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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute.

Copy No.: 1
Issue No.: 1

Test report No. D166-3/2013

DETERMINATION OF BACTERICIDAL (EN 13727) AND YEASTICIDAL (EN 13624) ACTIVITY OF THE PRODUCT **VIRUTON FORTE**

Sample ID: D166/2013

Sample name: **Viruton Forte**

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

Producer: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

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Incoming date:
8.11.2013

Delivery date:
9.6.2014

Hodonín, 9.6.2014



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Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013
Rep No: 43
Sample name: **Viruton Forte**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013
Sample delivered: 8.11.2013
Testing date: 16.5.2014-6.6.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33
Page: 2

Subject of testing:

Determination of bactericidal and yeasticidal activity of the product.

Identification of the sample:

Name of the product:	Viruton Forte
Batch number:	A-25-PAZ-33
Date of manufacture:	25.10.2013
Expiry date:	04.2016
Manufacturer:	Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Incoming date:	8.11.2013
Storage conditions:	stated by the manufacturer
Active ingredients, 100 g contains:	
CAS 2372-82-9 N-(3-Aminopropyl)-N-dodecylpropane-1,3-diamine	3,76 g
CAS 94667-33-1 N,N-Didecyl-N-methyl-poly(oxyethyl)ammonium propionate	3,39 g

Experiment conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 13727+A1)

Period of analysis:	27.5. – 28.5.2014
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	hard water
Appearance of the products:	yellow liquid
Test concentration:	0.5%
Contact time:	15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	<i>Pseudomonas aeruginosa</i> ATCC 15442 <i>Staphylococcus aureus</i> ATCC 6538 <i>Enterococcus hirae</i> ATCC 10541
Incubation conditions:	37 °C ± 1 °C, 24 hours

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:

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) (for hygienic hand wash at least a 3 lg reduction).

The standard:

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

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

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Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Pseudomonas aeruginosa* ATCC 15442

Tab No. 1.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})				Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)				Dilution neutralization control (C) Product conc.: 0.5%				
V_{c1}	42	$\Phi_{N_{V0}} = 43$		V_{c1}	37	$\Phi_A = 42.5$		V_{c1}	41	$\Phi_B = 42$		V_{c1}	43	$\Phi_C = 40$		
V_{c2}	44			V_{c2}	48			V_{c2}	43			V_{c2}	37			
$30 \leq \Phi_{N_{V0}} \leq 160$				$\Phi_A \geq 0.5 \Phi_{N_{V0}}$				$\Phi_B \geq 0.5 \Phi_{N_{V0}}$				$\Phi_C \geq 0.5 \Phi_{N_{V0}}$				
x	yes		no	x	yes		no	x	yes		no	x	yes		no	
Validation of suspension (N_{VB})				V_{c1}	43	V_{c2}	44	Φ_{NVB}	43.5			$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$				
												x	yes		no	

Tab No. 1.1.2 Verification of methodology, dirty conditions

Tab No. 1.1.2 Verification of methodology, dirty conditions																			
Validation of suspension (N_{V0})					Validation of selected experimental conditions (A)					Neutralizer toxicity control (B)				Dilution neutralization control (C) Product conc.: 0.5%					
V_{c1}	42	$\Phi_{N_{V0}}=43$			V_{c1}	39	$\Phi_A=40.5$			V_{c1}	41	$\Phi_B=42$			V_{c1}	40	$\Phi_C=42$		
V_{c2}	44				V_{c2}	42				V_{c2}	43				V_{c2}	44			
$30 \leq \Phi_{N_{V0}} \leq 160$					$\Phi_A \geq 0.5 \Phi_{N_{V0}}$					$\Phi_B \geq 0.5 \Phi_{N_{V0}}$				$\Phi_C \geq 0.5 \Phi_{N_{V0}}$					
x	yes			no	x	yes			no	x	yes			no	x	yes			no
Validation of suspension (N_{VB})					V_{c1}	43		V_{c2}	44		Φ_{NVB}	43.5			$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$				
															x	yes			no

