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Workplace nr. 1 - Ostrava
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L 1554

Testing protocol n. 1/2017/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 445

735 81 Bohumín

Czech Republic

Order n.:

Sample identification:

Product name: **CHIROSAN PLUS**

LOT n.: **014A160217**

Manufacturer: **SCHÜLKE CZ s.r.o**

Storage conditions: room temperature, dark
Diluent: tap water
Appearance and composition: Peracetic acid produced in situ
Active compounds:

Date of delivery: 9th January.2017

Dates of testing: 27th and 28th January 2017

Results: see attachments 1 - 3

Conclusion:

According to EN 14348, Product **CHIROSAN PLUS** LOT **014A160217** after dilution by hard water to 0,5% (m/m), proved **mycobactericidal** activity within **15 minutes** on temperature 20° C 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* was **5,03** ($\pm 0,037^*$) logarithmic orders. Second organism was tested once and proved higher reduction than *Mycobacterium avium*.

*S_R – reproducibility standard deviation

In Ostrava: 22th February 2017

Vít Ulmann, MSc.
Specialist supervisor for laboratory
of mycobacterial diagnostics and TB

Zdravotní ústav se sídlem v Ostravě
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Protocol attachment n. 1: 1/2017/SMU

EN 14348 (phase 2/stage 1), Product name: **CHIROSAN PLUS**

Manufacturer: **SCHÜLKE CZ s.r.o.**

LOT: **014A160217**

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

Number of seeded plates 2 ml, Neutralizer: Polysorbate 80 30, 0 g/l + natrium thiosulphate (Na₂S₂O₃) 15 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°C

Procedure: Product was diluted by hard water to final concentrations **0,5 a 1 % (m/m)**.

Date of the test: 27th January 2017

Elaborated by: Vít Ulmann

Controlled by: Vít Ulmann

Signature: 

Controls and validations:

Validation suspension (N _{v0})			Experimental conditions control (A)			Neutralizer control (B)			Validation (product control) (C)		
V _{c1}	75	X=78,5	V _{c1}	84	X=71,5	V _{c1}	70	X=77,5	V _{c1}	53	X=64
V _{c2}	82		V _{c2}	59		V _{c2}	85		V _{c2}	75	
39 ≤ x from N _{v0} ≤ 160 ? YES <input checked="" type="checkbox"/> NO			X z A ≥ 0,5 * x from N _{v0} ? YES <input checked="" type="checkbox"/> NO			X z B ≥ 0,5 * x from N _{v0} ? YES <input checked="" type="checkbox"/> NO			X z C ≥ 0,5 * x from N _{v0} ? 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 = 9,59 N ₀ = N/10 = lg 8,59 8,17 ≤ N ₀ ≤ 8, 70? YES <input checked="" type="checkbox"/> NO
	10 ⁻⁷	343	316	
	10 ⁻⁸	86	117	

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 nebo x _{wm} x 10)	Lg R (N ₀ = lg 8,59)	Exposure time (min)
0,5%	10 ⁰	111*	115*	3,05	5,54	15 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1%	10 ⁰	<14*	<14*	2,15	>6,45	15 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
1%	10 ⁰	>660	>660	5,46	3,14	5 min
	10 ⁻¹	>660	>660			
	10 ⁻²	271*	240*			
	10 ⁻³	53*	67*			

*Encountered values

Comments: N 10⁻⁷: 164 + 179; 145 + 171
10⁻⁸: 45 + 41; 61 + 56

N_{v0}: 43 + 32; 53 + 29
N_a: 0,5% : 56, 55; 67, 48
N_a: 1% 15 min: 2, 3; 1, 0
N_a: 1% 5 min: 129, 142; 112, 128; 26, 27; 39, 28

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_a)

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Protocol attachment n. 2: 1/2017/SMU

EN 14348 (phase 2/stage 1), Product name: **CHIROSAN PLUS**

LOT: **014A160217**

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) 15 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 concentrations **0, 5 a 1 % (m/m)**.

