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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025.

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Test report No. 35/2016

DETERMINATION OF BACTERICIDAL (EN 13727+A1), YEASTICIDAL (EN 13624), TUBERCULOCIDAL (EN 14348) AND VIRUCIDAL (EN 14476+A1) ACTIVITY OF THE PRODUCT **VITASEPT E75 GEL** HYGIENIC HANDRUB (EN 1500) SURGICAL HAND DISINFECTION (EN 12791)

Sample ID: D35/2016
Sample name: **VITASEPT E75 GEL**.
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Producer: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia

Incoming date:
29.1.2016

Delivery date:
3.5.2016

Hodonín, 3.5.2016

Ing. Jana Slitrová, Head of Laboratory

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Subject of testing:
Determination of bactericidal, yeasticidal, tuberculocidal and virucidal activity of the product. Hygienic handrub. Surgical hand disinfection.

Identification of the sample:

Name of the product:	**VITASEPT E75 GEL**
Batch number:	91215
Date of manufacture:	9.12.2015
Expiry date:	1.11.2018
Manufacturer:	Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Incoming date:	29.1.2016
Storage conditions:	stated by the manufacturer
Active compounds and concentrations in 100 g:	CAS 64-17-5 Ethanol 75 g Quarternary ammonium compounds

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13727:2012+A1)
Period of analysis:	23.2. – 24.2.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the products:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	30 s (0.5 min), 60 s (1 min) and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli K12* NCTC 10538
Incubation conditions:	37 °C ± 1 °C, 24 hours

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under defined conditions by at least 5 orders (10^5).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$
* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

The standard:
EN 13727:2012+A1 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1) November 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
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Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

The Number of CFU in the tested product **VITASEPT E75 GEL**: < 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Pseudomonas aeruginosa* ATCC 15442

Tab No. 1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 100%*		
V_{c1}	48	Φ_{NV0}= 49.5	V_{c1}	49	Φ_A = 51	V_{c1}	47	Φ_B = 48.5	V_{c1}	53	Φ_C = 49.5
V_{c2}	51		V_{c2}	53		V_{c2}	50		V_{c2}	46	
$30 \leq \Phi_{NV0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{NV0}$			$\Phi_B \geq 0.5\ \Phi_{NV0}$			$\Phi_C \geq 0.5\ \Phi_{NV0}$		
x	yes	no	x	Yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	46	V_{c2}	52	Φ_{NVB}	49	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 1.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 50 \times 10^8 = \lg 9.70$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.70$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	52	48	$7.17 \leq \lg N_0 \leq 7.70$
				x yes No

Tab No. 1.3 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Pseudomonas aeruginosa* ATCC 15442

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.70$)**
100*/0.5/clean	10^0	<14	<14	< 2.15	**≥ 5.55**
100*/1/clean	10^0	<14	<14	< 2.15	**≥ 5.55**
100*/3/clean	10^0	<14	<14	< 2.15	**≥ 5.55**

2. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Staphylococcus aureus* ATCC 6538

Tab No. 2.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 100%*		
V_{c1}	41	Φ_{NV0}= 43	V_{c1}	47	Φ_A = 43.5	V_{c1}	50	Φ_B = 43	V_{c1}	44	Φ_C = 41.5
V_{c2}	45		V_{c2}	40		V_{c2}	36		V_{c2}	39	
$30 \leq \Phi_{NV0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{NV0}$			$\Phi_B \geq 0.5\ \Phi_{NV0}$			$\Phi_C \geq 0.5\ \Phi_{NV0}$		
x	yes	no	x	Yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	40	V_{c2}	47	Φ_{NVB}	43.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 2.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 47 \times 10^8 = \lg 9.67$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.67$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	46	48	$7.17 \leq \lg N_0 \leq 7.70$
				x yes No

Tab No. 2.3 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Staphylococcus aureus* ATCC 6538

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.67$)**
100*/0.5/clean	10^0	<14	<14	< 2.15	**≥ 5.52**
100*/1/clean	10^0	<14	<14	< 2.15	**≥ 5.52**
100*/3/clean	10^0	<14	<14	< 2.15	**≥ 5.52**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_{V0} = the number of cfu/ml of the bacterial test suspension for validation, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation) R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a, * Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

3. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Enterococcus hirae* ATCC 10541

Tab No. 3.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 100%*		
V_{c1}	45	Φ_{NV0}= 47	V_{c1}	47	Φ_A = 46.5	V_{c1}	49	Φ_B = 45.5	V_{c1}	48	Φ_C = 44
V_{c2}	49		V_{c2}	46		V_{c2}	42		V_{c2}	40	
$30 \leq \Phi_{NV0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{NV0}$			$\Phi_B \geq 0.5\ \Phi_{NV0}$			$\Phi_C \geq 0.5\ \Phi_{NV0}$		
x	yes	no	x	Yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	43	V_{c2}	48	Φ_{NVB}	45.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 3.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 49.5 \times 10^8 = \lg 9.69$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.69$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	52	47	$7.17 \leq \lg N_0 \leq 7.70$
				x yes / No

Tab No. 3.3 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Enterococcus hirae* ATCC 10541

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.69$)**
100*/0.5/clean	10^0	<14	<14	< 2.15	**≥ 5.54**
100*/1/clean	10^0	<14	<14	< 2.15	**≥ 5.54**
100*/3/clean	10^0	<14	<14	< 2.15	**≥ 5.54**

4. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Escherichia coli K12* NCTC 10538

Tab No. 4.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 100%*		
V_{c1}	35	Φ_{NV0}= 30.5	V_{c1}	28	Φ_A = 30	V_{c1}	30	Φ_B = 28.5	V_{c1}	28	Φ_C = 28.5
V_{c2}	26		V_{c2}	32		V_{c2}	27		V_{c2}	29	
$30 \leq \Phi_{NV0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{NV0}$			$\Phi_B \geq 0.5\ \Phi_{NV0}$			$\Phi_C \geq 0.5\ \Phi_{NV0}$		
x	yes	no	x	Yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	29	V_{c2}	33	Φ_{NVB}	31	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 4.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 289 \times 10^7 = \lg 9.46$	10^{-7}	271	305	$\lg N_0 = \lg N/100 = \lg 7.46$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	29	31	$7.17 \leq \lg N_0 \leq 7.70$
				x yes / No

Tab No. 4.3 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Escherichia coli K12* NCTC 10538

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.46$)**
100*/0.5/clean	10^0	<14	<14	< 2.15	**≥ 5.31**
100*/1/clean	10^0	<14	<14	< 2.15	**≥ 5.31**
100*/3/clean	10^0	<14	<14	< 2.15	**≥ 5.31**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_{V0} = the number of cfu/ml of the bacterial test suspension for validation, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation) R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a, * Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

5. Evaluation of bactericidal activity of the product **VITASEPT E75 GEL**

Tab No. 5.1 The efficacy of chemical disinfectant **VITASEPT E75 GEL** on test strains – bactericidal activity

Bactericidal activity of the product (EN 13727:2012+A1)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13727:2012 +A1	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	0.5	100*	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	0.5	100*	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	0.5	100*	clean	≥ 5	**> 5**
Escherichia coli K12 NCTC 10538	20	0.5	100*	clean	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	1	100*	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	1	100*	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	1	100*	clean	≥ 5	**> 5**
Escherichia coli K12 NCTC 10538	20	1	100*	clean	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	3	100*	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	3	100*	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	3	100*	clean	≥ 5	**> 5**
Escherichia coli K12 NCTC 10538	20	3	100*	clean	≥ 5	**> 5**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_{V0} = the number of cfu/ml of the bacterial test suspension for validation, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation) R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a, * Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Hana Konevalíková, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624)
Period of analysis:	19.2. – 22.2.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the products:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	30 s (0.5 min), 60 s (1 min) and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Test organisms:	*Candida albicans* ATCC 10231
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least 4 orders (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least 4 orders (10^4).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$
* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

