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

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Issue No.: 1

Test report No. D166-3/2013

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

Sample ID: D166/2013
Sample name: **Quatrodes 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

Incoming date:
8.11.2013

Delivery date:
9.6.2014

Hodonín, 9.6.2014

Zuzana Maňušková, Head of Laboratory

Digitally signed by Palii Nina
Date: 2023.01.06 09:22:36 EET
Reason: MoldSign Signature
Location: Moldova

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

Sample ID: D166/2013
Rep No: 43
Sample name: **Quatrodes 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

Subject of testing:
Determination of bactericidal and yeasticidal activity of the product.

Identification of the sample:

Name of the product:	**Quatrodes 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:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* 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
Sample name: **Quatrodes 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

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

1. Testing the efficacy of chemical disinfectant **Quatrodes 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	Φ_{Nvo}= 43	V_{c1}	37	Φ_A = 42.5	V_{c1}	41	Φ_B = 42	V_{c1}	43	Φ_C = 40
V_{c2}	44		V_{c2}	48		V_{c2}	43		V_{c2}	37	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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

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	Φ_{Nvo}= 43	V_{c1}	39	Φ_A = 40.5	V_{c1}	41	Φ_B = 42	V_{c1}	40	Φ_C = 42
V_{c2}	44		V_{c2}	42		V_{c2}	43		V_{c2}	44	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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 $\Phi = 40.5 \times 10^7 = \lg 8.61$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 $\lg N_0 = \lg N/10 = \lg 7.61$ $7.17 \leq \lg N_0 \leq 7.70$		
	10^{-6}	> 330	> 330			
	10^{-7}	39	42			
				x	yes	no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Quatrodes 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: **Quatrodes 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

2. Testing the efficacy of chemical disinfectant **Quatrodes 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	Φ_{Nvo}= 46	V_{c1}	45	Φ_A = 43	V_{c1}	49	Φ_B = 44.5	V_{c1}	47	Φ_C = 47
V_{c2}	41		V_{c2}	41		V_{c2}	40		V_{c2}	47	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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	Φ_{Nvo}= 46	V_{c1}	43	Φ_A = 44.5	V_{c1}	49	Φ_B = 44.5	V_{c1}	42	Φ_C = 45
V_{c2}	41		V_{c2}	46		V_{c2}	40		V_{c2}	48	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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_{c1}	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 **Quatrodes 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: **Quatrodes Forte**
ampled: 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

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

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	Φ_{Nvo}= 31	V_{c1}	25	Φ_A = 30.5	V_{c1}	27	Φ_B = 27	V_{c1}	33	Φ_C = 30
V_{c2}	33		V_{c2}	36		V_{c2}	27		V_{c2}	27	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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

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	Φ_{Nvo}= 31	V_{c1}	31	Φ_A = 31.5	V_{c1}	27	Φ_B = 27	V_{c1}	30	Φ_C = 27.5
V_{c2}	33		V_{c2}	32		V_{c2}	27		V_{c2}	25	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
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 **Quatrodes 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 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: **Quatrodes 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

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

Tab No. 4.1 The efficacy of chemical disinfectant **Quatrodes 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**
Pseudomonas aeruginosa ATCC 15442	20	15	0.5	clean	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	0.5	clean	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	0.5	clean	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	0.5	dirty	≥ 5	**< 5**
Staphylococcus aureus ATCC 6538	20	15	0.5	dirty	≥ 5	**> 5**
Enterococcus hirae 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: **Quatrodes 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

Experimental conditions:

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

Period of analysis:	16.5. – 19.5.2014
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:	*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 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: **Quatrodes 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

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

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_{Nvo}= 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	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes		x	yes		x	yes		x	yes	
	no			no			no			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. 5.1.2 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	Φ_{Nvo}= 48	V_{c1}	42	Φ_A = 43.5	V_{c1}	50	Φ_B = 46	V_{c1}	44	Φ_C = 43.5
V_{c2}	50		V_{c2}	45		V_{c2}	42		V_{c2}	43	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes		x	yes		x	yes		x	yes	
	no			no			no			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_{c1}	Test suspension N_0 (time = 0)
Φ = 48.5 x 10^6 = lg 7.69	10^{-5}	> 330	> 330	lg N_0 = lg N/10 = lg 6.69
$7.17 \leq$ lg N ≤ 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 **Quatrodes Forte** on *Candida albicans* ATCC 10231

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	lg N_a = lg (Φ_a x 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 **Quatrodes Forte**

Tab No. 6.1 The efficacy of chemical disinfectant **Quatrodes 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**
Candida albicans ATCC 10231	20	15	0.5	clean	≥ 4	**> 4**
Candida albicans 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: **Quatrodes 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

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
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Sample name: **Quatrodes Forte**
Sampled: by client
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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

7. Testing the efficacy of chemical disinfectant **Quatrodes 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_{Nvo}= 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	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	41	V_{c2}	35	Φ_{NVB}	38	$30 \leq \Phi_{NVB}(N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 7.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	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 **Quatrodes 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 **Quatrodes Forte**

Tab No. 8.1 The efficacy of chemical disinfectant **Quatrodes 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**
Pseudomonas aeruginosa 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: **Quatrodes 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

Interpretation:
Results of tests are in Tabs.

According to EN 13727 the tested product **Quatrodes 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 °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 by at least 5 (lg) orders.

According to EN 13727 the tested product **Quatrodes 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 °C ± 1 °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 **Quatrodes 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 °C ± 1 °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 **Quatrodes 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 °C ± 1 °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 **Quatrodes 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 **Quatrodes 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 Šitrová, Leader of Study