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

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Test report No. D166/2013

DETERMINATION OF MYCOBACTERICIDAL AND TUBERCULOCIDAL (EN 14348) ACTIVITY OF THE PRODUCT **VIRUTON FORTE** DETERMINATION OF VIRUCIDAL ACTIVITY (EN 14476) OF THE PRODUCT **VIRUTON FORTE** AGAINST BVDV AND VACCINIA VIRUS

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

Incoming date:
8.11.2013

Delivery date:
7.4.2014

Hodonín, 7.4.2014

TESTING LABORATORY CERTIFIED BY CZECH ACCREDITATION INSTITUTE -2- Chemila, spol. s.r.o. Za Dráhou 4386/3 695 01 Hodonín CZ č. 1273

Zuzana Matušková, Head of Laboratory

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

ID: D166/2013
: 173
name: **Viruton Forte**
d: by client
g point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz
Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 6.11.2013
Sample delivered: 8.11.2013
Testing date: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

of testing:
nation of mycobactericidal and tuberculocidal activity of the product. Determination of virucidal activity roduct on BVDV and Vaccinia virus.

cation of the sample:

f the product:	**Viruton Forte**
umber:	A-25-PAZ-33
manufacture:	25.10.2013
date:	04.2016
cturer:	Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
g date:	8.11.2013
conditions:	stated by the manufacturer

ngredients, 100 g contains:
72-82-9 N-(3-Aminopropyl)-N-dodecylpropane-1,3-diamine 3,76 g
667-33-1 N,N-Didecyl-N-methyl-poly(oxyethyl)ammonium propionate 3,39 g

ental conditions:	**Quantitative suspension test for evaluation of mycobactericidal and tuberculocidal activity** SOP-M-19-00 (EN 14348)
f analysis:	11.12.2013 - 2.1.2014
perature:	20 °C ± 1 °C
hod:	dilution neutralization method
zation medium:	Dey-Engley Neutralizing Broth M 1062
diluent:	hard water
nce of the products:	yellow liquid
centration:	1% and 4%
time:	15 min, 30 min
ng substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
anisms:	*Mycobacterium terrae* ATCC 15755 *Mycobacterium avium* ATCC 15769
on conditions:	37 °C ± 1 °C, 21 days

cedure:
Preparation of test suspension
Preparation of product test solutions
Quantitative suspension test
Incubation and calculation
Expression and interpretation of results

tericidal activity – the capability of a product to produce a reduction in the number of viable cells of *terium terrae* and *Mycobacterium avium* under defined conditions by at least 4 orders (10^4).
ocidal activity - the capability of a product to produce a reduction in the number of viable cells of *terium terrae* under defined conditions by at least 4 orders (10^4).
N_a nebo $\lg R = \lg N_0 - \lg N_a$ the reduction in viability

dard:
48 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of
tericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test

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

Sample ID: D166/2013
Rep No: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

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

1. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Mycobacterium avium* ATCC 15769

Tab No. 1.1.1 Verification of metodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 4%		
V_{c1}	30	$\Phi_{Nvo} = 33$	V_{c1}	29	$\Phi_A = 30.5$	V_{c1}	31	$\Phi_B = 28$	V_{c1}	35	$\Phi_C = 33$
V_{c2}	36		V_{c2}	32		V_{c2}	25		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. 1.1.2 Verification of metodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 4%		
V_{c1}	30	$\Phi_{Nvo} = 33$	V_{c1}	30	$\Phi_A = 32$	V_{c1}	31	$\Phi_B = 28$	V_{c1}	28	$\Phi_C = 30.5$
V_{c2}	36		V_{c2}	34		V_{c2}	25		V_{c2}	33	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 1.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 174 \times 10^7 = \lg 9.24$	10^{-7}	167	182	$\lg N_0 = \lg N/10 = \lg 8.24$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	18	15	$8.17 \leq \lg N_0 \leq 8.70$
				x yes no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Mycobacterium avium* ATCC 15769

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 8.24$)**
1/30/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.09**
1/30/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.09**
4/15/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.09**
4/15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.09**

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: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

2. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Mycobacterium terrae* ATCC 15755