The standard:
EN 13624 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

6. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Candida albicans* ATCC 10231

Tab No. 6.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 100%*		
V_{c1}	39	Φ_{Nv0}= 42	V_{c1}	47	Φ_A = 43.5	V_{c1}	44	Φ_B = 40.5	V_{c1}	43	Φ_C = 40
V_{c2}	45		V_{c2}	40		V_{c2}	37		V_{c2}	37	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	41	V_{c2}	45	Φ_{NVB}	43	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 6.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 44 \times 10^7 = \lg 8.64$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 6.64$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	43	45	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 6.3 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Candida albicans* ATCC 10231

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.64$)**
100*/0.5/clean	10^0	<14	<14	< 2.15	**≥ 4.49**
100*/1/clean	10^0	<14	<14	< 2.15	**≥ 4.49**
100*/3/clean	10^0	<14	<14	< 2.15	**≥ 4.49**

7. Evaluation of yeasticidal activity of the product **VITASEPT E75 GEL**

Tab No. 7.1 The efficacy of chemical disinfectant **VITASEPT E75 GEL** on test strains – yeasticidal activity

Yeasticidal activity of the product (EN 13624)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13624	**lg R**
Candida albicans ATCC 10231	20	0.5	100*	clean	≥ 4	**> 4**
Candida albicans ATCC 10231	20	1	100*	clean	≥ 4	**> 4**
Candida albicans ATCC 10231	20	3	100*	clean	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_{V0} = the number of cfu/ml of the test suspension for validation, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), R = N_0/ N_a = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$, * Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Hana Konevalíková, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14348)
Period of analysis:	25.2. – 17.3.2015
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the products:	colourless liquid
Test concentration:	100 % (concentrated)*
Contact time:	30 s (0.5 min), 60 s (1 min) and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Test organisms:	*Mycobacterium terrae* ATCC 15755
Incubation conditions:	37 °C ± 1 °C, 21 days

Test procedure:

1. Preparation of the test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Mycobactericidal activity – the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* and *Mycobacterium avium* under defined conditions by at least 4 orders (10^4).
Tuberculocidal activity - the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* under defined conditions by at least 4 orders (10^4).
$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.

The standard:
EN 14348 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test method and requirements (phase 2, step 1) January 2005

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **I A E E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml
Page: 9

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8. Testing the efficacy of chemical disinfectant **I A E E75 GEL** on *Mycobacterium terrae* ATCC 15755

Tab No. 8.1 Verification of methodology, temperature 20 °C, clean conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	109	$\Phi_{Nv0} = 99$	V_{c1}	97	$\Phi_A = 94.5$	V_{c1}	94	$\Phi_B = 96.5$	V_{c1}	92	$\Phi_C = 96$
V_{c2}	89		V_{c2}	92		V_{c2}	99		V_{c2}	100	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x yes		no	x yes		no	x yes		no	x yes		no

Tab No. 8.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 275 \times 10^7 = \lg 9.44$	10^{-7}	262	288	$\lg N_0 = \lg N/100 = \lg 7.44$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	31	24	$7.17 \leq \lg N_0 \leq 7.70$
				x yes / no

Tab No. 8.3 Testing the efficacy of chemical disinfectant **I A E E75 GEL** on *Mycobacterium terrae* ATCC 15755

Test concentration (%)/contact time (min) /conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.44$)**
100*/0.5/clean	10^{-1}	<14	<14	< 3.15	**≥ 4.29**
100*/1/clean	10^{-1}	<14	<14	< 3.15	**≥ 4.29**
100*/3/clean	10^{-1}	<14	<14	< 3.15	**≥ 4.29**

9. Evaluation of tuberculocidal activity of the product **I A E E75 GEL**

Tab No. 9.1 The efficacy of chemical disinfectant **I A E E75 GEL** on test strain – tuberculocidal activity

Tuberculocidal activity of the product (EN 14348)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14348	**lg R**
Mycobacterium terrae ATCC 15755	20	0.5	100*	clean	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	1	100*	clean	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	3	100*	clean	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before the dilution neutralization method, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of survivors per ml in control tests (A – experimental conditions control, B – neutralization validation, C – method validation), $R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Prepared by: Ing. Eva Kremlová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	10.3. – 17.3.2016
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Appearance of the product:	colourless liquid
Test concentration:	100%* (concentrated)
Contact time:	30 s (0.5 min), 60 s (1 min) and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K46046503, expiry date: 2016/09/30
Test virus:	*Vacciniavirus* strain Ankara (MVA)ATCC VR-1508 (2nd passage)**
Cell lines:	BHK-21 cells (ATCC CCL-10)
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of cell culture
3. Preparation of the test virus suspension
4. Test of viral infectivity
5. Virus titration with interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for virucidal activity of product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders. The test for virucidal activity against enveloped virus Vaccinia virus strain Ankara will cover all enveloped viruses only (Annex A, standard EN 14476:2013+A1:2015)
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml

The standard:
EN 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) September 2015

10. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Tab No. 10.1 Table of results of product **VITASEPT E75 GEL** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 0.5 min	- $\log_{10}$ $TCID_{50}$ after 1 min	- $\log_{10}$ $TCID_{50}$ after 3 min	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
VITASEPT E75 GEL	100%*	clean	4.50***	4.50***	4.50***	4.50***	-	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤ 1.50	-	-	-	5.50	4.50	4.17
			Virus titration, time = 0						
Virus control	-	PBS	6.50	-	-	-	6.50	6.50	6.33
Virus control	-	clean	6.50	6.50	6.50	6.50	-	-	-

Tab No. 10.2 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
100%*	6.50	clean	0.5 min	4.50***	**2.00**
100%*	6.50	clean	1 min	4.50***	**2.00**
100%*	6.50	clean	3 min	4.50***	**2.00**

11. Evaluation of virucidal activity of the product **VITASEPT E75 GEL**

Tab No. 11.1 The efficacy of chemical disinfectant **VITASEPT E75 GEL** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476+A1	$\Delta\log_{10}$ $TCID_{50}$
Vacciniavirus strain Ankara (MVA) ATCC VR-1508 **	20	0.5	100*	clean	≥ 4	< 4***
Vacciniavirus strain Ankara (MVA) ATCC VR-1508 **	20	1	100*	clean	≥ 4	< 4***
Vacciniavirus strain Ankara (MVA) ATCC VR-1508 **	20	3	100*	clean	≥ 4	< 4***

Note:

$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.

** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml

***It is not possible to express the virucidal activity because the cytotoxicity of the product after using MicroSpin™ S 400 HR is $10^{4.5}$ $TCID_{50}$/ml and the virus suspension was $10^{6.5}$ $TCID_{50}$/ml

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	18.3. – 29.3.2016
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Appearance of the product:	colourless liquid
Test concentration:	100%* (concentrated)**
Contact time:	30 s (0.5 min), 60 s (1 min) and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K46046503, expiry date: 2016/09/30
Test virus:	*Vaccinia virus* strain Elstree CAMP V-160 (3rd passage)
Cell lines:	VERO cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of cell culture
3. Preparation of the test virus suspension
4. Test of viral infectivity
5. Virus titration with interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for virucidal activity of product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders. The test for virucidal activity against enveloped virus Vaccinia virus strain Ankara will cover all enveloped viruses only (Annex A, standard EN 14476:2013+A1:2015)
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because of the cytotoxicity of the product

The standard:
EN 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

12. Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Vaccinia virus* strain Elstree CAMP V-160

Tab No. 12.1 Table of results of product **VITASEPT E75 GEL** on *Vaccinia virus* strain Elstree CAMP V-160

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 0.5 min	- $\log_{10}$ $TCID_{50}$ after 1 min	- $\log_{10}$ $TCID_{50}$ after 3 min	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
VITASEPT E75 GEL	100%*	clean	4.50**	4.50	4.50	4.50	-	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤ 1.50	-	-	-	8.50	7.50	6.50
			Virus titration, time = 0						
Virus control	-	PBS	9.50	-	-	-	9.50	9.50	9.33
Virus control	-	clean	9.50	9.50	9.50	9.50	-	-	-

