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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2005.

Copy No.: 1
Issue No.: 2

Test report No. D271/2016

DETERMINATION OF BACTERICIDAL (EN 13727+A2, EN 14561), YEASTICIDAL (EN 13624, EN 14562), MYCOBACTERICIDAL AND TUBERCULOCIDAL (EN 14563), SPORICIDAL (EN 13704, EN 14347) ACTIVITY OF THE PRODUCT **DETROCID ACTIV** DETERMINATION OF VIRUCIDAL ACTIVITY (EN 14476+A1) OF THE PRODUCT **DETROCID ACTIV**

Sample ID: D271/2016 Page: 1
Sample name: **DETROCID ACTIV** From pages: 29
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Producer: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Incoming date: 17.10.2016
Delivery date: 22.1.2019

Hodonín, 22.1.2019

Ing. Jana Šilrová, Head of Laboratory

The test report No. D271/2016 was issued originally on 26.7.2017 with the name of the product **DETRO ACTIV** and it was reissued on 22.1.2019 upon the client´s request due to the change of client's name and the product name. The client claims that the products **DETRO ACTIV** and **DETROCID ACTIV** are identical products, which are the same in their composition and which we tested under the name **DETRO ACTIV**.

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

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

Identification of the sample:

Name of the product:	**DETROCID ACTIV**
Batch number:	3442016001
Date of manufacture:	10.10.2016
Expiry date:	10.10.2018
Manufacturer:	Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Incoming date:	17.10.2016
Storage conditions:	stated by the manufacturer

Active ingredients:
CAS 15630-87-4 Disodium carbonate compound with hydrogen peroxide ≥50%
CAS 77-92-9 Citric acid ≤15%
CAS 29329-71-3 (1-hydroxyethylidene)bisphosphonic acid, sodium salt <5%

Experimental conditions:

	Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 13727:2012+A2:2015)
Period of analysis:	23.2. – 24.2.2017
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 product:	white powder
Test concentration:	0.5%
Contact time:	5 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 *Escherichia coli* ATCC 10536
Incubation conditions:	37 °C ± 1 °C, 24 hours

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

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under defined conditions by at least 5 orders (10^5).
 The standard:
The standard:
EN 13727:2012+A2:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1) October 2015 .

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

Sample ID: D271/2016
Rep No: 257
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Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

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Page: 3

The Number of CFU in the tested product: $<10^1$ CFU/g

1. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Pseudomonas aeruginosa* ATCC 15442

Tab No. 1.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	46	Φ_{Nvo}= 47	V_{c1}	43	Φ_A = 46	V_{c1}	44	Φ_B = 47	V_{c1}	44	Φ_C = 42.5
V_{c2}	48		V_{c2}	49		V_{c2}	50		V_{c2}	41	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	Yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	40	V_{c2}	55	Φ_{NVB}	47.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 1.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 45.5 \times 10^7 = \lg 8.66$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.66$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	45	46	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** 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 ($\lg N_0 = \lg 7.66$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.51**

2. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Staphylococcus aureus* ATCC 6538

Tab No. 2.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	52	Φ_{Nvo}= 52	V_{c1}	54	Φ_A = 48	V_{c1}	53	Φ_B = 47	V_{c1}	54	Φ_C = 48
V_{c2}	52		V_{c2}	42		V_{c2}	41		V_{c2}	42	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	55	V_{c2}	50	Φ_{NVB}	52.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 2.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 49 \times 10^7 = \lg 8.69$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.69$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	56	42	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 2.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** 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 ($\lg N_0 = \lg 7.69$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.54**

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} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, 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 control, 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

3. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Enterococcus hirae* ATCC 10541

Tab No. 3.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	50	Φ_{Nvo}= 53.5	V_{c1}	50	Φ_A = 46	V_{c1}	56	Φ_B = 47.5	V_{c1}	52	Φ_C = 48
V_{c2}	57		V_{c2}	42		V_{c2}	39		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}	56	V_{c2}	46	Φ_{NVB}	51	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 3.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 49.5 \times 10^7 = \lg 8.69$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.69$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	56	43	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

