



Testing protocol n. 2/2016/SMU

EN 14 348 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants. Test methods and requirements (phase 2, step 1)

Applicant:

SCHÜLKE CZ s.r.o.

Lidická 326

735 81 Bohumín

Czech Republic

Order n.:

Sample identification:

Product name: **Desam EFFEKT+**

LOT n.: **017A160120**

Manufacturer: **SCHÜLKE CZ s.r.o**

Storage conditions: room temperature, dark

Dilution agent: drinking water

Active compounds: Quaternary Ammonium salts, bis (3-aminopropyl) dodecylamine, 2-fenoxyethanol

Product delivered: 1st February 2016

Dates of testing: 5th, 8th and 9th February 2016

Results: see attachments 1 - 3

Conclusion:

According to EN 14348, Product **Desam EFFEKT+** LOT **017A160120** after dilution by hard water to **0.5% (m/v)**, proved **mycobactericidal** activity within **60 minutes** on temperature 20° C, clean (bovine albumin 0,3 g/l) and dirty conditions (bovine albumin 3 g/l + sheep erythrocytes 3ml) for reference strains *Mycobacterium terrae* and *Mycobacterium avium*. Average reduction by six repetitions with limiting organism *Mycobacterium avium* in dirty conditions was **1,06 x 10⁴**. Second organism was tested once and proved higher reduction than *Mycobacterium avium*.

According to EN 14348, Product **Desam EFFEKT+** LOT **017A160120** after dilution by hard water to **1 and 1.5% (m/v)**, proved **mycobactericidal** activity within **30 minutes** on temperature 20° C, dirty conditions (bovine albumin 3 g/l + sheep erythrocytes 3ml) and clean (bovine albumin 0.3 g/l) conditions for reference strains *Mycobacterium terrae* and *Mycobacterium avium*.

In Ostrava: 29th February 2016

Vít Ulmann, MSc.

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of mycobacterial diagnostics and TB

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Protocol attachment n. 1: 2/2016/SMUEN 14348 (phase 2/stage 1), Product name: **Desam EFFEKT+**LOT: **017A160120**Manufacturer: **SCHÜLKE CZ s.r.o.**

Storage conditions (temperature etc.): room temperature, dark

Number of seeded plates 2 ml, Neutralizer: Polysorbate 80 30.0 g/l + natrium thiosulphate (Na₂S₂O₃) 5 g/l, L-histidine 1 g/l.

Test conditions: 20°C Load: High - Erythrocytes 3ml/l + Bovine albumin 3 g/l,

Tested organism: **Mycobacterium terrae DSM 43227**, Temperature of incubation 36°CProcedure: Product was diluted by hard water to final concentrations **0.5, 1, and 1.5 %** (m/V).Date of the test: 5th February 2016

Elaborated by: Vít Ulmann

Controlled by: Vít Ulmann

Signature:

Controls and validations:

Validation suspension (Nv ₀)			Experimental conditions control (A)			Neutralizer control (B)			Validation (product control) (C)		
V _{c1}	153	X=150	V _{c1}	101	X=90	V _{c1}	86	X=87,5	V _{c1}	101	X=89,5
V _{c2}	147		V _{c2}	90		V _{c2}	89		V _{c2}	78	
39 ≤ x from Nv ₀ ≤ 160 ? YES <input checked="" type="checkbox"/> NO			X z A ≥ 0,5 * x from Nv ₀ ? YES <input checked="" type="checkbox"/> NO			X z B ≥ 0,5 * x from Nv ₀ ? YES <input checked="" type="checkbox"/> NO			X z C ≥ 0,5 * x from Nv ₀ ? YES <input checked="" type="checkbox"/> NO		

Test suspension and test:

Test suspension control (N a N ₀)		N	V _{c1}	V _{c2}	X _{wm} = 500,00x 10 ⁷ = log = 9,66 N ₀ = N/10 = lg 8,66 8,17 ≤ N ₀ ≤ 8,70? YES <input checked="" type="checkbox"/> NO
		10 ⁻⁷	344	427	
		10 ⁻⁸	129	107	