Tab No. 12.2 Testing the efficacy of chemical disinfectant **VITASEPT E75 GEL** on *Vaccinia virus* strain Elstree CAMP V-160

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
100%*	9.50	clean	0.5 min	4.50	**5.00**
100%*	9.50	clean	1 min	4.50	**5.00**
100%*	9.50	clean	3 min	4.50	**5.00**

13. Evaluation of virucidal activity of the product **VITASEPT E75 GEL**

Tab No. 13.1 The efficacy of chemical disinfectant **VITASEPT E75 GEL** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476+A1	$\Delta\log_{10}$ $TCID_{50}$
Vaccinia virus strain Elstree CAMP V-160	20	0.5	100*	clean	≥ 4	> 4
Vaccinia virus strain Elstree CAMP V-160	20	1	100*	clean	≥ 4	> 4
Vaccinia virus strain Elstree CAMP V-160	20	3	100*	clean	≥ 4	> 4

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because of the cytotoxicity of the product

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml
Page: 14

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 1500)
Period of analysis:	2.3. – 3.3.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Appearance of the products:	colourless liquid
The test concentration:	100% (concentrated)
The volume of the product:	3 ml / person
The application time:	30 s
The soap:	soft soap from linseed oil 200 g/l
Reference item:	2-Propanol p.a., CAS 67-63-0, batch number: K46556334514, expiry date: 2020/03/31, concentration: 60% (V/V)
The volume of the reference propan-2-ol used per person:	2 x 3 ml, according to reference handrub procedure, the total application volume is 6 ml
The application time:	2 x 30 s, according to reference handrub procedure, the total application time is 1 min
Test organism:	*Escherichia coli* K 12 NCTC 10538 2.1×10^8 CFU/ml
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Treatment procedure:	hygienic handrub disinfection in accordance with the standard handrub procedure also include the instructions to keep hands wet with the product for a given time

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the test suspension of *Escherichia coli*
3. Determination of the number of viable cells of *Escherichia coli*
4. Prevalue – number of cfu sampled after the contamination with *Escherichia coli*
5. Postvalue – number of cfu sampled after the treatment with the disinfectant
6. Reduction factor – ratio of prevalues and postvalues, generally expressed by decimal logarithms

The standard:
EN 1500 Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2) April 2013

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

14. Preparation of the test suspension of *Escherichia coli* K 12 NCTC 10538 and verification of methodology

Tab No. 14.1 Verification of methodology

Validation of suspension (N_{V0})			Validation of suspension (N_{VB})			Neutralizer toxicity control (B)			Method validation (C)		
V_{c1}	120	$\Phi_{N_{V0}} = 122$	V_{c1}	117	$\Phi_{NVB} = 121.5$	V_{c1}	123	$\Phi_B = 116.5$	V_{c1}	115	$\Phi_C = 118.5$
V_{c2}	124		V_{c2}	126		V_{c2}	110		V_{c2}	122	
$30 \leq \Phi_{N_{V0}} \leq 160$			$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$			$\Phi_B \geq 0.0005\ \Phi_{NVB}$			$\Phi_C \geq 0.5\ \Phi_{N_{V0}}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 14.2 Test suspension

Test suspension N $\Phi = 210 \times 10^6 = 2.1 \times 10^8$ $\lg 2.1 \times 10^8 = 8.32$ $8.17 \leq \lg N \leq 8.70$				N	V_{c1}	V_{c1}	Weighted mean (Ø)		
				10^{-6}	203	215			
				10^{-7}	21	23	for N	$5 \leq Ø \leq 15$	9.50
				10^{-8}	<14	<14			
x	yes		no						

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

15. Experimental results

Tab. No. 15.1 Hygienic handrub – experimental results for RP and PP

Propan-2-ol 60% (V/V) 2×3 ml/person (6 ml), 2×0.5 min (1 min) – RP								**VITASEPT E75 GEL, 3 ml/person, 0.5 min – PP**							
Volunteer		**Number of CFU/plate from dilution 10^x**						**Volunteer**		**Number of CFU/plate from dilution 10^x**					
No.	**Hand**	**Prevalues**			**Postvalues**			**No.**	**Hand**	**Prevalues**			**Postvalues**		
		10^{-3}	**10^{-4}**	**10^{-5}**	**10^{0}**	**10^{-1}**	**10^{-2}**			**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{0}**	**10^{-1}**	**10^{-2}**
1	left	>330	239	26	104	<14	<14	1	left	>330	138	14	≤14	<14	<14
	right	>330	216	23	132	<14	<14		right	>330	154	15	≤14	<14	<14
2	left	>330	204	21	153	15	<14	2	left	>330	143	15	15	<14	<14
	right	>330	178	19	185	17	<14		right	>330	160	16	19	<14	<14
3	left	>330	92	<14	151	15	<14	3	left	>330	>330	144	≤14	<14	<14
	right	>330	78	<14	177	18	<14		right	>330	>330	168	≤14	<14	<14
4	left	>330	57	<14	>330	38	<14	4	left	>330	>330	156	≤14	<14	<14
	right	>330	75	<14	>330	34	<14		right	>330	>330	164	≤14	<14	<14
5	left	174	17	<14	106	<14	<14	5	left	>330	155	14	≤14	<14	<14
	right	158	16	<14	90	<14	<14		right	>330	143	14	≤14	<14	<14
6	left	>330	56	<14	156	16	<14	6	left	>330	>330	162	≤14	<14	<14
	right	>330	73	<14	172	17	<14		right	>330	>330	140	≤14	<14	<14
7	left	>330	251	25	14	<14	<14	7	left	>330	>330	189	≤14	<14	<14
	right	>330	242	22	16	<14	<14		right	>330	>330	215	≤14	<14	<14
8	left	>330	154	15	19	<14	<14	8	left	>330	262	27	≤14	<14	<14
	right	>330	162	18	15	<14	<14		right	>330	246	24	≤14	<14	<14
9	left	155	15	<14	92	<14	<14	9	left	>330	53	<14	15	<14	<14
	right	128	14	<14	79	<14	<14		right	>330	35	<14	15	<14	<14
10	left	155	16	<14	21	<14	<14	10	left	>330	>330	45	27	<14	<14
	right	131	<14	<14	27	<14	<14		right	>330	>330	35	33	<14	<14
11	left	>330	89	<14	235	23	<14	11	left	>330	>330	79	≤14	<14	<14
	right	>330	74	<14	252	27	<14		right	>330	>330	93	≤14	<14	<14
12	left	>330	81	<14	>330	46	<14	12	left	>330	123	<14	≤14	<14	<14
	right	>330	93	<14	>330	33	<14		right	>330	99	<14	≤14	<14	<14
13	left	>330	278	27	>330	203	21	13	left	>330	104	<14	≤14	<14	<14
	right	>330	261	24	>330	177	18		right	>330	134	<14	≤14	<14	<14
14	left	>330	213	20	>330	105	<14	14	left	>330	245	25	≤14	<14	<14
	right	>330	231	24	>330	123	<14		right	>330	213	23	≤14	<14	<14
15	left	>330	275	28	>330	164	16	15	left	>330	253	26	≤14	<14	<14
	right	>330	300	31	>330	181	18		right	>330	231	22	≤14	<14	<14
16	left	>330	192	21	>330	183	19	16	left	>330	205	19	≤14	<14	<14
	right	>330	216	22	>330	169	16		right	>330	234	22	≤14	<14	<14
17	left	167	16	<14	>330	45	<14	17	left	>330	199	17	≤14	<14	<14
	right	143	14	<14	>330	37	<14		right	>330	193	18	≤14	<14	<14
18	left	173	15	<14	57	<14	<14	18	left	>330	156	15	≤14	<14	<14
	right	147	14	<14	66	<14	<14		right	>330	141	14	≤14	<14	<14
19	left	328	35	<14	>330	42	<14	19	left	>330	>330	51	25	<14	<14
	right	309	33	<14	>330	34	<14		right	>330	>330	37	31	<14	<14
20	left	>330	34	<14	297	33	<14	20	left	>330	>330	48	32	<14	<14
	right	>330	38	<14	314	38	<14		right	>330	>330	60	36	<14	<14

underlined = count used for further computation, >330 = not countable

Prepared by: Mgr. Alena Rýdlová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Tab. No. 15.2 List of computed lg values (means of left and right hands) and lg reductions