Tab No. 2.1.1 Verification of metodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 4%		
V_{c1}	30	$\Phi_{Nvo} = 31.5$	V_{c1}	29	$\Phi_A = 31$	V_{c1}	37	$\Phi_B = 34.5$	V_{c1}	32	$\Phi_C = 35$
V_{c2}	33		V_{c2}	33		V_{c2}	32		V_{c2}	38	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 2.1.2 Verification of metodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 4%		
V_{c1}	30	$\Phi_{Nvo} = 31.5$	V_{c1}	32	$\Phi_A = 31$	V_{c1}	37	$\Phi_B = 34.5$	V_{c1}	28	$\Phi_C = 30.5$
V_{c2}	33		V_{c2}	30		V_{c2}	32		V_{c2}	33	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 2.2 Test suspensions

Test suspension N $\Phi = 190 \times 10^7 = \lg 9.28$ $9.17 \leq \lg N \leq 9.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0) $\lg N_0 = \lg N/10 = \lg 8.28$ $8.17 \leq \lg N_0 \leq 8.70$			
	10^{-7}	183	193				
	10^{-8}	20	22				
				x	yes		no

Tab No. 2.3 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Mycobacterium terrae* ATCC 15755

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 8.28$)**
1/30/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.13**
1/30/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.13**
4/15/clean	10^{-1}	<14	<14	< 3.15	**≥ 5.13**
4/15/dirty	10^{-1}	<14	<14	< 3.15	**≥ 5.13**

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: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

3. Evaluation of tuberculocidal activity of the product **Viruton Forte**

Tab No. 3.1 The efficacy of chemical disinfectant **Viruton Forte** on test strain – mycobactericidal and tuberculocidal activity

Mycobactericidal and tuberculocidal activity of the product (EN 14348)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14348	**lg R**
Mycobacterium avium ATCC 15769	20	30	1	clean	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	30	1	clean	> 4	**> 4**
Mycobacterium avium ATCC 15769	20	30	1	dirty	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	30	1	dirty	> 4	**> 4**
Mycobacterium avium ATCC 15769	20	15	4	clean	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	15	4	clean	> 4	**> 4**
Mycobacterium avium ATCC 15769	20	15	4	dirty	> 4	**> 4**
Mycobacterium terrae ATCC 15755	20	15	4	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: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33
Page: 6

Experiment conditions:	**Quantitative test for evaluation of virucidal activity** SOP-M-19-00 (EN 14476)
Period of analysis:	15. 11. - 21. 11. 2013
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Appearance of the products:	yellow liquid
Product diluent:	hard water
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)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14

Procedure to stop action of product: The virucidal activity is immediately suppressed by transfer of the sample into 9 volumes of ice-cold diluent. The dillutions are transferred into cell culture units-wells of micro titre plates. For the quantal test are inoculated 6 units with each dilution. For validation is used reference item. Incubation: 36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h, 48 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method. The reduction of virus inactivation are calculated from differences of lg virus titres before and after treatment with test product – virucidal effect.

Test virus:	*Vaccinia virus* strain Elstree CAMP V-160 (2^{th} passage)
Cell lines:	VERO cells

Titre values are calculated according to Spearman and Kärber.

Preparation of the test

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

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The 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: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

4. Testing the efficacy of chemical disinfectant **Viruton Forte** on *Vaccinia virus* strain Elstree CAMP V-160

Tab No. 4.1 Table of results of product **Viruton Forte** on *Vaccinia virus* strain Elstree CAMP V-160

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
Viruton Forte	0.5%	clean	2.50	4.00	-
Viruton Forte	0.5%	dirty	2.50	4.50	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	6.67
			Virus titration, time = 0		
Virus control	-	PBS	8.50	-	8.50
Virus control	-	clean	8.50	8.50	-
Virus control	-	dirty	8.50	8.50	-

Tab No. 4.2 Testing the efficacy of chemical disinfectant **Viruton Forte** on *Vaccinia virus* strain Elstree CAMP V-160

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
0.5%	8.50	clean	15 min	4.00	**4.50**
0.5%	8.50	dirty	15 min	4.50	**4.00**

