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**Determination of sporicidal activity
of ChiroSan[®] plus (sample no. 2819)
according to the EN 17126**

Summary Report

Laboratory expertise no. 191288/2019

Date of the expert report: November 19, 2019

Sporicidal activity


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Characteristics of presented sample

Contracting authority: Schulke CZ, s.r.o.,
Lidická 445, 735 81 Bohumín

Manufacturer: Schulke CZ, s.r.o.,
Lidická 445, 735 81 Bohumín

Product description: (according to manufacturer's specification)

Product ChiroSan[®] plus (sample no. 2819) is white powder

Package type: plastic container

Unit shipments in original undamaged packaging: 1 piece

Total amount of supplied product: about 250 g

Date of sampling: August 28, 2019

Test start: October 2019

Test end: October 2019

Product composition: The active substance is peroxyacetic acid, which is generated in the aqueous environment from the ingredients - sodium percarbonate and tetraacetylenediamine (TAED).

Product contains Sodium percarbonate (CAS: 15630-89-4, EC: 239-707-6) ≤ 50 %;

Citric acid (CAS: 77-92-9, EC: 201-069-1) < 15 %; (1-hydroxyethylidene)

bisphosphonic acid, sodium salt (CAS: 29329-71-3, ES: 249-559-4) < 5 %; Potassium carbonate (CAS: 584-08-7, ES: 209-529-3) < 1 %

Use of product: Concentrated powder disinfectant based on generated peroxyacetic acid, suitable for single-phase disinfection, second and higher disinfection of medical devices, surgical, anesthesiological and dental instruments and endoscopes. Also suitable for disinfecting surfaces of medical devices.

Documentation included:

Safety Data Sheet in Czech language.

Subject of expertise was the interpretation of results of the sporicidal efficiency of the submitted samples in laboratory experiments.

Interpretation of laboratory tests results

Product ChiroSan® plus (Laboratory sample No. 2819) demonstrated the sporicidal activity against *Clostridium difficile* (According to the EN 17126) at the concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l), for products used in the Medical area.

Product ChiroSan® plus (Laboratory sample No. 2819) demonstrated the sporicidal activity (According to the EN 17126) at the concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l), for products used in the Medical area.

The samples were not evaluated in terms of cleaning performance, corrosivity, toxicity, irritability and safety. This report applies only to the samples submitted and the conclusions drawn from this expertise can be applied to other products of the same kind only if their composition, contents and properties completely match the samples under testing.

The presented test results relate only to the samples referred to in this protocol and are not intended to replace other official manufacturer's documentation.

The protocol can be reproduced only in complete form with the written consent of the testing laboratory.

Determination of sporicidal activity of ChiroSan® plus (sample no. 2819) according to the EN 17126

The microbicidal effectiveness of disinfectants is determined by the following methods:

Non accredited methods

1. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants and antiseptics according to the EN 17126 in the medical area.

The test method used for evaluation of the sporicidal activity of disinfectants is processed based on selected methods according to the EN 14885 (EN 17126). This is a quantitative method. The method is not accredited according to the EN ISO/IEC 17025:2005.

National Reference Laboratory for disinfection and sterilization is "testing laboratory no. 1206.4 accredited by Czech Accreditation Institute according to the standard EN ISO / IEC 17025:2005".

Results – Non accredited methods

More information you will find in the report of the outcome of Non accredited methods - Annex.

The sporicidal activity against *Clostridium difficile* for the product ChiroSan® plus (Laboratory sample No. 2819), determined for general purposes according to the EN 17126 standard (obligatory conditions) under clean conditions is:

According to the EN 17126, the product ChiroSan® plus (Laboratory sample No. 2819), demonstrates sporicidal activity against *Clostridium difficile* in concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l).

A reduction of spores of 4 log was noticed. This reduction guarantees according to the EN 17126 sporicidal activity against *Clostridium difficile* for products used in the Medical area.

The sporicidal activity against *Clostridium difficile* was tested on test organism *Clostridium difficile* spores.

The sporicidal activity for the product ChiroSan® plus (Laboratory sample No. 2819), determined for general purposes according to the EN 17126 standard (obligatory conditions) under clean conditions is:

According to the EN 17126, the product ChiroSan® plus (Laboratory sample No. 2819), demonstrates sporicidal activity in concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l).