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

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.69$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.54**

4. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Escherichia coli* ATCC 10536

Tab No. 4.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	44	Φ_{Nvo}= 48	V_{c1}	50	Φ_A = 46.5	V_{c1}	54	Φ_B = 47.5	V_{c1}	50	Φ_C = 46
V_{c2}	52		V_{c2}	43		V_{c2}	41		V_{c2}	42	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	51	V_{c2}	46	Φ_{NVB}	48.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$		
							x	yes	no

Tab No. 4.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 49 \times 10^7 = \lg 8.69$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.69$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	48	50	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 4.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Escherichia coli* ATCC 10536

Test concentration (%)/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.69$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.54**

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} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, 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 control, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 – \lg N_a$

5. Evaluation of bactericidal activity of the product **DETROCID ACTIV**

Tab No. 5.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – bactericidal activity

Bactericidal activity of the product (EN 13727:2012+A2:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13727:2012 +A2:2015	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	5	0.5	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	5	0.5	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	5	0.5	dirty	≥ 5	**> 5**
Escherichia coli ATCC 10536	20	5	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} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions for validation in the test mixture A, B, C at the beginning of the contact time = 0, 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 control, 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers SOP-M-22-12 (EN 14561:2006)

Period of analysis:	27.4. – 28.4.2017
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 product:	white powder
Test concentration:	0.5%
Contact time:	5 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
	Escherichia coli ATCC 10536
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 14561:2006 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*

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Sampling date: 10.10.2016
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Batch No: 3442016001

6. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Pseudomonas aeruginosa* ATCC 15442 on carriers

Tab No. 6.1 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	62	$\Phi_{Nvo} = 65$	V_{c1}	56	$\Phi_A = 64$	V_{c1}	52	$\Phi_B = 59$	V_{c1}	66	$\Phi_C = 63$
V_{c2}	68		V_{c2}	72		V_{c2}	66		V_{c2}	60	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 6.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 275 \times 10^7 = \lg 9.44$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	268	279	
	10^{-8}	27	31	
				x yes no

Tab No. 6.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1960 \times 10^4 = \lg 7.29$ $\lg N_W = \lg 7.29$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.14$
	10^{-4}	190	202	
				x yes no

Tab No. 6.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Pseudomonas aeruginosa* ATCC 15442 on carriers, dirty conditions

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.29$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.14**

7. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Staphylococcus aureus* ATCC 6538 on carriers

Tab No. 7.1 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	30	$\Phi_{Nvo} = 37$	V_{c1}	42	$\Phi_A = 38.5$	V_{c1}	37	$\Phi_B = 39$	V_{c1}	39	$\Phi_C = 36$
V_{c2}	44		V_{c2}	35		V_{c2}	41		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. 7.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 290 \times 10^7 = \lg 9.46$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	281	293	
	10^{-8}	30	33	
				x yes no

Tab No. 7.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 1955 \times 10^4 = \lg 7.29$ $\lg N_W = \lg 7.29$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.16$
	10^{-4}	202	189	
				x yes no

Tab No. 7.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Staphylococcus aureus* ATCC 6538 on carriers, dirty conditions

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.29$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.14**

8. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Enterococcus hirae* ATCC 10541 on carriers

Tab No. 8.1 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	83	$\Phi_{Nvo} = 86.5$	V_{c1}	84	$\Phi_A = 81$	V_{c1}	92	$\Phi_B = 81$	V_{c1}	95	$\Phi_C = 83$
V_{c2}	90		V_{c2}	78		V_{c2}	70		V_{c2}	71	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 8.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 296 \times 10^7 = \lg 9.47$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	288	303	
	10^{-8}	29	32	
				x yes no

Tab No. 8.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 990 \times 10^5 = \lg 8.00$ $\lg N_W = \lg 8.00$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.17$
	10^{-5}	106	92	
				x yes no