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 nebo x _{wm} x 10)	Lg R (N _w = lg 8,66)	Exposure time (min)
0,5	10 ⁰	>660	>660	4,34	4,32	60 min
	10 ⁻¹	226*	214*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1	10 ⁰	352	369	3,44	5,22	30 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1,5	10 ⁰	34	38	2,56	6,10	30 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			

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Test conditions: 20°C Load: Low - Bovine albumin 0.3 g/l,
 Tested organism: *Mycobacterium terrae* DSM 43227, Temperature of incubation 36°C
 Procedure: Product was diluted by hard water to final concentrations 0.5, 1, and 1.5 % (m/V).
 Date of the test: 5th February 2016

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 nebo x _{wm} x 10)	Lg R (N _w = lg 8,70)	Exposure time (min)
0.5	10 ⁰	259	247	3,40	5,26	60 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1	10 ⁰	119	120	3,08	5,58	30 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1.5	10 ⁰	<14	<14	2,15	> 6,51	30 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			

*Encountered values

Comments: N 10⁻⁷ : 182 + 162; 219 + 208
 10⁻⁸ : 61 + 68; 59 + 48

Nvo: 88 + 65; 58 + 89

Explanatory notes: V_c = count per ml, x = average V_{c1} a V_{c2} (1. + 2) duplicate determination, X_{wm} = weighted average x, R reduction
 (lg R = Lg N₀ - Lg N_t)

Protocol attachment n. 2: 2/2016/SMUEN 14348 (phase 2/stage 1), Product name: **Desam EFFEKT+**LOT: **017A160120**Manufacturer: **SCHÜLKE CZ s.r.o**

Storage conditions (temperature etc.): room temperature, dark

Number of seeded plates 2 ml, Neutralizer: Polysorbate 80 30, 0 g/l + natrium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) 5 g/l, L-histidine 1 g/l.

Test conditions: 20°C Load: High: Erythrocytes 3ml/l + Bovine albumin 3 g/l

Tested organism: **Mycobacterium avium DSM 44157**, Temperature of incubation 36°CProcedure: Product was diluted by hard water to final concentrations **0.5, 1, and 1.5 % (m/v)**.Date of the test: 8th February 2016

Elaborated by: Vít Ulmann

Controlled by: Vít Ulmann

Signature:

Controls and validations:

Validation suspension (Nv0)			Experimental conditions control (A)			Neutralizer control (B)			Validation (product control) (C)		
Vc1	153	X=129	Vc1	79	X=73,5	Vc1	81	X=84	Vc1	67	X=67
Vc2	105		Vc2	68		Vc2	87		Vc2	67	
39 ≤ x from Nv0 ≤ 160 ? YES <input checked="" type="checkbox"/> NO			X z A ≥ 0,5 * x from Nv0 ? YES <input checked="" type="checkbox"/> NO			X z B ≥ 0,5 * x from Nv0 ? YES <input checked="" type="checkbox"/> NO			X z C ≥ 0,5 * x from Nv0 ? YES <input checked="" type="checkbox"/> NO		

Test suspension and test:

Test suspension control (N a NO)		N	V_{c1}	V_{c2}	$X_{wm} = 500,00 \times 10^7 = \log = 9,65$ $N_0 = N/10 = \lg 8,65$ $8,17 \leq N_0 \leq 8,70$? YES <input checked="" type="checkbox"/> NO
		10^{-7}	381	358	
		10^{-8}	113	128	

Products concentration %	Dilution step	V_{c1}	V_{c2}	$\lg N_a = \lg (x \times 10 \text{ or } x_{wm} \times 10)$	$\lg R (N_w = \lg 8,65)$	Exposure time (min)
0.5	10^0	>660	>660	4,61	4,04**	60 min
	10^{-1}	293*	267*			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
1	10^0	211*	249*	3,48	5,17	30 min
	10^{-1}	46*	85*			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			
1.5	10^0	124*	112*	3,14	5,51	30 min
	10^{-1}	33*	34*			
	10^{-2}	<14	<14			
	10^{-3}	<14	<14			

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Test conditions: 20°C Load: Low: Bovine albumin 0, 3 g/l

Tested organism: *Mycobacterium avium* DSM 44157, Temperature of incubation 36°C

Procedure: Product was diluted by hard water to final concentrations 0.5, 1, and 1.5 % (m/V).