5. Evaluation of virucidal activity of the product **Viruton Forte**

Tab No. 5.1 The efficacy of chemical disinfectant **Viruton Forte** on test viruses – virucidal activity

Virucidal activity of the product						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476	$\Delta\log_{10}$ $TCID_{50}$
Vaccinia virus strain Elstree CAMP V-160	20	15	0.5	clean	≥ 4	**> 4**
Vaccinia virus strain Elstree CAMP V-160	20	15	0.5	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: 173
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: 15.11.2013-2.1.2014
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:	28. 11. - 5. 12. 2013
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Appearance of the products:	yellow liquid
Product diluent:	hard water
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)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K44006603245, expiry date: 30.11.14

Procedure to stop action of product: The virucidal activity is immediately suppressed by transfer of the sample into 9 volumes of ice-cold diluent. The dillutions are transferred into cell culture units-wells of micro titre plates. For the quantal test are inoculated 6 units with each dilution. For validation is used reference item. Incubation: 36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 24 h, 48 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method. The reduction of virus inactivation are calculated from differences of lg virus titres before and after treatment with test product – virucidal effect.

Test virus:	*BVDV* strain NADL ATCC-VR-534 (6^{th} passage)
Cell lines:	MDBK cells

Titre values are calculated according to Spearman and Kärber.

Preparation of the test

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

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The 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: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33

6. Testing the efficacy of chemical disinfectant **Viruton Forte** on *BVDV* strain NADL ATCC-VR-534

Tab No. 6.1 Table of results of product **Viruton Forte** on *BVDV* strain NADL ATCC-VR-534

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min
Viruton Forte	0.5%	clean	2.50	3.67	-
Viruton Forte	0.5%	dirty	2.50	4.00	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	6.00
			Virus titration, time = 0		
Virus control	-	PBS	8.50	-	8.50
Virus control	-	clean	8.50	8.50	-
Virus control	-	dirty	8.50	8.50	-

Tab No. 6.2 Testing the efficacy of chemical disinfectant **Viruton Forte** on *BVDV* strain NADL ATCC-VR-534

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
0.5%	8.50	clean	15 min	3.67	**4.83**
0.5%	8.50	dirty	15 min	4.00	**4.50**

7. Evaluation of virucidal activity of the product **Viruton Forte**

Tab No. 7.1 The efficacy of chemical disinfectant **Viruton Forte** on test viruses – virucidal activity

Virucidal activity of the product						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476	$\Delta\log_{10}$ $TCID_{50}$
BVDV strain NADL ATCC-VR-534	20	15	0.5	clean	≥ 4	**> 4**
BVDV strain NADL ATCC-VR-534	20	15	0.5	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
* Product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: D166/2013
Rep No: 173
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: 15.11.2013-2.1.2014
Delivered amount: 250 ml
Batch No: A-25-PAZ-33
Page: 10

Interpretation:
Results of tests are in Tabs.

The tested product **Viruton 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 clean and dirty conditions at temperature 20 °C ± 1 °C by the dilution-neutralization method **decreased** the number of alive microbes *Mycobacterium avium* ATCC 15769 and *Mycobacterium terrae* ATCC 15755 by 4 (lg) orders (EN 14348).

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 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtiter plates to reduce the number of infectious *Vaccinia virus* strain Elstree CAMP V-160 particles under defined conditions by at least 4 (lg) orders (EN 14476).

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 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtiter plates to reduce the number of infectious *BVDV* strain NADL ATCC-VR-534 particles under defined conditions by at least 4 (lg) orders (EN 14476).

Conclusion:
The product **Viruton Forte** is capable of reducing the number of viable mycobacterial cells of the relevant organism under defined conditions to the declared values, and consequently, may be called mycobactericidal and tuberculocidal.
The product **Viruton Forte** is capable of reducing the number of infectious *Vaccinia virus* strain Elstree CAMP V-160 particles under defined conditions to the declared values, and consequently, can be called virucidal on *Vaccinia virus*. The product **Viruton Forte** is capable of reducing the number of infectious *BVDV* strain NADL ATCC-VR-534 particles under defined conditions to the declared values, and consequently, can be called virucidal on *BVDV*.

7.4.2014, Hodonín

Ing. Jana Šitrová, Leader of Study