Tab No. 8.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Enterococcus hirae* ATCC 10541 on carriers, dirty conditions

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 8.00)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.85**

9. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Escherichia coli* ATCC 10536 on carriers

Tab No. 9.1 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 0.5%		
V_{c1}	55	$\Phi_{Nvo} = 52.5$	V_{c1}	42	$\Phi_A = 50$	V_{c1}	56	$\Phi_B = 49.5$	V_{c1}	49	$\Phi_C = 51$
V_{c2}	50		V_{c2}	58		V_{c2}	43		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	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 9.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 211 \times 10^7 = \lg 9.32$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	192	225	
	10^{-8}	25	22	
				x yes no

Tab No. 9.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 620 \times 10^5 = \lg 7.79$ $\lg N_W = \lg 7.79$ $7.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.02$
	10^{-5}	58	66	
				x yes no

Tab No. 9.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Escherichia coli* ATCC 10536 on carriers, dirty conditions

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 7.79)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.64**

10. Evaluation of bactericidal activity of the product **DETROCID ACTIV** on carriers

Tab No. 10.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – bactericidal activity on carriers

Bactericidal activity of the product on carriers (EN 14561:2006)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14561:2006	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	5	0.5	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	5	0.5	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	5	0.5	dirty	≥ 5	**> 5**
Escherichia coli ATCC 10536	20	5	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_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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method
SOP-M-19-00 (EN 13624:2013)

Period of analysis:	28.11. – 2.12.2016
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 product:	white powder
Test concentration:	0.5%
Contact time:	5 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 test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Note:
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least 4 orders (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least 4 orders (10^4).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

11. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Candida albicans* ATCC 10231

Tab No. 11.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 0.5%		
V_{c1}	48	Φ_{Nvo}= 45	V_{c1}	42	Φ_A = 44.5	V_{c1}	45	Φ_B = 42.5	V_{c1}	46	Φ_C = 42
V_{c2}	42		V_{c2}	47		V_{c2}	40		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

Validation of suspension (N_{VB})	V_{c1}	44	V_{c2}	49	Φ_{NVB}	46.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160			
							x	yes		no

Tab No. 11.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 44.5 \times 10^6 = \lg 7.65$	10^{-5}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 6.65$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	41	48	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 11.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Candida albicans* ATCC 10231

Test concentration (%)/contact time (min)	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a$ = $\lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.65$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 4.50**

12. Evaluation of yeasticidal activity of the product **DETROCID ACTIV**

Tab No. 12.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – yeasticidal activity

Fungicidal activity of the product (EN 13624:2013)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13624:2013	**lg R**
Candida albicans ATCC 10231	20	5	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 test suspension, N_{V0} = the number of cfu/ml of the test suspension for validation, N_a = the number of survivors per ml in the test mixture, A, B, C = the number of survivors per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), R = N_0/ N_a = the reduction in viability, or lg R = lg N_0 – lg N_a

Prepared by: Hana Konevalíková, Lab Technician

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers SOP-M-22-12 (EN 14562:2006)

Period of analysis:	20.2. – 23.2.2017
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 product:	white powder
Test concentration:	0.5%
Contact time:	5 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 14562:2006 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

13. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Candida albicans* ATCC 10231 on carriers

Tab No. 13.1 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc. 0.5%		
V_{c1}	132	$\Phi_{Nvo} = 145$	V_{c1}	154	$\Phi_A = 138$	V_{c1}	128	$\Phi_B = 137$	V_{c1}	129	$\Phi_C = 120.5$
V_{c2}	158		V_{c2}	122		V_{c2}	146		V_{c2}	112	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 13.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 311 \times 10^6 = \lg 8.49$ $8.17 \leq \lg N \leq 8.70$
	10^{-6}	311	307	
	10^{-7}	35	31	
				x yes no

Tab No. 13.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 240 \times 10^4 = \lg 6.38$ $\lg N_W = \lg 6.38$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 7.19$
	10^{-4}	24	24	
				x yes no