Volunteer	Chronological Sequence	Reference handrub (RP) (Propan-2-ol 60% V/V)			Handrub with tested product (PP) (VITASEPT E75 GEL)		
		lg prevalues	lg postvalues	lg R	lg prevalues	lg postvalues	lg R
1	RP → PP	6.36	2.07	4.29	6.16	1.15	5.01
2	RP → PP	6.28	2.23	4.05	6.18	1.23	4.95
3	RP → PP	5.93	2.21	3.72	7.19	1.15	6.04
4	RP → PP	5.82	2.56	3.26	7.20	1.15	6.06
5	RP → PP	5.22	1.99	3.23	6.17	1.15	5.02
6	RP → PP	5.81	2.21	3.60	7.18	1.15	6.03
7	RP → PP	6.39	1.18	5.21	7.31	1.15	6.16
8	RP → PP	6.20	1.23	4.97	6.40	1.15	5.25
9	RP → PP	5.15	1.93	3.22	5.64	1.18	4.47
10	RP → PP	5.16	1.38	3.78	6.60	1.48	5.12
11	PP → RP	5.91	2.39	3.52	6.93	1.15	5.78
12	PP → RP	5.94	2.60	3.34	6.05	1.15	4.90
13	PP → RP	6.43	3.28	3.15	6.08	1.15	4.93
14	PP → RP	6.35	3.06	3.29	6.36	1.15	5.21
15	PP → RP	6.46	3.24	3.22	6.38	1.15	5.23
16	PP → RP	6.31	3.25	3.06	6.34	1.15	5.19
17	PP → RP	5.19	2.61	2.58	6.29	1.15	5.14
18	PP → RP	5.20	1.79	3.41	6.17	1.15	5.02
19	PP → RP	5.51	2.58	2.93	6.64	1.45	5.19
20	PP → RP	5.56	2.49	3.07	6.73	1.53	5.20
Ø	Overall	5.86	2.31	3.54	6.50	1.20	5.30
s		0.49	0.63	0.66	0.46	0.12	0.46
n		20	20	20	20	20	20
Ø	RP → PP	5.83	1.90	3.93	6.60	1.19	5.41
s		0.50	0.47	0.71	0.58	0.10	0.60
n		10	10	10	10	10	10
Ø	PP → RP	5.88	2.73	3.16	6.40	1.21	5.18
s		0.50	0.48	0.27	0.29	0.15	0.24
n		10	10	10	10	10	10

lg R = decimal log reduction; RP → PP sequence: first RP, second PP; PP → RP sequence: first PP, second RP; Ø = mean; s = standard deviation; n = number of values (= volunteers)
Difference of mean Rs (RP → PP): 3.93 - 5.41 = -1.48; Difference of mean Rs (PP → RP): 3.16 - 5.18 = -2.02; Absolute difference of differences: |(-1.48)-(-2.02)| = 0.54 (hence less than 2.00)

Acceptance criteria for test results:
Only if the results of the test procedure fulfil the following requirements, they shall be accepted for further evaluation, otherwise the test shall be repeated:
a) A complete set of results from at least 18 volunteers shall be available. All complete sets of results shall be used for further evaluation.
b) The overall means of the lg prevalues for RP and PP shall be both at least 5.00.
c) Not more than three individual lg reductions less than 3.00 shall occur in RP.
d) The absolute diference of mean differences between lg reductions of RP and PP of group RP → PP and group PP → RP shall be less than 2.00.
e) All quotients of weighted mean counts between 5 and 15.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Tab. No. 15.3.1 Computation of individual differences of lg Rs of RP – PP

Volunteer	lg reduction (R)		Difference RP – PP
	Reference procedure (RP)	Product procedure (PP)	
1	4.29	5.01	-0.72
2	4.05	4.95	-0.90
3	3.72	6.04	-2.32
4	3.26	6.06	-2.80
5	3.23	5.02	-1.79
6	3.60	6.03	-2.43
7	5.21	6.16	-0.95
8	4.97	5.25	-0.28
9	3.22	4.47	-1.25
10	3.78	5.12	-1.34
11	3.52	5.78	-2.26
12	3.34	4.90	-1.56
13	3.15	4.93	-1.78
14	3.29	5.21	-1.92
15	3.22	5.23	-2.01
16	3.06	5.19	-2.13
17	2.58	5.14	-2.56
18	3.41	5.02	-1.61
19	2.93	5.19	-2.26
20	3.07	5.20	-2.13

Tab. No. 15.3.2 Computation for Hodges-Lehmann 97.5% upper confidence limits

	Sorted differences	Mean pairwise differences $(d_i+d_{ii})/2$									
		-0.28	-0.72	-0.90	-0.95	-1.25	-1.34	-1.56	-1.61	-1.78	-1.79
1	-0.28	-0.28^{1}									
2	-0.72	-0.50^{2}	-0.72^{5}								
3	-0.90	-0.59^{3}	-0.81^{8}	-0.90^{10}							
4	-0.95	-0.62^{4}	-0.84^{9}	-0.93^{12}	-0.95^{14}						
5	-1.25	-0.77^{6}	-0.99^{15}	-1.08^{19}	-1.10^{21}	-1.25^{31}					
6	-1.34	-0.81^{7}	-1.03^{17}	-1.12^{22}	-1.15^{25}	-1.30^{39}	-1.34^{42}				
7	-1.56	-0.92^{11}	-1.14^{23}	-1.23^{29}	-1.26^{34}	-1.41^{49}	-1.45	-1.56			
8	-1.61	-0.95^{13}	-1.17^{26}	-1.26^{33}	-1.28^{37}	$\mathbf{-1.43^{53}}$	-1.48	-1.59	-1.61		
9	-1.78	-1.03^{16}	-1.25^{30}	-1.34^{41}	-1.37^{46}	-1.52	-1.56	-1.67	-1.70	-1.78	
10	-1.79	-1.04^{18}	-1.26^{32}	-1.35^{43}	-1.37^{47}	-1.52	-1.57	-1.68	-1.70	-1.79	-1.79
11	-1.92	-1.10^{20}	-1.32^{40}	-1.41^{48}	-1.44	-1.59	-1.63	-1.74	-1.77	-1.85	-1.86
12	-2.01	-1.15^{24}	-1.37^{45}	-1.46	-1.48	-1.63	-1.68	-1.79	-1.81		
13	-2.13	-1.21^{27}	-1.43^{51}	-1.52	-1.54	-1.69	-1.74	-1.85			
14	-2.13	-1.21^{28}	-1.43^{52}	-1.52	-1.54	-1.69	-1.74	-1.85			
15	-2.26	-1.27^{35}	-1.49	-1.58	-1.61	-1.76	-1.80				
16	-2.26	-1.27^{36}	-1.49	-1.58	-1.61	-1.76	-1.80				
17	-2.32	-1.30^{38}	-1.52	-1.61	-1.64	-1.79	-1.83				
18	-2.43	-1.36^{44}	-1.58	-1.67	-1.69	-1.84					
19	-2.56	-1.42^{50}	-1.64	-1.73	-1.76						
20	-2.80	-1.54	-1.76	-1.85							