Tab No. 1.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0		
$\Phi = 40.5 \times 10^7 = \lg 8.61$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.61$		
$8.17 \leq \lg N \leq 8.70$	10^{-7}	39	42	$7.17 \leq \lg N_0 \leq 7.70$		
				x	yes	no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Pseudomonas aeruginosa* ATCC 15442

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	$\lg R$ ($\lg N_0 = \lg 7.61$)
0.5/15/clean	10^0	<14	<14	< 2.15	≥ 5.46
0.5/15/dirty	10^{-1}	132	150	4.15	3.46

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_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, 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 $\lg R = \lg N_0 - \lg N_a$

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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2. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Staphylococcus aureus* ATCC 6538

Tab No. 2.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C) Product conc.: 0.5%		
V_{c1}	51	$\Phi_{N_{V0}}=46$	V_{c1}	45	$\Phi_A=43$	V_{c1}	49	$\Phi_B=44.5$	V_{c1}	47	$\Phi_C=47$
V_{c2}	41		V_{c2}	41		V_{c2}	40		V_{c2}	47	
$30 \leq \Phi_{N_{V0}} \leq 160$			$\Phi_A \geq 0.5 \Phi_{N_{V0}}$			$\Phi_B \geq 0.5 \Phi_{N_{V0}}$			$\Phi_C \geq 0.5 \Phi_{N_{V0}}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no
Validation of suspension (N_{VB})			V_{c1}	45	V_{c2}	47	Φ_{NVB}		46		$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$
									x yes no		

Tab No. 2.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C) Product conc.: 0.5%		
V_{c1}	51	$\Phi_{N_{V0}}=46$	V_{c1}	43	$\Phi_A=44.5$	V_{c1}	49	$\Phi_B=44.5$	V_{c1}	42	$\Phi_C=45$
V_{c2}	41		V_{c2}	46		V_{c2}	40		V_{c2}	48	
$30 \leq \Phi_{N_{V0}} \leq 160$			$\Phi_A \geq 0.5 \Phi_{N_{V0}}$			$\Phi_B \geq 0.5 \Phi_{N_{V0}}$			$\Phi_C \geq 0.5 \Phi_{N_{V0}}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no
Validation of suspension (N_{VB})			V_{c1}	45	V_{c2}	47	Φ_{NVB}	46	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
									x	yes	no

Tab No. 2.2 Test suspensions

Test suspension N		N		V_{c1}		V_{c2}		Test suspension N_0	
$\Phi = 47 \times 10^7 = \lg 8.67$		10^{-6}		> 330		> 330		$\lg N_0 = \lg N/10 = \lg 7.67$	
$8.17 \leq \lg N \leq 8.70$		10^{-7}		47		47		$7.17 \leq \lg N_0 \leq 7.70$	
								x yes no	

Tab No. 2.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Staphylococcus aureus* ATCC 6538

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	$\lg R$ ($\lg N_0 = \lg 7.67$)
0.5/15/clean	10^0	< 14	< 14	< 2.15	≥ 5.52
0.5/15/dirty	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_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, 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 $\lg R = \lg N_0 - \lg N_a$

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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3. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Enterococcus hirae* ATCC 10541

Tab No. 3.1.1 Verification of methodology, clean conditions

Tab No. 3.1.1 Verification of methodology, clean conditions															
Validation of suspension (N_{V0})				Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)				Dilution neutralization control (C) Product conc.: 0.5%			
V_{c1}	29	$\Phi_{N_{V0}} = 31$		V_{c1}	25	$\Phi_A = 30.5$		V_{c1}	27	$\Phi_B = 27$		V_{c1}	33	$\Phi_C = 30$	
V_{c2}	33			V_{c2}	36			V_{c2}	27			V_{c2}	27		
$30 \leq \Phi_{N_{V0}} \leq 160$				$\Phi_A \geq 0.5 \Phi_{N_{V0}}$				$\Phi_B \geq 0.5 \Phi_{N_{V0}}$				$\Phi_C \geq 0.5 \Phi_{N_{V0}}$			
x	yes		no	x	yes		no	x	yes		no	x	yes		no
Validation of suspension (N_{VB})				V_{c1}	28	V_{c2}	33	Φ_{NVB}	30.5			$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$			
												x	yes		no