Tab No. 13.3.1 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Candida albicans* ATCC 10231 on carriers, dirty conditions

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 6.38$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 4.23**

14. Evaluation of yeasticidal activity of the product **DETROCID ACTIV** on carriers

Tab No. 14.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – yeasticidal activity on carriers

Fungicidal activity of the product on carriers (EN 14562:2006)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14562:2006	**lg R**
Candida albicans ATCC 10231	20	5	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 fungal test suspension, N_W = the number of cfu/ml of the control fungal 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 fungal 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers SOP-M-22-12 (EN 14563:2008)

Period of analysis:	28.11. – 19.12.2016
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 product:	white powder
Test concentration:	2.0%
Contact time:	5 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 14563:2008 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

15. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Mycobacterium avium* ATCC 15769

Tab No. 15.1. Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C), product conc.: 2.0%		
V_{c1}	42	$\Phi_{Nvo} = 36$	V_{c1}	18	$\Phi_A = 21$	V_{c1}	28	$\Phi_B = 25$	V_{c1}	26	$\Phi_C = 26.5$
V_{c2}	30		V_{c2}	24		V_{c2}	22		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

Tab No. 15.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c2}	$\Phi = 37.5 \times 10^8 = \lg 9.57$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	> 330	> 330	
	10^{-8}	36	39	
				x yes no

Tab No. 15.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 540 \times 10^5 = \lg 7.73$ $\lg N_W = \lg 7.73$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.27$
	10^{-5}	43	65	
				x yes no

Tab No. 15.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Mycobacterium avium* ATCC 15769 on carriers, dirty conditions

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.73$)**
2.0/5/dirty	10^{-1}	<14	<14	< 3.15	**≥ 4.58**

16. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Mycobacterium terrae* ATCC 15755

Tab No. 16.1 Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Dilution neutralization control (C), product conc.: 2.0%		
V_{c1}	55	$\Phi_{Nvo} = 49.5$	V_{c1}	49	$\Phi_A = 49.5$	V_{c1}	37	$\Phi_B = 39.5$	V_{c1}	35	$\Phi_C = 36$
V_{c2}	44		V_{c2}	50		V_{c2}	42		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

Tab No. 16.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c2}	$\Phi = 48 \times 10^8 = \lg 9.68$ $9.17 \leq \lg N \leq 9.70$
	10^{-7}	> 330	> 330	
	10^{-8}	54	42	
				x yes no

Tab No. 16.2.1 The control test suspension, dirty conditions

Test suspension (N_W)	N_W	V_{c1}	V_{c2}	$\Phi \times 10 = 410 \times 10^5 = \lg 7.61$ $\lg N_W = \lg 7.61$ $6.15 \leq \lg N_w \leq (\lg N - 1.3)\ 8.38$
	10^{-5}	33	49	
				x yes no

Tab No. 16.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Mycobacterium terrae* ATCC 15755 on carriers, dirty conditions

Test concentration (%) / contact time (min) / conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_W = \lg 7.61$)**
2.0/5/dirty	10^{-1}	<14	<14	< 3.15	**≥ 4.46**

17. Evaluation of mycobactericidal and tuberculocidal activity of the product **DETROCID ACTIV**

Tab No. 17.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strain – mycobactericidal and tuberculocidal activity on carriers

Mycobactericidal and tuberculocidal activity of the product (EN 14563:2008)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14563:2008	**lg R**
Mycobacterium avium ATCC 15769	20	5	2.0	dirty	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	5	2.0	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_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

Prepared by: Ing. Eva Kremlová, Lab Technician

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method
SOP-M-19-00 (EN 13704:2002)

Period of analysis:	24.2. – 27.2.2017
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 product:	white powder
Test concentration:	2.0%
Contact time:	10 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Test organisms:	*Bacillus subtilis* ATCC 6633
Incubation conditions:	30 °C ± 1 °C, minimum 3 and maximum 7 days

Test procedure:

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

Note:
Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* under defined conditions by at least 3 orders (10^3).
$\lg R = \lg [(N \times 10^{-1})/N_a]$

The standard:
EN 13704:2002 Chemical disinfectants – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1) February 2002

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001
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18. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus subtilis* ATCC 6633

Tab No. 18.1 Verification of methodology, clean conditions

Validation of suspension (N_v) x 10^1			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 2.0%		
V_{c1}	311	$\Phi_{Nv} = 304$	V_{c1}	284	$\Phi_A = 290$	V_{c1}	292	$\Phi_B = 285.5$	V_{c1}	272	$\Phi_C = 284$
V_{c2}	297		V_{c2}	296		V_{c2}	279		V_{c2}	296	
$60 \leq \Phi_{Nv} \leq 300$			$\Phi_A \geq 0.05\ \Phi_{Nv}$			$\Phi_B \geq 0.05\ \Phi_{Nv}$			$\Phi_C \geq 0.5\ \Phi_B$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 18.2 Test suspension

Test suspension (N)	N	V_{c1}	V_{c1}	$\Phi = 37 \times 10^5 = \lg 6.57$ $6.17 \leq \lg N \leq 6.70$
	10^{-4}	> 330	> 330	
	10^{-5}	33	41	
				x yes no

Tab No. 18.3 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus subtilis* ATCC 6633

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 = lg 6.57)**
2.0/10/clean	10^0	<14	<14	< 2.15	**≥ 3.42**

19 Evaluation of sporicidal activity of the product **DETROCID ACTIV**

Tab No. 19.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – sporicidal activity

Sporicidal activity of the product (EN 13704)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 13704	**lg R**
Bacillus subtilis ATCC 6633	20	10	2.0	clean	≥ 3	**> 3**

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 spore test suspension, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_v = the number of cfu/ml of the spore validation test suspension, A,B,C = the number of cfu/ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), lg $R = \lg [(N \times 10^{-1})/N_a]$ = the reduction in viability

Prepared by: Hana Konevalíková, Lab Technician

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method
SOP-M-19-00 (EN 14347:2005)

Period of analysis:	24.2. – 27.2.2017
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Product diluent:	distilled water
Appearance of the product:	white powder
Test concentration:	2.0%
Contact time:	10 min
Interfering substances:	no interfering substance (distilled water)
Test organisms:	*Bacillus subtilis* ATCC 6633
	Bacillus cereus ATCC 12826
Incubation conditions:	37 °C ± 1 °C, minimum 4 and maximum 7 days

Test procedure:

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

Note:
Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* and *Bacillus cereus* under defined conditions by at least 4 orders (10^4).
$R = N_w / N_a$ nebo $\lg R = \lg N_w - \lg N_a$ the reduction in viability

The standard:
EN 14347:2005 Chemical disinfectants and antiseptics - Basic sporicidal activity - Test method and requirements (phase 1, step 1) January 2005