The median is between the 10th and 11th value: [(-1.79) + (-1.92)]/2 = -1.86
The mean pairwise differences that do not exceed the median (here: -1.86) are computed. From table (see Table E.5 in EN 1500) of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n=20 and a one-sided 0.025 level of significance, the critical value of 52 is found. **Hence c=52+1=53.** The pairwise differences are sorted in descending order (small exponents). **The 53rd value is -1.43.** Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is -1.43, which is less than the agreed inferiority margin of 0.6. Therefore the hypothesis of inferiority of PP is rejected and it can be concluded the test preparation PP is non-inferior to RP.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 12791)
Period of analysis:	7.3. – 18.3.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Appearance of the product:	colourless liquid
Concentration:	100% (concentrated)
The volume of the product used per person:	2 x 3 ml, according to information provided by the manufacturer, the total application volume is 6 ml
The application time:	1.5 min, according to information provided by the manufacturer
The soap:	soft soap from linseed oil
Reference item:	CAS 71-23-8 1-Propanol p.a., batch number: K45273097410, expiry date: 31.1.2019, 60% (V/V)
The volume of the reference propan-1-ol used per person:	2 x 3 ml, according to reference surgical hand disinfection procedure, the total application volume is 6 ml
The application time:	2 x 1.5 min, according to reference surgical hand disinfection procedure, the total application time is 3 min
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Surgical hand disinfection procedure with product:	surgical hand disinfection in accordance with the standard (rubbing hands) 3-hour effect - reduction of the release of skin flora from hands as assessed after wearing surgical gloves for 3 h following disinfection sustained effect - 3-hour effect of the product significantly larger than that of a reference disinfection procedure with propan-1-ol 60% (V/V)

Test procedure:

1. Determination of the presence of microorganisms in the product
2. Determination of the prevalue – number of cfu sampled immediately before treatment from the hand
3. Determination of the postvalue – number of cfu sampled after treatment from one hand immediately and from other hand after wearing surgical gloves for 3 h following disinfection
4. Expression and interpretation of results - reduction factor – ratio of prevalue and postvalue, generally expressed by decimal logarithms

The standard:
BS EN 12791 Chemical disinfectants and antiseptics – Surgical hand disinfection - Test method and requirements (phase 2/step 2) February 2016

CONFIDENTIAL

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

16. Experimental results

Tab. No. 16.1.1 Surgical hand disinfection procedure – experimental results for RP
(Propan-1-ol 60% V/V – 2 x 3 ml, 2 x 1.5 min)

Volunteer			Number of CFU per plate from dilution 10^x								
			Prevalues			Immediate postvalues			3 h postvalues		
No	Sequence	Hand (left or right)	10^{-1}	10^{-2}	10^{-3}	10^{0}	10^{-1}	10^{-2}	10^{0}	10^{-1}	10^{-2}
1	RP → PP	l	>330	33	2	>330	38	4			
		r	238	25	1				>330	34	3
2	RP → PP	l	304	28	3				150	16	1
		r	285	26	3	217	24	2			
3	RP → PP	l	>330	>330	66	>330	>330	51			
		r	>330	>330	70				>330	>330	35
4	RP → PP	l	>330	>330	63	>330	>330	56			
		r	>330	>330	66				>330	249	27
5	RP → PP	l	>330	>330	59	189	21	0			
		r	>330	271	25				189	20	2
6	RP → PP	l	>330	>330	46	>330	47	5			
		r	>330	92	8				203	17	1
7	RP → PP	l	>330	>330	71				>330	85	9
		r	>330	>330	65	>330	63	6			
8	RP → PP	l	>330	>330	67	>330	>330	58			
		r	>330	>330	68				>330	152	14
9	RP → PP	l	>330	237	27	>330	151	14			
		r	>330	94	10				>330	34	4
10	RP → PP	l	>330	81	8				>330	50	6
		r	>330	299	35	>330	238	26			
11	RP → PP	l	>330	95	9	>330	46	5			
		r	>330	86	7				>330	45	5
12	RP → PP	l	>330	91	8	>330	60	7			
		r	>330	84	8				>330	52	6
13	PP → RP	l	>330	75	7				199	21	3
		r	>330	50	4	285	26	3			
14	PP → RP	l	>330	75	8	>330	42	4			
		r	>330	69	6				258	25	2
15	PP → RP	l	197	23	2				226	26	1
		r	219	24	2	198	23	1			
16	PP → RP	l	268	29	3				204	16	2
		r	167	19	1	149	17	0			
17	PP → RP	l	>330	92	9	>330	296	34			
		r	>330	>330	67				>330	>330	64
18	PP → RP	l	>330	103	11	>330	195	24			
		r	>330	>330	45				>330	>330	56
19	PP → RP	l	>330	228	25	204	17	3			
		r	>330	>330	43				>330	54	6
20	PP → RP	l	>330	288	29				>330	33	3
		r	>330	172	15	228	26	2			
21	PP → RP	l	>330	>330	53				>330	206	24
		r	>330	147	18	>330	>330	87			
22	PP → RP	l	>330	>330	65				>330	234	19
		r	>330	104	10	>330	>330	45			
23	PP → RP	l	>330	>330	78	223	18	2			
		r	>330	>330	85				267	29	3
24	PP → RP	l	>330	>330	63	>330	78	6			
		r	>330	>330	68				>330	75	8

underlined = count used for further computation, >330 = not countable

Prepared by: Mgr. Alena Rýdlová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

CONFIDENTIAL

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Tab. No. 16.1.2 Surgical hand disinfection procedure – experimental results for PP
(VITASEPT E75 GEL – 2 x 3 ml, 1.5 min)

Volunteer			Number of CFU per plate from dilution 10^x								
			Prevalues			Immediate postvalues			3 h postvalues		
No	Sequence	Hand (left or right)	10^{-1}	10^{-2}	10^{-3}	10^{0}	10^{-1}	10^{-2}	10^{0}	10^{-1}	10^{-2}
1	RP → PP	l	153	14	2	0	0	0			
		r	>330	>330	53				>330	69	7
2	RP → PP	l	167	19	2	0	0	0			
		r	>330	>330	66				>330	34	3
3	RP → PP	l	>330	297	35				0	0	0
		r	>330	276	31	0	0	0			
4	RP → PP	l	>330	283	25	0	0	0			
		r	>330	262	24				0	0	0
5	RP → PP	l	>330	>330	76				268	28	3
		r	>330	>330	62	0	0	0			
6	RP → PP	l	>330	>330	72				206	24	2
		r	>330	>330	42	0	0	0			
7	RP → PP	l	>330	>330	36	0	0	0			
		r	>330	304	27				248	27	2
8	RP → PP	l	>330	>330	59	0	0	0			
		r	>330	>330	46				307	33	4
9	RP → PP	l	>330	>330	47				>330	48	5
		r	227	25	2	0	0	0			
10	RP → PP	l	>330	103	9	0	0	0			
		r	>330	>330	39				>330	45	4
11	RP → PP	l	>330	197	22	0	0	0			
		r	>330	>330	40				179	21	1
12	RP → PP	l	>330	64	6	0	0	0			
		r	>330	305	25				204	16	2
13	PP → RP	l	>330	>330	68				>330	157	17
		r	>330	>330	72	0	0	0			
14	PP → RP	l	>330	>330	49				>330	83	9
		r	>330	>330	69	0	0	0			
15	PP → RP	l	>330	203	16	0	0	0			
		r	>330	>330	34				0	0	0
16	PP → RP	l	>330	>330	58				0	0	0
		r	>330	73	6	0	0	0			
17	PP → RP	l	>330	287	30				0	0	0
		r	>330	>330	65	0	0	0			
18	PP → RP	l	>330	>330	49	0	0	0			
		r	>330	297	34				0	0	0
19	PP → RP	l	>330	68	7	0	0	0			
		r	>330	239	26				0	0	0
20	PP → RP	l	>330	82	9	0	0	0			
		r	>330	172	15				0	0	0
21	PP → RP	l	>330	321	34				0	0	0
		r	>330	>330	66	0	0	0			
22	PP → RP	l	>330	>330	53	0	0	0			
		r	>330	45	5				0	0	0
23	PP → RP	l	>330	>330	82				167	20	2
		r	>330	>330	159	0	0	0			
24	PP → RP	l	>330	>330	68				148	18	0
		r	>330	>330	91	0	0	0			

underlined = count used for further computation, >330 = not countable
Prepared by: Mgr. Alena Rýdlová, Lab Technician