Tab No. 3.1.2 Verification of methodology, dirty conditions

Tab No. 3.1.2 Verification of methodology, dirty conditions												
Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)			Dilution neutralization control (C) Product conc.: 0.5%		
V_{c1}	29	$\Phi_{N_{V0}} = 31$	V_{c1}	31	$\Phi_A = 31.5$	V_{c1}	27	$\Phi_B = 27$	V_{c1}	30	$\Phi_C = 27.5$	
V_{c2}	33		V_{c2}	32		V_{c2}	27		V_{c2}	25		
$30 \leq \Phi_{N_{V0}} \leq 160$			$\Phi_A \geq 0.5 \Phi_{N_{V0}}$			$\Phi_B \geq 0.5 \Phi_{N_{V0}}$			$\Phi_C \geq 0.5 \Phi_{N_{V0}}$			
x	yes	no	x	yes	no	x	yes	no	x	yes	no	
Validation of suspension (N_{VB})			V_{c1}	28	V_{c2}	33	Φ_{NVB}	30.5	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$			
									x	yes	no	

Tab No. 3.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0		
$\Phi = 266 \times 10^6 = \lg 8.42$	10^{-6}	266	271	$\lg N_0 = \lg N/10 = \lg 7.42$		
$8.17 \leq \lg N \leq 8.70$	10^{-7}	27	22	$7.17 \leq \lg N_0 \leq 7.70$		
				x	yes	no

Tab No. 3.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Enterococcus hirae* ATCC 10541

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	$\lg R$ ($\lg N_0 = \lg 7.42$)
0.5/15/clean	10^0	<14	<14	< 2.15	≥ 5.27
0.5/15/dirty	10^0	<14	<14	< 2.15	≥ 5.27

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_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_v = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation N_{V0} at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, N_a = the number of survivors per ml in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, 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 $\lg R = \lg N_0 - \lg N_a$

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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4. Evaluation of bactericidal activity of the product **Viruton Forte**

Tab No. 4.1 The efficacy of chemical disinfectant **Viruton Forte** on test strains – bactericidal activity

Bactericidal activity of the product (EN 13727)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13727	lg R
<i>Pseudomonas aeruginosa</i> ATCC 15442	20	15	0.5	clean	≥ 5	> 5
<i>Staphylococcus aureus</i> ATCC 6538	20	15	0.5	clean	≥ 5	> 5
<i>Enterococcus hirae</i> ATCC 10541	20	15	0.5	clean	≥ 5	> 5
<i>Pseudomonas aeruginosa</i> ATCC 15442	20	15	0.5	dirty	≥ 5	< 5
<i>Staphylococcus aureus</i> ATCC 6538	20	15	0.5	dirty	≥ 5	> 5
<i>Enterococcus hirae</i> ATCC 10541	20	15	0.5	dirty	≥ 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_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, 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 $\lg R = \lg N_0 - \lg N_a$

Prepared by: Hana Konevalíková, Lab Technician

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

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Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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Experimental conditions:

Period of analysis:

Test temperature:

Test method:

Neutralization medium:

Product diluent:

Appearance of the products:

Test concentration:

Contact time:

Interfering substances:

Test organisms:

Incubation conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 13624)

16.5. – 19.5.2014

20 °C ± 1 °C

dilution neutralization method

Dey-Engley Neutralizing Broth M 1062

hard water

yellow liquid

0.5%

15 min

0.3 g/l BSA (clean conditions)

3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)

Candida albicans

ATCC 10231

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 vegetative yeast cells belonging to reference strain *Candida albicans* under defined conditions by at least 4 orders (10^4) (for hygienic hand wash at least a 2 lg reduction).

