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

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Test report No. D166-2/2013

DETERMINATION OF BACTERICIDAL (EN 14561), FUNGICIDAL (EN 14562), MYCOBACTERICIDAL AND TUBERCULOCIDAL (EN 14563) ACTIVITY OF THE PRODUCT **QUATRODES FORTE** DETERMINATION OF VIRUCIDAL (EN 14476) 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:
15.4.2014

Hodonín, 15.4.2014

Zuzana Matušková, Head of Laboratory

Digitally signed by Palii Nina
Date: 2023.01.06 09:22:57 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Subject of testing:
Determination of bactericidal, fungicidal, mycobactericidal and tuberculocidal activity of the product on carriers.
Determination of virucidal 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

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 14561)
Period of analysis:	4.2. – 5.2.2014
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%, 1.0%
Contact time:	15 and 30 min
Interfering substances:	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 the product test solutions
3. Quantitative carrier test
4. Incubation and calculation
5. Expression and interpretation of the results

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms on carriers under defined conditions by at least 5 orders (10^5). The drying time: 30-35 min
$R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

The standard:
EN 14561 Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2) May 2006

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: 27.1.2014-1.4.2014
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 on carriers

Tab No. 1.1 Verification of methodology, temperature 20 °C, 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}	60	$\Phi_{Nv0} = 67.5$	V_{c1}	62	$\Phi_A = 60$	V_{c1}	58	$\Phi_B = 59.5$	V_{c1}	60	$\Phi_C = 61$
V_{c2}	75		V_{c2}	58		V_{c2}	61		V_{c2}	62	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 1.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 241 \times 10^7 = \lg 9.38$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	240	233	
	10^{-8}	26	31	
				x yes no

Tab No. 1.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1170 \times 10^5 = \lg 8.07$ $\lg N_W = \lg 8.07$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.08$
	10^{-5}	114	120	
				x yes no

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

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_W = lg 8.07)**
0.5/30/dirty	10^0	<14	<14	< 2.15	**≥ 5.92**
1.0/15/dirty	10^0	<14	<14	< 2.15	**≥ 5.92**

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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralizer toxicity validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

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

Tab No. 2.1 Verification of methodology, temperature 20 °C, 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}	50	$\Phi_{Nv0} = 46$	V_{c1}	37	$\Phi_A = 45$	V_{c1}	32	$\Phi_B = 41.5$	V_{c1}	54	$\Phi_C = 46$
V_{c2}	42		V_{c2}	53		V_{c2}	51		V_{c2}	38	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 2.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 162 \times 10^7 = \lg 9.21$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	160	155	
	10^{-8}	20	22	
				x yes no

Tab No. 2.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 525 \times 10^5 = \lg 7.72$ $\lg N_W = \lg 7.72$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 7.91$
	10^{-5}	52	53	
				x yes no

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

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.72$)**
0.5/30/dirty	10^0	<14	<14	< 2.15	**≥ 5.57**
1.0/15/dirty	10^0	<14	<14	< 2.15	**≥ 5.57**

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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralizer toxicity validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

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

Tab No. 3.1 Verification of methodology, temperature 20 °C, 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}	53	$\Phi_{Nv0} = 51$	V_{c1}	44	$\Phi_A = 47.5$	V_{c1}	49	$\Phi_B = 49$	V_{c1}	50	$\Phi_C = 46.5$
V_{c2}	49		V_{c2}	51		V_{c2}	49		V_{c2}	43	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 3.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 192 \times 10^7 = \lg 9.28$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	192	188	
	10^{-8}	20	22	
				x yes no

Tab No. 3.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1520 \times 10^4 = \lg 7.18$ $\lg N_W = \lg 7.18$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 7.98$
	10^{-4}	136	168	
				x yes no

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

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.18$)**
0.5/30/dirty	10^0	<14	<14	< 2.15	**≥ 5.03**
1.0/15/dirty	10^0	<14	<14	< 2.15	**≥ 5.03**

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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralizer toxicity validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

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

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

Bactericidal activity of the product on carriers (EN 14561)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14561	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	30	0.5	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	30	0.5	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	30	0.5	dirty	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	1.0	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	1.0	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralizer toxicity validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 14562)
Period of analysis:	27.1. – 30.1.2014
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%, 1.0%
Contact time:	15 and 30 min
Interfering substances:	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 the test suspension
2. Preparation of the product test solutions
3. Quantitative carrier test
4. Incubation and calculation
5. Expression and interpretation of the results

Note:
Fungicidal activity – the capability of a product to produce a reduction in the number of relevant organisms on carriers 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 fungi belonging to reference strain *Candida albicans* on carriers under defined conditions by at least 4 orders (10^4).
The drying time: 30-35 min
$R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

The standard:
EN 14562 Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2) May 2006