Tab. No. 16.2.1 List of computed lg values and lg reductions – immediate effect

Volunteer	Chronological Sequence	Reference handrub (RP) (Propan-1-ol 60% V/V)			Handrub with tested product (PP) (VITASEPT E75 GEL)		
		lg prevalues	lg postvalues	lg R	lg prevalues	lg postvalues	lg R
1	RP → PP	3.52	2.58	0.94	3.18	0.00	3.18
2	RP → PP	3.45	2.34	1.11	3.23	0.00	3.23
3	RP → PP	4.82	3.71	1.11	4.45	0.00	4.45
4	RP → PP	4.80	3.75	1.05	4.45	0.00	4.45
5	RP → PP	4.77	2.28	2.49	4.79	0.00	4.79
6	RP → PP	4.66	2.67	1.99	4.62	0.00	4.62
7	RP → PP	4.81	2.80	2.01	4.56	0.00	4.56
8	RP → PP	4.83	3.76	1.07	4.77	0.00	4.77
9	RP → PP	4.38	3.18	1.20	3.36	0.00	3.36
10	RP → PP	4.48	3.38	1.10	4.01	0.00	4.01
11	RP → PP	3.98	2.66	1.32	4.30	0.00	4.30
12	RP → PP	3.96	2.78	1.18	3.81	0.00	3.81
13	PP → RP	3.70	2.45	1.25	4.86	0.00	4.86
14	PP → RP	3.88	2.62	1.26	4.84	0.00	4.84
15	PP → RP	3.34	2.30	1.04	4.30	0.00	4.30
16	PP → RP	3.23	2.18	1.05	3.86	0.00	3.86
17	PP → RP	3.96	3.48	0.48	4.81	0.00	4.81
18	PP → RP	4.01	3.30	0.71	4.69	0.00	4.69
19	PP → RP	4.36	2.30	2.06	3.83	0.00	3.83
20	PP → RP	4.23	2.36	1.87	3.91	0.00	3.91
21	PP → RP	4.18	3.94	0.24	4.82	0.00	4.82
22	PP → RP	4.02	3.65	0.37	4.72	0.00	4.72
23	PP → RP	4.89	2.34	2.55	5.20	0.00	5.20
24	PP → RP	4.80	2.89	1.91	4.96	0.00	4.96
Ø	Overall	4.21	2.90	1.31	4.35	0.00	4.35
s		0.52	0.58	0.62	0.57	0.00	0.57
n		24	24	24	24	24	24
Ø	RP → PP	4.37	2.99	1.38	4.13	0.00	4.13
s		0.52	0.54	0.50	0.60	0.00	0.60
n		12	12	12	12	12	12
Ø	PP → RP	4.05	2.82	1.23	4.57	0.00	4.57
s		0.50	0.62	0.73	0.47	0.00	0.47
n		12	12	12	12	12	12

lg R = decimal log reduction; RP → PP sequence: first RP, second PP; PP → RP sequence: first PP, second RP;
Ø = mean; s = standard deviation; n = number of values (= volunteers)
Difference of mean Rs (RP → PP): 1.38 - 4.13 = -2.75; Difference of mean Rs (PP → RP): 1.23 - 4.57 = -3.34;
Absolute difference of differences: |(-2.75) - (-3.34)| = 0.59

Acceptance criteria for test results:
Only if the results of the test procedure fulfil the following requirements, they shall be accepted for further evaluation, otherwise the test shall be repeated:
a) A complete set of results from at least 23 volunteers shall be available. All complete sets of results shall be used for further evaluation.
b) The overall means of the lg prevalues for RP and PP shall be both at least 3.50.
c) The absolute difference of mean differences between lg reductions of RP and PP of group RP → PP and group PP → RP shall be less than 2.00.
d) All quotients of weighted mean counts between 5 and 15.

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Tab. No. 16.2.2 List of computed lg values and lg reductions – 3 h effect

Volunteer	Chronological Sequence	Reference handrub (RP) (Propan-1-ol 60% V/V)			Handrub with tested product (PP) (VITASEPT E75 GEL)		
		lg prevalues	lg postvalues	lg R	lg prevalues	lg postvalues	lg R
1	RP → PP	3.38	2.53	0.85	4.72	2.84	1.88
2	RP → PP	3.48	2.18	1.30	4.82	2.53	2.29
3	RP → PP	4.85	3.54	1.31	4.48	0.00	4.48
4	RP → PP	4.82	3.40	1.42	4.41	0.00	4.41
5	RP → PP	4.43	2.28	2.15	4.88	2.43	2.45
6	RP → PP	3.96	2.30	1.66	4.86	2.32	2.54
7	RP → PP	4.85	2.93	1.92	4.48	2.40	2.08
8	RP → PP	4.83	3.18	1.65	4.66	2.49	2.17
9	RP → PP	3.97	2.53	1.44	4.67	2.68	1.99
10	RP → PP	3.91	2.70	1.21	4.59	2.65	1.94
11	RP → PP	3.93	2.65	1.28	4.60	2.26	2.34
12	RP → PP	3.92	2.72	1.20	4.48	2.30	2.18
13	PP → RP	3.88	2.30	1.58	4.83	3.20	1.63
14	PP → RP	3.84	2.41	1.43	4.69	2.92	1.77
15	PP → RP	3.30	2.36	0.94	4.53	0.00	4.53
16	PP → RP	3.43	2.30	1.13	4.76	0.00	4.76
17	PP → RP	4.83	3.81	1.02	4.46	0.00	4.46
18	PP → RP	4.65	3.75	0.90	4.48	0.00	4.48
19	PP → RP	4.63	2.73	1.90	4.38	0.00	4.38
20	PP → RP	4.46	2.52	1.94	4.23	0.00	4.23
21	PP → RP	4.72	3.32	1.40	4.51	0.00	4.51
22	PP → RP	4.81	3.36	1.45	3.65	0.00	3.65
23	PP → RP	4.93	2.43	2.50	4.91	2.23	2.68
24	PP → RP	4.83	2.88	1.95	4.83	2.18	2.65
Ø	Overall	4.28	2.80	1.48	4.58	1.48	3.10
s		0.56	0.50	0.42	0.27	1.29	1.15
n		24	24	24	24	24	24
Ø	RP → PP	4.20	2.74	1.45	4.64	2.08	2.56
s		0.54	0.44	0.35	0.16	0.98	0.90
n		12	12	12	12	12	12
Ø	PP → RP	4.36	2.85	1.51	4.52	0.88	3.64
s		0.58	0.57	0.49	0.34	1.32	1.15
n		12	12	12	12	12	12

lg R = decimal log reduction; RP → PP sequence: first RP, second PP; PP → RP sequence: first PP, second RP;
Ø = mean; s = standard deviation; n = number of values (= volunteers)
Difference of mean Rs (RP → PP): 1.45 - 2.56 = -1.11; Difference of mean Rs (PP → RP): 1.51 - 3.64 = -2.13;
Absolute difference of differences: |(-1.11) - (-2.13)| = 1.02

Acceptance criteria for test results:
Only if the results of the test procedure fulfil the following requirements, they shall be accepted for further evaluation, otherwise the test shall be repeated:
a) A complete set of results from at least 23 volunteers shall be available. All complete set sof results shall be used for further evaluation.
b) The overall means of the lg prevalues for RP and PP shall be both at least 3.50.
c) The absolute difference of mean differences between lg reductions of RP and PP of group RP → PP and group PP → RP shall be less than 2.00.
d) All quotients of weighted mean counts between 5 and 15.