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

20. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus subtilis* ATCC 6633

Tab No. 20.1 Verification of methodology

Test suspension N1			Validation suspension Nv			Neutralizer control (B)			Method validation (C) Product conc. 2.0%		
Dilution	V_{c1}	V_{c2}	Dilution	V_{c1}	V_{c2}	Dilution	10^{-6}	10^{-6}	Dilution	10^{-3}	10^{-3}
10^{-6}	>330	>330	10^{-2}	>330	>330	V_{c1}	58	$\Phi_B = 54$	V_{c1}	50	$\Phi_C = 54$
10^{-7}	66	56	10^{-3}	62	51	V_{c2}	50		V_{c2}	58	
lg N1	$61 \times 10^7 = lg\ 8.79$		lg Nv	$56.5 \times 10^3 = lg\ 4.75$		lg B	$54 \times 10^6 = lg\ 7.73$		lg C	$54 \times 10^3 = lg\ 4.73$	
Norm	$8.48 \leq lg\ N1 \leq 9.00$		Norm	$4.48 \leq lg\ Nv \leq 5.00$		Norm	$lg\ B \geq lg\ Nw$		Norm	$4.48 \leq lg\ C \leq 5.00$	
Test suspension N2			Water control Nw			ONT (original neutralization tube)			The weighted mean count – quotient Φ		
Dilution	V_{c1}	V_{c2}	Dilution	V_{c1}	V_{c2}				N	Norm	Φ
10^0	>330	>330	10^{-5}	>330	>330				N1	$5 \leq \Phi \leq 15$	-
10^{-1}	51	59	10^{-6}	48	58				N2	$5 \leq \Phi \leq 15$	-
lg N2	$55 \times 10^1 = lg\ 2.74$		lg Nw	$53 \times 10^6 = lg\ 7.72$		Percept	Visible growth		Nv	$5 \leq \Phi \leq 15$	-
Norm	$2.48 \leq lg\ N2 \leq 3.00$		Norm	$7.48 \leq lg\ Nw \leq 8.00$		Norm	Visible growth		Nw	$5 \leq \Phi \leq 15$	-

Tab No. 20.2 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus subtilis* ATCC 6633

Test concentration (%) / contact time (min)/ conditions	Dilution after test procedure	V_{c1}	V_{c2}	$lg\ N_a = lg\ (\Phi_a \times 10)$	**lg R ($lg\ N_w = lg\ 7.72$)**
2.0/10/-	10^{-1}	<14	<14	< 3.15	**≥ 4.57**

21. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus cereus* ATCC 12826

Tab No. 21.1 Verification of methodology

Test suspension N1			Validation suspension Nv			Neutralizer control (B)			Method validation (C) Product conc. 2.0%		
Dilution	V_{c1}	V_{c2}	Dilution	V_{c1}	V_{c2}	Dilution	10^{-6}	10^{-6}	Dilution	10^{-3}	10^{-3}
10^{-6}	>330	>330	10^{-2}	>330	>330	V_{c1}	35	$\Phi_B =$	V_{c1}	39	$\Phi_C = 42$
10^{-7}	42	45	10^{-3}	38	44	V_{c2}	40	37.5	V_{c2}	45	
lg N1	$43.5 \times 10^7 = lg\ 8.64$		lg Nv	$41 \times 10^3 = lg\ 4.61$		lg B	$37.5 \times 10^6 = lg\ 7.57$		lg C	$42 \times 10^3 = lg\ 4.62$	
Norm	$8.48 \leq lg\ N1 \leq 9.00$		Norm	$4.48 \leq lg\ Nv \leq 5.00$		Norm	$lg\ B \geq lg\ Nw$		Norm	$4.48 \leq lg\ C \leq 5.00$	
Test suspension N2			Water control Nw			ONT (original neutralization tube)			The weighted mean count – quotient Φ		
Dilution	V_{c1}	V_{c2}	Dilution	V_{c1}	V_{c2}				N	Norm	Φ
10^0	>330	>330	10^{-5}	>330	>330				N1	$5 \leq \Phi \leq 15$	-
10^{-1}	31	48	10^{-6}	33	39				N2	$5 \leq \Phi \leq 15$	-
lg N2	$39.5 \times 10^1 = lg\ 2.60$		lg Nw	$36 \times 10^6 = lg\ 7.56$		Percept	Visible growth		Nv	$5 \leq \Phi \leq 15$	-
Norm	$2.48 \leq lg\ N2 \leq 3.00$		Norm	$7.48 \leq lg\ Nw \leq 8.00$		Norm	Visible growth		Nw	$5 \leq \Phi \leq 15$	-

Tab No. 21.2 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Bacillus cereus* ATCC 12826

Test concentration (%)* / contact time (min)/ conditions	Dilution after test procedure	V_{c1}	V_{c2}	$lg\ N_a = lg\ (\Phi_a \times 10)$	**lg R ($lg\ N_w = lg\ 7.56$)**
2.0/10/-	10^{-1}	<14	<14	< 3.15	**≥ 4.41**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N1 = the number of cfu/ml of the bacterial test suspension, N2 = the number of cfu/ml of the bacterial test suspension after dilution, N_v = the number of cfu/ml of the bacterial test suspension for validation, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_w = the number of cfu/ml of the bacterial test suspension in water control, B and C = the number of survivors per ml in control tests (B – neutralizer control, 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