$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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: D166/2013
 Rep No: 43
 Sample name: **Viruton Forte**
 Sampled: by client
 Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz
 Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013
 Sample delivered: 8.11.2013
 Testing date: 16.5.2014-6.6.2014
 Delivered amount: 250 ml
 Batch No: A-25-PAZ-33
 Page: 8

5. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Candida albicans* ATCC 10231

Tab No. 5.1.1 Verification of methodology, clean conditions

Tab No. 5.1.1 Verification of methodology, clean conditions																							
Validation of suspension (N _{V0})				Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)				Dilution neutralization control (C) Product conc.: 0.5%											
V _{c1}		46		Φ _{N_{V0}} = 48	V _{c1}		48		Φ _A = 46.5	V _{c1}		50		Φ _B = 46	V _{c1}		41		Φ _C = 47				
V _{c2}		50			V _{c2}		45			V _{c2}		42			V _{c2}		53						
30 ≤ Φ _{N_{V0}} ≤ 160					Φ _A ≥ 0.5 Φ _{N_{V0}}					Φ _B ≥ 0.5 Φ _{N_{V0}}					Φ _C ≥ 0.5 Φ _{N_{V0}}								
x	yes				no	x	yes				no	x	yes				no						
Validation of suspension (N _{VB})																							
Validation of suspension (N _{VB})				V _{c1}		41		V _{c2}		55		Φ _{N_{VB}}		48		30 ≤ Φ _{N_{VB}} (N _{VB} /1000) ≤ 160							
												x		yes					no				

Tab No. 5.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C) Product conc.: 0.5%				
V_{c1}	46	$\Phi_{N_{V0}} = 48$	V_{c1}	42	$\Phi_A = 43.5$	V_{c1}	50	$\Phi_B = 46$	V_{c1}	44	$\Phi_C = 43.5$		
V_{c2}	50		V_{c2}	45		V_{c2}	42		V_{c2}	43			
$30 \leq \Phi_{N_{V0}} \leq 160$			$\Phi_A \geq 0.5 \Phi_{N_{V0}}$			$\Phi_B \geq 0.5 \Phi_{N_{V0}}$			$\Phi_C \geq 0.5 \Phi_{N_{V0}}$				
x	yes		no	x	yes		no	x	yes		no		
Validation of suspension (N_{VB})													
V_{c1}			41	V_{c2}		55	Φ_{NVB}		48	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$			
										x	yes		no

Tab No. 5.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c2}	Test suspension N_0 (time = 0)
$\Phi = 48.5 \times 10^6 = \lg 7.69$	10^{-5}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 6.69$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	47	50	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Candida albicans* ATCC 10231

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	$\lg R$ ($\lg N_0 = \lg 6.69$)
0.5/15/clean	10^0	<14	<14	< 2.15	≥ 4.54
0.5/15/dirty	10^0	<14	<14	< 2.15	≥ 4.54

6. Evaluation of yeasticidal activity of the product **Viruton Forte**

Tab No. 6.1 The efficacy of chemical disinfectant **Viruton Forte** on test strains – yeasticidal activity

Fungicidal activity of the product (EN 13624)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\lg R$ EN 13624	$\lg R$
<i>Candida albicans</i> ATCC 10231	20	15	0.5	clean	≥ 4	> 4
<i>Candida albicans</i> ATCC 10231	20	15	0.5	dirty	≥ 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 fungicidal test suspension, N_0 = the number of cfu/ml of the fungicidal 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 neutralizer toxicity method, N_v = the number of cfu/ml of the fungicidal test suspension for validation, N_{v0} = the number of cfu/ml of the fungicidal 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 – neutralizer toxicity validation, C – method validation)

$R = N_0 / N_a$ nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability.

Prepared by: Hana Konevalíková, Lab Technician

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

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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Experiment conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 13727+A1)

Period of analysis:

5.6. – 6.6.2014

Test temperature:

20 °C ± 1 °C

Test method:

dilution neutralization method

Neutralization medium:

Dey-Engley Neutralizing Broth M 1062

Product diluent:

hard water

Appearance of the products:

yellow liquid

Test concentration:

1.0%

Contact time:

15 min

Interfering substances:

3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)

Test organisms:

Pseudomonas aeruginosa ATCC 15442

Incubation conditions:

37 °C ± 1 °C, 24 hours

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:

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) (for hygienic hand wash at least a 3 lg reduction).