Tab. No. 16.3 Individual differences of lg Rs between RP and PP for immediate and 3 h effects

Volunteer	lg R immediate effect			lg R 3 h effect		
	RP	PP	Difference RP-PP	RP	PP	Difference RP-PP
1	0.94	3.18	-2.24	0.85	1.88	-1.03
2	1.11	3.23	-2.12	1.30	2.29	-0.99
3	1.11	4.45	-3.34	1.31	4.48	-3.17
4	1.05	4.45	-3.40	1.42	4.41	-2.99
5	2.49	4.79	-2.30	2.15	2.45	-0.30
6	1.99	4.62	-2.63	1.66	2.54	-0.88
7	2.01	4.56	-2.55	1.92	2.08	-0.16
8	1.07	4.77	-3.70	1.65	2.17	-0.52
9	1.20	3.36	-2.16	1.44	1.99	-0.55
10	1.10	4.01	-2.91	1.21	1.94	-0.73
11	1.32	4.30	-2.98	1.28	2.34	-1.06
12	1.18	3.81	-2.63	1.20	2.18	-0.98
13	1.25	4.86	-3.61	1.58	1.63	-0.05
14	1.26	4.84	-3.58	1.43	1.77	-0.34
15	1.04	4.30	-3.26	0.94	4.53	-3.59
16	1.05	3.86	-2.81	1.13	4.76	-3.63
17	0.48	4.81	-4.33	1.02	4.46	-3.44
18	0.71	4.69	-3.98	0.90	4.48	-3.58
19	2.06	3.83	-1.77	1.90	4.38	-2.48
20	1.87	3.91	-2.04	1.94	4.23	-2.29
21	0.24	4.82	-4.58	1.40	4.51	-3.11
22	0.37	4.72	-4.35	1.45	3.65	-2.20
23	2.55	5.20	-2.65	2.50	2.68	-0.18
24	1.91	4.96	-3.05	1.95	2.65	-0.70

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Tab. No. 16.4.1 Computation for Hodges-Lehmann 97.5% upper confidence limit for the **immediate effect**

	Sorted differences	Mean pairwise differences $(d_i+d_{ii})/2$											
		-1.77	**-2.04**	**-2.12**	**-2.16**	**-2.24**	**-2.30**	**-2.55**	**-2.63**	**-2.63**	**-2.65**	**-2.81**	**-2.91**
1	-1,77	$-1,77^{1}$											
2	-2,04	$-1,91^{2}$	$-2,04^{7}$										
3	-2,12	$-1,95^{3}$	$-2,08^{8}$	$-2,12^{10}$									
4	-2,16	$-1,97^{4}$	$-2,10^{9}$	$-2,14^{12}$	$-2,16^{14}$								
5	-2,24	$-2,01^{5}$	$-2,14^{11}$	$-2,18^{16}$	$-2,20^{19}$	$-2,24^{23}$							
6	-2,30	$-2,04^{6}$	$-2,17^{15}$	$-2,21^{21}$	$-2,23^{22}$	$-2,27^{24}$	$-2,30^{27}$						
7	-2,55	$-2,16^{13}$	$-2,30^{26}$	$-2,34^{31}$	$-2,36^{33}$	$-2,40^{40}$	$-2,43^{44}$	$-2,55^{61}$					
8	-2,63	$-2,20^{17}$	$-2,34^{29}$	$-2,38^{35}$	$-2,40^{38}$	$-2,44^{45}$	$-2,47^{49}$	$-2,59^{68}$	$-2,63^{74}$				
9	-2,63	$-2,20^{18}$	$-2,34^{30}$	$-2,38^{36}$	$-2,40^{39}$	$-2,44^{46}$	$-2,47^{50}$	$-2,59^{69}$	$-2,63^{75}$	$-2,63^{76}$			
10	-2,65	$-2,21^{20}$	$-2,35^{32}$	$-2,39^{37}$	$-2,41^{42}$	$-2,45^{47}$	$-2,48^{52}$	$-2,60^{70}$	$-2,64^{78}$	$-2,64^{79}$	$\mathbf{-2,65^{82}}$		
11	-2,81	$-2,29^{25}$	$-2,43^{43}$	$-2,47^{48}$	$-2,49^{53}$	$-2,53^{57}$	$-2,56^{63}$	-2,68	-2,72	-2,72	-2,73	-2,81	-2,91
12	-2,91	$-2,34^{28}$	$-2,48^{51}$	$-2,52^{56}$	$-2,54^{58}$	$-2,58^{65}$	$-2,61^{73}$	-2,73	-2,77	-2,77	-2,78	-2,86	-2,95
13	-2,98	$-2,38^{34}$	$-2,51^{54}$	$-2,55^{60}$	$-2,57^{64}$	$-2,61^{72}$	$-2,64^{77}$	-2,77	-2,81	-2,81	-2,82	-2,90	
14	-3,05	$-2,41^{41}$	$-2,55^{59}$	$-2,59^{67}$	$-2,61^{71}$	$-2,65^{81}$	-2,68	-2,80	-2,84	-2,84	-2,85	-2,93	
15	-3,26	$-2,52^{55}$	$-2,65^{80}$	-2,69	-2,71	-2,75	-2,78	-2,91	-2,95	-2,95			
16	-3,34	$-2,56^{62}$	-2,69	-2,73	-2,75	-2,79	-2,82	-2,95					
17	-3,40	$-2,59^{66}$	-2,72	-2,76	-2,78	-2,82	-2,85						
18	-3,58	-2,68	-2,81	-2,85	-2,87	-2,91	-2,94						
19	-3,61	-2,69	-2,83	-2,87	-2,89	-2,93							
20	-3,70	-2,74	-2,87	-2,91	-2,93								
21	-3,98	-2,88											
22	-4,33												
23	-4,35												
24	-4,58												

The median is between the 12th and 13th value: [(-2.91 + (-2.98)]/2 = -2.95 The mean pairwise differences that do not exceed the median (here: -2.95) are computed. From table D.1 in EN 12791 of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n =24 and a one-sided 0.025 level of significance, the critical value of 81 is found. **Hence c=81+1=82.** The pairwise differences are sorted in descending order (small exponents). **The 82nd value is -2.65.** Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is -2.65, which is less than the agreed inferiority margin of 0.75. Therefore the hypothesis of the immediate effect of PP versus RP can be rejected.

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml
Page: 26

Tab. No. 16.4.2 Computation for Hodges-Lehmann 97.5% upper confidence limit for the **3 h effect**

