISEPT G COLOR** (Batch No. 71020215), in ready to use conditions, with the contact times: 30, 60, 90 and 120 sec. The sampling method[1] 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 COLOR 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:**

[1] 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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*Kesklabor*

Health Protection Inspectorate
*Central Laboratory*

Registreerimisnumber L013

Labor on volitatud Põllumajandusministri käskkirjaga nr.203 29.06.2000 toidutoorme ja toidu järelvalveks võetud proovide analüüsimiseks



**MIKROBIOLOOGILINE UURING NR. 162**

**Uuritav materjal:** CHEMISEPT COLOR/ CHEMISEPT G COLOR ( värvainega naha antiseptikum kirurgiliseks kasutamiseks )

**Tootja:** AS Chemi-Pharm Põllu 132 Tallinn 10917

**Proovi võtmise kuupäev, kellaaeg:** 18.03.2008

**Säilitamise tingimused:** toatemperatuuril pimedas

**Toimaine:** etanool

**Katsemeetod ja valideerimine:** lahjendamis-neutraliseerimismeetod EN 12054:2001* järgi

**Katsetamise aeg:** 28.05.2008- 02.06.2008

**Toote kontsentratsioon:** lahjendamata

**Katsetamise temperatuur:** $20^0C \pm 1^0C$

**Kontaktajad:** 30 sek, 1 min

**Uurimise tulemused:** Vt lisa 1 ja 2

**Kokkuvõte:** Vastavalt EN 12054 nõuetele katsetamisel $20^0C$ juures lahjendamata toode CHEMISEPT COLOR/ CHEMISEPT G COLOR omas kontaktaegade 30 sek, 1 min puhul bakteritsiidset toimet (reduktsioon oli suurem kui $10^5$) referentstüvede *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 8043 ja *Pseudomonas aeruginosa* ATCC 15442 suhtes ning vastas esitatud nõuetele.

Tallinn, 02.06.2008 Vanemspetsialist I.Bekergun

**Katsetulemused kehtivad uuritud proovide kohta. Protokolli tohib paljundada ainult tervikuna.**
**Tärniga (*) tähistatud meetod ei kuulu akrediteerimisulatusse.**

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Lisa 1

**Lahjendamis-neutraliseerimise meetodi valideerimine CHEMISEPT COLOR / CHEMISEPT G COLOR katsetamisel**

| Test mikroorganism | Kolooniate arv Petri tassil (kolooniate keskmine arv CFU) | | |
|---|---|---|---|
| | Bakterite testimise suspensioon ($N_v$) | Neutraliseerimise valideerimine | |
| | | Neutraliseerimisvedeliku toksilisuse kontroll (B) | Katsetingimuste testimine (C) |
| *Escherichia coli* | 152,168 (N=160) | 143,165 (B=154) | 148,148 (C=148) |
| *Staphylococcus aureus* | 112,130 (N=121) | 106,125 (B=116) | 120,92 (C=106) |
| *Enterococcus hirae* | 126,134 (N=130) | 114,120 (B=117) | 90,108 (C=100) |
| *Pseudomonas aeruginosa* | 176,188 (N=182) | 172,186 (B=180) | 148,166 (C=157) |
| Testitud tüvede puhul:<br>$N_v$ ja B peavad olema 100 CFU kuni 300 CFU;<br>B peab olema suurem kui 0,5 x N;<br>C peab olema suurem kui 0,5 x B; | | | |
| Kasutatud neutraliseerimisvedelik on neutraliseerimiseks kõlblik | | | |

Lisa 2

**Toote CHEMISEPT COLOR/ CHEMISEPT G COLOR katsetamine lahjendamis-neutraliseerimise meetodil**

| Katsetatav mikroorganism | Bakterite testimise alg-suspensioon | Neutrali-seeritud segu külvi-kogus | Lahjendamata toote erinevatel kontaktaegadel katsetamise tulemused | |
|---|---|---|---|---|
| | | | eluvõimeliste arv CFU/ml | |
| | | | 30 sek | 1 min |
| *Escherichia coli* | $1,9x10^{8}$ | $1,0\ 10^{-1}$ | $<1,5x10^{1}$ | $<1,5x10^{1}$ |
| *Staphylococcus aureus* | $1,6x10^{8}$ | $1,0\ 10^{-1}$ | $<1,5x10^{1}$ | $<1,5x10^{1}$ |
| *Enterococcus hirae* | $1,6x10^{8}$ | $1,0\ 10^{-1}$ | $<1,5x10^{1}$ | $<1,5x10^{1}$ |
| *Pseudomonas aeruginosa* | $1,8x10^{8}$ | $1,0\ 10^{-1}$ | $<1,5x10^{1}$ | $<1,5x10^{1}$ |

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Mikrobioloogia Kesklabor • Central Laboratory of Microbiology

Akrediteeritud L013

**MIKROBIOLOOGILINE UURING NR. 4302**

**Toote nimi:** kiirdesinfektant CHEMISEPT G COLOR

**Tootja:** AS Chemi-Pharm

**Katsetamisele suunav asutus, isik:** AS Chemi-Pharm, labori juhataja M. Milner

**Proovi võtmise koht:** AS Chemi-Pharm Põllu 132 Tallinn 10917

**Proovi võtmise kuupäev, kellaaeg:** 22.12..2004

**Uuringu eesmärk:** desinfitseerivate omaduste määramine

**Laborisse saabumise aeg:** 23.12.2004 kell 09.30

**Proovi seisund laborisse saabumisel:** ilma iseärasusteta proov tootja pakendis

**Laboris säilitamise tingimused:** toatemperatuuril ja pimedas

**Mikrobioloogilise uuringu algus:** 27.12.2004 kell 11.00

**Uurimismeetod:** lahjendamis-neutraliseerimismeetod *

**Katsetamise aeg:** 27.12.2004-03.01.2005

**Katsetingimused:** Katsetatud temperatuuril 20±1$^0$C

**Uuringu tulemused:**

| Lahuse kontsentratsioon | Toimeaeg | *Escherichia coli* ATCC 10538 | *Staphylococcus aureus* ATCC 6538 | *Enterococcus hirae* ATCC 10541 | *Pseudomonas aeruginosa* ATCC 15442 | *Candida albicans* ATCC 10231 |
|---|---|---|---|---|---|---|
| 0 | Testitava tüve kasvu kontroll | + | + | + | + | + |
| Lahjendamata | 30 sek | - | - | - | - | - |

Tähistused: + Testkultuur kasvab, lahusel puudub bakteritsiidne toime
- Testkultuuri kasv puudub, lahus toimib bakteritsiidselt.

**Uuringu tulemused kehtivad antud proovi kohta.**

**Katseprotokolli paljundamine on lubatud ainult tervikuna.**

03.01.2005 Mikrobioloog K. Birk

* – EAK poolt akrediteerimata

Kotka 2, 11315 Tallinn Tel. 372 6 943 670; Fax 372 6943 951