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

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

Tab No. 5.1 Verification of methodology, temperature 20 °C, 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}	57	$\Phi_{Nv0} = 54.5$	V_{c1}	59	$\Phi_A = 54.5$	V_{c1}	51	$\Phi_B = 54$	V_{c1}	54	$\Phi_C = 54$
V_{c2}	52		V_{c2}	50		V_{c2}	57		V_{c2}	54	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 5.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 49.5 \times 10^7 = \lg 8.69$ $8.17 \leq \lg N \leq 8.70$
	10^{-6}	>330	>330	
	10^{-7}	55	44	
				x yes no

Tab No. 5.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 970 \times 10^4 = \lg 6.99$ $\lg N_W = \lg 6.99$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 7.39$
	10^{-4}	95	99	
				x yes no

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

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 6.99$)**
0.5/30/dirty	10^0	<14	<14	< 2.15	**≥ 4.84**
1.0/15/dirty	10^0	<14	<14	< 2.15	**≥ 4.84**

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_W = the number of cfu/ml of the control test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, 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 validation, B – neutralizer toxicity validation, C – method validation), R = N_W / N_a nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

6. Evaluation of fungicidal activity of the product **Quatrodes Forte** on carriers

Tab No. 6.1 The efficacy of chemical disinfectant **Quatrodes Forte** on test strains – fungicidal activity on carriers

Fungicidal activity of the product on carriers (EN 14562)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14562	**lg R**
Candida albicans ATCC 10231	20	30	0.5	dirty	≥ 4	**> 4**
Candida albicans ATCC 10231	20	15	1.0	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 test suspension, N_W = the number of cfu/ml of the control test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, 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 validation, B – neutralizer toxicity validation, C – method validation), R = N_W / N_a nebo lg R = lg N_W – 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 14563)
Period of analysis:	11.3.2014 - 1.4.2014
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% and 4%
Contact time:	15 min, 30 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Mycobacterium terrae* ATCC 15755
	Mycobacterium avium ATCC 15769
Incubation conditions:	37 °C ± 1 °C, 21 days

Test procedure:

1. Preparation of test suspension
2. Preparation of product test solutions
3. Quantitative carrier 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). The drying time: 30-35 min

The standard:
EN 14563 Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area - Test method and requirements (phase 2, step 2) November 2008

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

7. Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Mycobacterium avium* ATCC 15769

Tab No. 7.1 Verification of methodology, temperature 20 °C, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C), product conc.:4.0%		
V_{c1}	49	$\Phi_{Nvo} = 52$	V_{c1}	52	$\Phi_A = 55.5$	V_{c1}	40	$\Phi_B = 44$	V_{c1}	42	$\Phi_C = 48.5$
V_{c2}	55		V_{c2}	59		V_{c2}	48		V_{c2}	55	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 7.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c2}	$\Phi = 50 \times 10^8 = \lg 9.70$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	>330	>330	
	10^{-8}	50	50	
				x yes no

Tab No. 7.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1855 \times 10^5 = \lg 8.27$ $\lg N_W = \lg 8.27$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.40$
	10^{-5}	178	193	
				x yes no

Tab No. 7.3 Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Mycobacterium avium* ATCC 15769 on carriers

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 8.27$)**
1.0/30/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.12**
4.0/15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.12**

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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralization validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

8. Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Mycobacterium terrae* ATCC 15755

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

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C), product conc.:4.0%		
V_{c1}	33	$\Phi_{Nvo} = 33$	V_{c1}	27	$\Phi_A = 28$	V_{c1}	32	$\Phi_B = 29$	V_{c1}	25	$\Phi_C = 28$
V_{c2}	33		V_{c2}	29		V_{c2}	26		V_{c2}	31	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 8.2 Test suspension, temperature 20 °C

Test suspension (N)	N	V_{c1}	V_{c2}	$\Phi = 35.5 \times 10^8 = \lg 9.55$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	>330	>330	
	10^{-8}	33	38	
				x yes / no

Tab No. 8.2.1 The control test suspension, temperature 20 °C, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1455 \times 10^5 = \lg 8.16$ $\lg N_W = \lg 8.16$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.25$
	10^{-5}	170	121	
				x yes / no

Tab No. 8.3 Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Mycobacterium terrae* ATCC 15755 on carriers

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 8.16$)**
1.0/30/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.01**
4.0/15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.01**

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_W = the number of cfu/ml of the control bacterial test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_{v0}= the number of cfu/ml of the bacterial 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 validation, B – neutralization validation, C – method validation), $R = N_W / N_a$ nebo $\lg R = \lg N_W - \lg N_a$ = the reduction in viability

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

9. Evaluation of mycobactericidal and tuberculocidal activity of the product **Quatrodes Forte**

Tab No. 9.1 The efficacy of chemical disinfectant **Quatrodes Forte** on test strain – mycobactericidal and tuberculocidal activity on carriers

Mycobactericidal and tuberculocidal activity of the product (EN 14563)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14563	**lg R**
Mycobacterium avium ATCC 15769	20	30	1.0	dirty	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	30	1.0	dirty	> 4	**> 4**
Mycobacterium avium ATCC 15769	20	15	4.0	dirty	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	15	4.0	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 bacterial test suspension, N_0 = the number of cfu/ml of the bacterial 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 bacterial test suspension for validation, N_{v0}= the number of cfu/ml of the bacterial 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