Date of the test: 8th February 2016

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 or x _{wm} x 10)	Lg R (N _w = lg 8,70)	Exposure time (min)
0.5	10 ⁰	>660	>660	4,48	4,17	60 min
	10 ⁻¹	293*	267*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1	10 ⁰	211*	249*	3,37	5,28	30 min
	10 ⁻¹	46*	85*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1.5	10 ⁰	124*	112*	2,82	5,83	30 min
	10 ⁻¹	33*	34*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			

*Encountered values

Comments: N 10⁻⁷ : 171 + 210; 184 + 174

N_{vo}: 85 + 68; 49 + 56

10⁻⁸ : 64 + 67; 73 + 55

Explanatory notes: V_c = count per ml, x = average V_{c1} a V_{c2} (1. + 2) duplicate determination, X_{wm} = weighted average x, R reduction (lg R = Lg N_o - Lg N_t)

Protocol attachment n. 3: 2/2016/SMU

EN 14348 (phase 2/stage 1), Product name: Desam EFTEKT+

LOT: 017A160120

Manufacturer: SCHÜLKE CZ s.r.o

Storage conditions (temperature etc.): room temperature, dark

Number of seeded plates 2 ml, Neutralizer: Polysorbate 80 30, 0 g/l + natrium thiosulphate (Na₂S₂O₃) 5 g/l, L-histidine 1 g/l.

Test conditions: 20°C Load: High: Erythrocytes 3ml/l + Bovine albumin 3 g/l

Tested organism: *Mycobacterium avium* DSM 44157, Temperature of incubation 36°C

Procedure: Product was diluted by hard water to final concentration 0.5 % (m/V). Each test was provided with newly prepared culture suspension and product dilution.

Dates of the test: 9th February 2016

Elaborated by: Vít Ulmann

Controlled by: Vít Ulmann

Signature:

Repetitions with organism:

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 or x wm x 10)	Lg R	Exposure time (min)
9. 2. 2016	1			4,58	N ₀ = lg 8,64	60 min
0,5	10 ⁰	>660	>660		4,06**	
	10 ⁻¹	374*	392*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
9. 2. 2016	2			4,68	N ₀ = lg 8,70	60 min
0,5	10 ⁰	>660	>660		4,02**	
.	10 ⁻¹	519*	442*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
9. 2. 2016	3			4,56	N ₀ = lg 8,57	60 min
0,5	10 ⁰	>660	>660		4,01**	
	10 ⁻¹	374*	358*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
9. 2. 2016	4			4,75	N ₀ = lg 8,70	60 min
0,5	10 ⁰	>660	>660		3,95**	
	10 ⁻¹	518*	599*			
	10 ⁻²	36*	43*			
	10 ⁻³	<14	<14			
9. 2. 2016	5			4,28	N ₀ = lg 8,34	60 min
0,5	10 ⁰	>660	>660		4,06**	
	10 ⁻¹	208*	175*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
** Average reduction at six repetitions:				1,06 x 10 ⁴		

*Encountered values

Comments: V_{c1} V_{c2}

1 N 10 ⁻⁷ : 398; 358	N = 2,9x10 ⁹ Lg N = 9,64
10 ⁻⁸ : 115; 94	No = 2,9x10 ⁸ Lg No = 8,64
2 N 10 ⁻⁷ : 392; 405	N = 4,9x10 ⁹ Lg N = 9,70
10 ⁻⁸ : 151; 148	No = 4,9x10 ⁸ Lg No = 8,70
3 N 10 ⁻⁷ : 320; 289	N = 3,7x10 ⁹ Lg N = 9,57
10 ⁻⁸ : 110; 98	No = 3,7x10 ⁸ Lg No = 8,57
4 N 10 ⁻⁷ : 410; 395	N = 5,0x10 ⁹ Lg N = 9,70
10 ⁻⁸ : 145; 158	No = 5,0x10 ⁸ Lg No = 8,70
5 N 10 ⁻⁷ : 215; 184	N = 2,7x10 ⁹ Lg N = 9,34
10 ⁻⁸ : 48; 36	No = 2,7x10 ⁸ Lg No = 8,34

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