The standard:

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

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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7. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Pseudomonas aeruginosa* ATCC 15442

Tab No.7.1 Verification of methodology, dirty conditions

Validation of suspension (N _{V0})				Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)				Dilution neutralization control (C) Product conc.: 1.0%											
V _{c1}		41		Φ _{N_{V0}} = 37		V _{c1}		36		Φ _A = 38		V _{c1}		33		Φ _B = 36		V _{c1}		34		Φ _C = 38.5	
V _{c2}		33				V _{c2}		40				V _{c2}		39				V _{c2}		43			
30 ≤ Φ _{N_{V0}} ≤ 160				Φ _A ≥ 0.5 Φ _{N_{V0}}				Φ _B ≥ 0.5 Φ _{N_{V0}}				Φ _C ≥ 0.5 Φ _{N_{V0}}											
x		yes				no		x		yes				no		x		yes				no	
Validation of suspension (N _{VB})				V _{c1}		41		V _{c2}		35		Φ _{N_{VB}}		38		30 ≤ Φ _{N_{VB}} (N _{VB} /1000) ≤ 160							
																x		yes				no	

Tab No. 7.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c2}	Test suspension N_0	
$\Phi = 34.5 \times 10^7 = \lg 8.54$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.54$	
$8.17 \leq \lg N \leq 8.70$	10^{-7}	34	35	$7.17 \leq \lg N_0 \leq 7.70$	
				x	yes
					no

Tab No. 7.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Pseudomonas aeruginosa* ATCC 15442

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	$\lg R$ ($\lg N_0 = \lg 7.54$)
1.0/15/dirty	10^0	<14	<14	< 2.15	≥ 5.39

8. Evaluation of bactericidal activity of the product **Viruton Forte**

Tab No. 8.1 The efficacy of chemical disinfectant **Viruton Forte** on test strains – bactericidal activity

Bactericidal activity of the product (EN 13727)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\lg R$ EN 13727	$\lg R$
<i>Pseudomonas aeruginosa</i> ATCC 15442	20	15	1.0	dirty	≥ 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_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_V = the number of cfu/ml of the bacterial test suspension for validation N_{V0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_{VB} = the number of cfu/ml of the bacterial test suspension for the neutralizer control, 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 $\lg R = \lg N_0 - \lg N_a$

Prepared by: Hana Konevalíková, Lab Technician

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D166/2013

Rep No: 43

Sample name: **Viruton Forte**

Sampled: by client

Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013

Sample delivered: 8.11.2013

Testing date: 16.5.2014-6.6.2014

Delivered amount: 250 ml

Batch No: A-25-PAZ-33

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Interpretation:

Results of tests are in Tabs.

According to EN 13727 the tested product **Viruton Forte**, batch No. A-25-PAZ-33, in the concentration 0.5%, diluted in hard water, and the contact time 15 min under clean conditions at temperature $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ by the dilution-neutralization method **decreased** the number of alive microbes *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 by at least 5 (lg) orders.

According to EN 13727 the tested product **Viruton Forte**, batch No. A-25-PAZ-33, in the concentration 0.5%, diluted in hard water, and the contact time 15 min under dirty conditions at temperature $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ by the dilution-neutralization method **decreased** the number of alive microbes *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 by at least 5 (lg) orders.

According to EN 13624 the tested product **Viruton Forte**, batch No. A-25-PAZ-33 in the concentration 0.5%, diluted in hard water, and the contact time 15 min under clean and dirty conditions at temperature $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ by the dilution-neutralization method **decreased** the number of alive microbes *Candida albicans* ATCC 10231 by at least 4 (lg) orders.

According to EN 13727 the tested product **Viruton Forte**, batch No. A-25-PAZ-33, in the concentration 1.0%, diluted in hard water, and the contact time 15 min under dirty conditions at temperature $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ by the dilution-neutralization method **decreased** the number of alive microbes *Pseudomonas aeruginosa* ATCC 15442 by at least 5 (lg) orders.

Conclusion:

The product **Viruton Forte** is capable of reducing the number of viable bacterial cells of the relevant organism when used in the concentration 0.5% in the contact time 15 min under clean conditions and in the concentration 1.0% in the contact time 15 min under dirty conditions, and consequently, may be called bactericidal.

The product **Viruton Forte** is capable of reducing the number of viable vegetative yeast cells of the relevant organism when used in the concentration 0.5% in the contact time 15 min under clean and dirty conditions, and consequently, may be called yeasticidal.

9.6.2014, Hodonín



Ing. Jana Šlitrová, Leader of Study