22. Evaluation of sporicidal activity of the product **DETROCID ACTIV**

Tab No. 22.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test strains – sporicidal activity

Sporicidal activity of the product (EN 14347:2005)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R EN 14347:2005	**lg R**
Bacillus subtilis ATCC 6633	20	10	2.0	distilled water	≥ 4	**> 4**
Bacillus cereus ATCC 12826	20	10	2.0	distilled water	≥ 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), N1 = the number of cfu/ml of the bacterial test suspension, N2 = the number of cfu/ml of the bacterial test suspension after dilution, N_v = the number of cfu/ml of the bacterial test suspension for validation, N_a = the number of survivors per ml in the test mixture at the end of the contact time, N_w = the number of cfu/ml of the bacterial test suspension in water control, B and C = the number of survivors per ml in control tests (B – neutralizer control, 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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experiment conditions: **Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)

Period of analysis:	16.2. – 23.2.2017
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 product:	white powder
Test concentration:	2.0%
Contact time:	10 min
Interfering substances:	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: K47740803613, expiry date: 31.3.2018
Test virus:	*Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 (6^{th} passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 96 h and additional period of 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of 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 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

23. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Tab No. 23.1 Table of results of product **DETROCID ACTIV** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 10 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
DETROCID ACTIV	2.0%	dirty	3.50	4.83	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	5.83	5.17
			Virus titration, time = 0			
Virus control	-	PBS	9.00	-	9.00	9.17
Virus control	-	dirty	9.00	9.00	-	-

Tab No. 23.2 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** 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	$\Delta\log_{10}$ $TCID_{50}$
2.0%	9.00	dirty	10 min	4.83	**4.17**

24. Evaluation of virucidal activity of the product **DETROCID ACTIV**

Tab No. 24.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	10	2.0	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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	2.2. – 10.2.2017
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 product:	white powder
Test concentration:	2.0%
Contact time:	10 min
Interfering substances:	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: K47740803613, expiry date: 31.3.2018
Test virus:	*Murine norovirus (MNV)* strain S99, RVB-651 (2nd passage)
Cell lines:	RAW 264.7 *Murine macrophage* cell line
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 96 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

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

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

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

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

25. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Murine norovirus (MNV)* strain S99, RVB-651

Tab No. 25.1 Table of results of product **DETROCID ACTIV** on *Murine norovirus (MNV)* strain S99, RVB-6515

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
DETROCID ACTIV	2.0%	dirty	3.50	5.33	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	7.33	6.17
			Virus titration, time = 0			
Virus control	-	PBS	9.50	-	9.50	9.33
Virus control	-	dirty	9.50	9.50	-	-

Tab No. 25.2 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Murine norovirus (MNV)* strain S99, RVB-651

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
2.0%	9.50	dirty	10 min	5.33	**4.17**

26. Evaluation of virucidal activity of the product **DETROCID ACTIV**

Tab No. 26.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Murine norovirus (MNV) strain S99, RVB-651	20	10	2.0	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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	28.4. – 5.5.2017
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 product:	white powder
Test concentration:	2.0%
Contact time:	10 min
Interfering substances:	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: K47740803613, expiry date: 31.3.2018
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 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 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

27. Testing the efficacy of chemical disinfectant **DETROCID ACTIV** on *Poliovirus* type 1, LSc-2ab

Tab No. 27.1 Table of results of product **DETROCID ACTIV** on *Poliovirus* type 1, LSc-2ab

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 10 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
DETROCID ACTIV	2.0%	dirty	3.50	5.33	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	7.67	6.33
			Virus titration, time = 0			
Virus control	-	PBS	10.00	-	10.00	10.00
Virus control	-	dirty	10.00	10.00	-	-