Prepared by: Mgr. Mirka Horáková, Ph.D., 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Experiment conditions:	**Quantitative test for evaluation of virucidal activity** SOP-M-19-00 (EN 14476)
Period of analysis:	19.2.-26.2.2014
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the products:	yellow liquid
Test concentration:	1%
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)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14
Test virus:	*Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 (2^{nd} passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h or 48 h or 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 the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the 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 standard:
EN 14476 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

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

10. Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Tab No. 10.1 Table of results of product **Quatrodes Forte** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
Quatrodes Forte	1.0%	clean	3.50	3.50	-
Quatrodes Forte	1.0%	dirty	3.50	4.00	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	5.00
			Virus titration, time = 0		
Virus control	-	PBS	8.00	-	8.00
Virus control	-	clean	8.00	8.00	-
Virus control	-	dirty	8.00	8.00	-

Tab No.10.2 Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
1.0%	8.00	clean	15 min	3.50	**4.50**
1.0%	8.00	dirty	15 min	4.00	**4.00**

11. Evaluation of virucidal activity of the product **Quatrodes Forte**

Tab No. 11.1 The efficacy of chemical disinfectant **Quatrodes Forte** 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	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	1.0	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	1.0	dirty	≥ 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

Prepared by: Bc. Iva Čížová, 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Experiment conditions:	**Quantitative test for evaluation of virucidal activity** SOP-M-19-00 (EN 14476)
Period of analysis:	11.2.-18.2.2014
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the products:	yellow liquid
Test concentration:	4%
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)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14
Test virus:	*Poliovirus* type 1, LSc-2ab (5th passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h or 48 h or 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 the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the 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 standard:
EN 14476 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

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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

12. Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Poliovirus* type 1, LSc-2ab

Tab No. 12.1 Table of results of product **Quatrodes Forte** on *Poliovirus* type 1, LSc-2ab

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
Quatrodes Forte	4.0%	clean	3.50	3.50	-
Quatrodes Forte	4.0%	dirty	3.50	3.50	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	5.67
			Virus titration, time = 0		
Virus control	-	PBS	8.00	-	8.00
Virus control	-	clean	8.00	8.00	-
Virus control	-	dirty	8.00	8.00	-

Tab No.12.2 Testing the efficacy of chemical disinfectant **Quatrodes Forte** on *Poliovirus* type 1, LSc-2ab

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
4.0%	8.00	clean	15 min	3.50	**4.50**
4.0%	8.00	dirty	15 min	3.50	**4.50**

13. Evaluation of virucidal activity of the product **Quatrodes Forte**

Tab No. 13.1 The efficacy of chemical disinfectant **Quatrodes Forte** 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	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	15	4.0	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	4.0	dirty	≥ 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

Prepared by: Bc. Iva Čížová, 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: 27.1.2014-1.4.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

Interpretation:
Results of tests are in Tabs.

The tested product **Quatrodes Forte**, batch No. A-25-PAZ-33, in the concentration 0.5%, diluted in hard water, and the contact time 30 min and in the concentration 1%, 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** on carriers the number of alive microbes *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 by at least 5 (lg) orders (EN 14561).

The tested product **Quatrodes Forte**, batch No. A-25-PAZ-33 in the concentration 0.5%, diluted in hard water, and the contact time 30 min and in the concentration 1%, 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** on carriers the number of alive microbes *Candida albicans* ATCC 10231 by at least 4 (lg) orders (EN 14562).

The tested product **Quatrodes Forte**, batch No. A-25-PAZ-33, in the concentration 1%, diluted in hard water, and the contact time 30 min and in the concentration 4%, 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** on carriers the number of alive microbes *Mycobacterium avium* ATCC 15769 and *Mycobacterium terrae* ATCC 15755 by 4 (lg) orders (EN 14563).

According to EN 14476 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 clean and dirty 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 *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 particles under defined conditions by 4 (lg) orders.

According to EN 14476 the tested product **Quatrodes Forte**, batch No. A-25-PAZ-33, in the concentration 4.0%, diluted in hard water, and the contact time 15 min under clean and dirty 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 *Poliovirus* type 1, LSc-2ab particles under defined conditions by 4 (lg) orders.

Conclusion:
The product **Quatrodes Forte** is capable of reducing the number of viable bacterial and vegetative yeast and mycobacterial cells of the relevant organism under defined conditions to the declared values, and consequently, may be called bactericidal and yeasticidal and mycobactericidal and tuberculocidal on carriers.
The product **Quatrodes Forte** is capable of reducing the number of infectious *Adenovirus* and *Poliovirus* particles under defined conditions to the declared values, and consequently, may be called virucidal on *Adenovirus* and *Poliovirus*.

15.4.2014, Hodonín

Ing. Jana Skiřová, Leader of Study