Date of the test: 27th January 2016

Elaborated by: Vít Ulmann

Controlled by: Vít Ulmann

Signature: 

Controls and validations:

Validation suspension (N _{v0})			Experimental conditions control (A)			Neutralizer control (B)			Validation (product control) (C)		
V _{c1}	103	X=118	V _{c1}	80	X=66,5	V _{c1}	74	X=81,5	V _{c1}	75	X=80
V _{c2}	133		V _{c2}	53		V _{c2}	89		V _{c2}	85	
39 ≤ x from N _{v0} ≤ 160 ? YES <input checked="" type="checkbox"/> NO			X z A ≥ 0, 5 * x from N _{v0} ? YES <input checked="" type="checkbox"/> NO			X z B ≥ 0, 5 * x from N _{v0} ? YES <input checked="" type="checkbox"/> NO			X z C ≥ 0, 5 * x from N _{v0} ? 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 = 9,70 N ₀ = N/10 = lg 8,70 8, 17 ≤ N ₀ ≤ 8, 70? YES <input checked="" type="checkbox"/> NO
	10 ⁻⁷	358	375	
	10 ⁻⁸	180	186	

Products concentration %	Dilution step	V _{c1}	V _{c2}	Lg N _a = lg (x x 10 or x _{wm} x 10)	Lg R (N ₀ = lg 8,70)	Exposure time (min)
1%	10 ⁰	>660	>660	6,20	2,50	5 min
	10 ⁻¹	>660	>660			
	10 ⁻²	>660	>660			
	10 ⁻³	155*	163*			
1%	10 ⁰	40*	36*	2,58	6,12	15 min
	10 ⁻¹	<14	<14			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
0,5%	10 ⁰	382*	303*	3,59	5,11**	15 min
	10 ⁻¹	72*	99*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			

*Encountered values

Comments: N 10⁻⁷: 182 + 176; 191 + 184
10⁻⁸: 95 + 85; 92 + 94

N_{v0}: 45 + 58; 91 + 69
Na: 1% 5min: 76, 79; 94, 69
Na: 1% 15 min: 22, 18; 17, 19
Na: 0, 5% 15 min: 195,187; 145,158; 43, 29; 45, 54

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_a)

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Protocol attachment n. 3: 1/2017/SMU

EN 14348 (phase 2/stage 1), Product name: **CHIROSAN PLUS**

LOT: **014A160217**

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) 15 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/m). Each test was provided with newly prepared culture suspension and product dilution.

Dates of the test: 28th January 2017

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)
0,5	1			3,51	N ₀ = lg 8,54	15 min
	10 ⁰	320*	260*		5,03**	
	10 ⁻¹	62*	67*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
0,5	2			3,63	N ₀ = lg 8,57	15 min
	10 ⁰	381*	337*		4,94**	
	10 ⁻¹	97*	119*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
0,5	3			3,61	N ₀ = lg 8,62	15 min
	10 ⁰	386*	336*		5,01**	
	10 ⁻¹	90*	74*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
0,5	4			3,52	N ₀ = lg 8,64	15 min
	10 ⁰	300*	267*		5,12**	
	10 ⁻¹	74*	87*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
0,5	5			3,51	N ₀ = lg 8,60	15 min
	10 ⁰	285*	279*		5,09**	
	10 ⁻¹	63*	83*			
	10 ⁻²	<14	<14			
	10 ⁻³	<14	<14			
** Average reduction by six repetitions with standard deviation:					5,05 ± 0,037 lg order	

*Encountered values

Comments: V_{c1} V_{c2}

1 N 10 ⁻⁷ : 284; 290	N = 3,4x10 ⁹ Lg N = 9,54
10 ⁻⁸ : 88; 95	No = 3,4x10⁸ Lg No = 8,54
2 N 10 ⁻⁷ : 298; 310	N = 3,7x10 ⁹ Lg N = 9,57
10 ⁻⁸ : 95; 110	No = 3,7x10⁸ Lg No = 8,57
3 N 10 ⁻⁷ : 358; 355	N = 4,2x10 ⁹ Lg N = 9,62
10 ⁻⁸ : 110; 92	No = 4,2x10⁸ Lg No = 8,62
4 N 10 ⁻⁷ : 348; 381	N = 4,4x10 ⁹ Lg N = 9,64
10 ⁻⁸ : 112; 128	No = 4,4x10⁸ Lg No = 8,64
5 N 10 ⁻⁷ : 358; 298	N = 4,0x10 ⁹ Lg N = 9,60
10 ⁻⁸ : 99; 115	No = 4,0x10⁸ Lg No = 8,60

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