Tab No. 27.2 Testing the efficacy of chemical disinfectant **DETROCID ACTIV** 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	$\Delta\log_{10}$ $TCID_{50}$
2.0%	10.00	dirty	10 min	5.33	**4.67**

28. Evaluation of virucidal activity of the product **DETROCID ACTIV**

Tab No. 28.1 The efficacy of chemical disinfectant **DETROCID ACTIV** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	10	2.0	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: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001

Interpretation:
Results of tests are in Tabs.
According to EN 13727:2012+A2:2015 the tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 0.5%, diluted in hard water, and in the contact time 5 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, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536 by at least 5 (lg) orders.

The tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 0.5%, diluted in hard water, and in the contact time 5 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, *Escherichia coli* ATCC 10536 by at least 5 (lg) orders (EN 14561:2006).

According to EN 13624:2013 the tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 0.5%, diluted in hard water, and in the contact time 5 min under 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.

The tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 0.5%, diluted in hard water, and in the contact time 5 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 14562:2006).

The tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 5 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, *Mycobacterium terrae* ATCC 15755 by at least 4 (lg) orders (EN 14563:2008).

The tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 10 min under clean conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of bacterial spores *Bacillus subtilis* ATCC 6633 by at least 3 (lg) orders (EN 13704).

The tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 10 min under distilled water conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of alive microbes *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 12826 by at least 4 (lg) orders (EN 14347:2005).

According to EN 14476:2013+A1:2015 the tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 10 min under 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 at least 4 (lg) orders.

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

Sample ID: D271/2016
Rep No: 257
Sample name: **DETROCID ACTIV**
Sampled: by client
Sampling point: Detroks Kim.ve Sag.Ürn.Ürt.Dag.San.Tic.Ltd.Sti, Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY
Client: Detro Healthcare Kimya San. A.S., Atatürk Mah. Adnan Menderes Cad. No.7 Esenyurt / Istanbul, TURKEY

Sampling date: 10.10.2016
Sample delivered: 17.10.2016
Testing date: 28.11.2016–5.5.2017
Delivered amount: 3 x 0.5 l
Batch No: 3442016001
Page: 29

Interpretation:
Results of tests are in Tabs.
According to EN 14476:2013+A1:2015 the tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 10 min under 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 *Murine norovirus (MNV)* strain S99, RVB-651 particles under defined conditions by at least 4 (lg) orders.

According to EN 14476:2013+A1:2015 the tested product **DETROCID ACTIV,** batch No. 3442016001, in the concentration 2.0%, diluted in hard water, and in the contact time 10 min under 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 at least 4 (lg) orders.

Conclusion:
The product **DETROCID ACTIV** is capable of reducing the number of viable bacterial and vegetative yeast cells of the relevant organisms in the suspension and on carriers under defined conditions to the declared values, and consequently, may be called bactericidal and yeasticidal.
The product **DETROCID ACTIV** is capable of reducing the number of viable mycobacterial cells of the relevant organisms on carriers under defined conditions to the declared values, and consequently, may be called mycobactericidal and tuberculocidal on carriers.
The product **DETROCID ACTIV** is capable of reducing the number of bacterial spores of the relevant organisms under defined conditions to the declared values, and consequently, may be called sporicidal.
The product **DETROCID ACTIV** is capable of reducing the number of infectious *Adenovirus*, *Poliovirus* and *Murine norovirus* particles under defined conditions to the declared values, and consequently, may be called virucidal.
The test report No. D271/2016 was issued originally on 26.7.2017 with the name of the product **DETRO ACTIV** and it was reissued on 22.1.2019 upon the client´s request due to the change of client's name and the product name. The client claims that the products **DETRO ACTIV** and **DETROCID ACTIV** are identical products, which are the same in their composition and which we tested under the name **DETRO ACTIV**.

22.1.2019, Hodonín

Ing. Barbora Stoklásková, Leader of Study

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