	Sorted differences	Mean pairwise differences $(d_i+d_{ii})/2$											
		-0.05	**-0.16**	**-0.18**	**-0.30**	**-0.34**	**-0.52**	**-0.55**	**-0.70**	**-0.73**	**-0.88**	**-0.98**	**-0.99**
1	-0,05	$-0,05^{1}$											
2	-0,16	$-0,11^{2}$	$-0,16^{4}$										
3	-0,18	$-0,12^{3}$	$-0,17^{5}$	$-0,18^{7}$									
4	-0,30	$-0,18^{6}$	$-0,23^{9}$	$-0,24^{10}$	$-0,30^{15}$								
5	-0,34	$-0,20^{8}$	$-0,25^{11}$	$-0,26^{12}$	$-0,32^{16}$	$-0,34^{18}$							
6	-0,52	$-0,29^{13}$	$-0,34^{17}$	$-0,35^{19}$	$-0,41^{24}$	$-0,43^{27}$	$-0,52^{39}$						
7	-0,55	$-0,30^{14}$	$-0,36^{20}$	$-0,37^{21}$	$-0,43^{26}$	$-0,45^{30}$	$-0,54^{43}$	$-0,55^{44}$					
8	-0,70	$-0,38^{22}$	$-0,43^{25}$	$-0,44^{28}$	$-0,50^{33}$	$-0,52^{38}$	$-0,61^{55}$	$-0,63^{58}$	$-0,70^{69}$				
9	-0,73	$-0,39^{23}$	$-0,45^{29}$	$-0,46^{31}$	$-0,52^{37}$	$-0,54^{42}$	$-0,63^{57}$	$-0,64^{60}$	$-0,72^{71}$	$-0,73^{72}$			
10	-0,88	$-0,47^{32}$	$-0,52^{36}$	$-0,53^{40}$	$-0,59^{50}$	$-0,61^{54}$	$-0,70^{68}$	$-0,72^{70}$	$-0,79^{80}$	**$-0,81^{82}$**	-0,88		
11	-0,98	$-0,52^{34}$	$-0,57^{46}$	$-0,58^{48}$	$-0,64^{59}$	$-0,66^{62}$	$-0,75^{73}$	$-0,77^{75}$	-0,84	-0,86	-0,93	-0,98	-0,99
12	-0,99	$-0,52^{35}$	$-0,58^{47}$	$-0,59^{49}$	$-0,65^{61}$	$-0,67^{64}$	$-0,76^{74}$	$-0,77^{76}$	-0,85	-0,86	-0,94	-0,99	-1,01
13	-1,03	$-0,54^{41}$	$-0,60^{51}$	$-0,61^{53}$	$-0,67^{63}$	$-0,69^{66}$	$-0,78^{77}$	$-0,79^{79}$	-0,87	-0,88	-0,96	-1,01	
14	-1,06	$-0,56^{45}$	$-0,61^{52}$	$-0,62^{56}$	$-0,68^{65}$	$-0,70^{67}$	$-0,79^{78}$	$-0,81^{81}$	-0,88	-0,90	-0,97		
15	-2,20												
16	-2,29												
17	-2,48												
18	-2,99												
19	-3,11												
20	-3,17												
21	-3,44												
22	-3,58												
23	-3,59												
24	-3,63												

The median is between the 12th and 13th value: [(-0.99 + (-1.03)]/2 = -1.01 The mean pairwise differences that do not exceed the median (here: -1.01) are computed. From table D.1 in EN 12791 of critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n =24 and a one-sided 0.025 level of significance, the critical value of 81 is found. **Hence c=81+1=82.** The pairwise differences are sorted in descending order (small exponents). **The 82nd value is -0.81.** Hence the Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is -0.81, which is less than the agreed inferiority margin of 0.85. Therefore the hypothesis of the immediate effect of PP versus RP can be rejected.

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml

Interpretation:
Results of tests are in Tabs.
The tested concentrated* product **VITASEPT E75 GEL**, batch No. 91215, in contact times 30 s, 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli K12* NCTC 10538 by at least 5 (lg) orders (EN 13727:2012+A1).

The tested concentrated* product **VITASEPT E75 GEL**, batch No. 91215, in contact times 30 s, 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Candida albicans* ATCC 10231 by at least 4 (lg) orders (EN 13624).

The tested concentrated* product **VITASEPT E75 GEL**, batch No. 91215, in contact times 30 s, 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Mycobacterium terrae* ATCC 15755 by 4 (lg) orders (EN 14348).
* The product can only be tested at a concentration of 97% (RTU) or less as some dilution is always produced by adding the test organisms and the interfering substance.

The tested concentrated* product **VITASEPT E75 GEL**, batch No. 91215, in contact times 30 s, 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C **did not prove***** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 ** particles under defined conditions by 4 (lg) orders (EN 14476:2013 +A1:2015).
* The product can only be tested at a concentration of 97% (RTU) or less as some dilution is always produced by adding the test organisms and the interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml
***It is not possible to express the virucidal activity because the cytotoxicity of the product after using MicroSpin™ S 400 HR is $10^{4.5}$ $TCID_{50}$/ml and the virus suspension was $10^{6.5}$ $TCID_{50}$/ml only.

The tested concentrated* product **VITASEPT E75 GEL**, batch No. 91215, in contact times 30 s, 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Vaccinia virus* strain Elstree CAMP V-160 particles under defined conditions by at least 4 (lg) orders (EN 14476:2013 +A1:2015).
* The product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because of the cytotoxicity of the product

Hygienic handrub
The acceptance criteria for the test results were met.
The product **VITASEPT E75 GEL**, batch No. 91215, was tested according to EN 1500 under test conditions: application volume 3 ml/person and application time 30 s. The Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is smaller (**-1.43**) than the agreed inferiority margin of 0.6. Therefore the hypothesis of inferiority of **VITASEPT E75 GEL** is rejected and it can be concluded the test preparation **VITASEPT E75 GEL** is non-inferior to **propan-2-ol 60%**.

CONFIDENTIAL

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D35/2016
Rep No: 39
Sample name: **VITASEPT E75 GEL**
Sampled: by client
Sampling point: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Client: Goodpoint Chemicals OÜ, Vabaõhumuuseumi tee 3, Tallinn 13522, Estonia
Batch No: 91215

Sampling date: 25.1.2016
Sample delivered: 29.1.2016
Testing date: 19.2. – 29.3.2016
Delivered amount: 2 x 500 ml
Page: 28

Interpretation:
Results of tests are in Tabs.
Surgical hand disinfection
The acceptance criteria for the test results were met.
The product **VITASEPT E75 GEL**, batch No. 91215, was tested according to BS EN 12791 for **immediate effect** under test conditions: application volume 2 x 3 ml/person and application time 1.5 min.
For statistical evaluation of the immediate effect of PP critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n = 24 and a one-sided p = 0.025 level of significance, the critical value of 81 is found. **Hence c = 81+1 = 82.** The pairwise differences are sorted in descending order (small exponents). **The 82nd value is -2.65.**
The Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is smaller (-2.65) than the agreed inferiority margin of 0.75. Therefore the hypothesis of inferiority of **VITASEPT E75 GEL** can be rejected and it can be concluded that the test preparation **VITASEPT E75 GEL** is non-inferior to **propan-1-ol 60%.**

The product **VITASEPT E75 GEL**, batch No. 91215, was tested according to BS EN 12791 for **3 h effect** under test conditions: application volume 2 x 3 ml/person and application time 1.5 min.
For statistical evaluation of the 3 h effect of PP critical values for Wilcoxon's matched-pairs signed-ranks test the entry for n = 24 and a one-sided p = 0.025 level of significance, the critical value of 81 is found. **Hence c = 81+1 = 82.** The pairwise differences are sorted in descending order (small exponents). **The 82nd value is -0.81.**
The Hodges-Lehmann upper one-sided 97.5% confidence limit for the difference in lg Rs between RP and PP is smaller (-0.81) than the agreed inferiority margin of 0.85. Therefore the hypothesis of inferiority of **VITASEPT E75 GEL** can be rejected and it can be concluded that the test preparation **VITASEPT E75 GEL** is non-inferior to **propan-1-ol 60%.**

Conclusion:
The product **VITASEPT E75 GEL** is capable of reducing the number of viable bacterial, vegetative yeast and mycobacterial cells of the relevant organisms in the suspension and on carriers under defined conditions to the declared values, and consequently, may be called bactericidal, yeasticidal and tuberculocidal.
The product **VITASEPT E75 GEL** is capable of reducing the number of infectious *Vaccinia virus* strain Elstree particles under defined conditions to the declared values, and consequently, may be called virucidal on *Vaccinia virus* strain Elstree.
The product **VITASEPT E75 GEL** is deemed suitable to be used as medical hygienic handrub under conditions: application volume 3 ml/person and application time 30 s.
The product **VITASEPT E75 GEL** is suitable to be used as surgical hand disifection for both **immediate and 3 h effect** under test conditions: application volume 2 x 3 ml/person and application time 1.5 min.

3.5.2016, Hodonín

Ing. Eva Kremlová, Leader of Study