A reduction of spores of 4 log was noticed. This reduction guarantees according to the EN 17126 sporicidal activity for products used in the Medical area.

The sporicidal activity was tested on test organisms *Bacillus subtilis* spores and *Bacillus cereus* spores.

References:

1. EN 17126 (December 2018) - Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area - Test method and requirements (phase 2, step 1)
2. EN 14885 (August 2015) – Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics

Annex: Non accredited method - testing the efficiency of products used in the medical area

Test result – Quantitative sporicidal suspension test

EN 17126 (phase 2, step 1)

Product name: ChiroSan[®] plus

Designation batch: 037A190318, Expiration: 2021-03-18

Client and Manufacturer: Schulke CZ, s.r.o., Lidická 445, 735 81 Bohumín, Czech Republic

Appearance of the product: white powder

Storage conditions: Store in original, tightly closed containers. Store in a dry place. Do not store in direct sunlight and together with flammable materials. Store away from food, drinks, feed. Storage temperature: - 10 to + 25 ° C.

Dilution neutralization method – yes, Spread plate – yes, Number of plates 2/ml

Neutralizer: Tween 80 – 0,3 %, cystein – 0,1 %, histidin – 0,5 %,

sodium thiosulphate – 0,5 % in diluent

Test temperature: 20±1 °C

Contact time: 10 and 15 min ± 10 s (Concentration: 2 %), 30 min ± 10 s (Concentration: 1 %), 60 min ± 10 s (Concentration: 0,5 %)

Interfering substance: bovine albumin 0,3 g/l (clean conditions)

Test organisms:

Clostridium difficile R027 spores (NCTC 13366); Incubation temperature: 36±1 °C

Bacillus subtilis spores (ATCC 6633); Incubation temperature: 36±1 °C

Bacillus cereus spores (CIP 105151); Incubation temperature: 36±1 °C

Laboratory sample No.: 2819

Date of test: October 9 - 14, 2019

Responsible person: Ing. Jan Urban, Ph.D.

Signature: 

Diluent used for product test solutions: hard water

Appearance of the product test solutions: liquid with a weak white haze

Table 1

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)											
organism: <i>Clostridium difficile</i> spores (NCTC 13366)											
Non accredited SOP-NRL/DS-01, method R											
Laboratory sample No. 2819: ChiroSan® plus											
Validation and Controls											
Validation suspension (N_{V0})			Experimental Conditions Control (A)			Neutralizer control (B)			Method validation (C) Product conc.: 10 ml/l		
V_{C1}	74 (35+39)	$\bar{x} = 69$	V_{C1}	65 (34+31)	$\bar{x} = 61,5$	V_{C1}	56 (31+25)	$\bar{x} = 60,5$	V_{C1}	52 (27+25)	$\bar{x} = 55$
V_{C2}	64 (36+28)		V_{C2}	58 (27+31)		V_{C2}	65 (33+32)		V_{C2}	58 (31+27)	
$30 \leq \bar{x} \text{ of } N_{V0} \leq 160?$			$\bar{x} \text{ of } A \text{ is } \geq 0,5 \times \bar{x} \text{ of } N_{V0}?$			$\bar{x} \text{ of } B \text{ is } \geq 0,5 \times \bar{x} \text{ of } N_{V0}$ or $\bar{x} \text{ of } N_{VB}/1000?$			$\bar{x} \text{ of } C \text{ is } \geq 0,5 \times \bar{x} \text{ of } N_{V0}?$		
x yes no			x yes no			x yes no			x yes no		
Validation suspension (N_{VB})			$V_{C1} = 70$ (34+36)	$V_{C2} = 65$ (29+36)	$\bar{x} = 67,5$		$30 \leq \bar{x} \text{ of } N_{VB}/1000 \leq 160?$ x yes no				

Table 2

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)						
organism: <i>Clostridium difficile</i> spores (NCTC 13366)						
Non accredited SOP-NRL/DS-01, method R						
Laboratory sample No. 2819: ChiroSan® plus						
Test suspension						
Test-suspension (N and N_0):	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 195,45 \times 10^5 = \lg 7,29$		
	10^{-5}	188	203	$N_0 = N / 10 = \lg 6,29$		
	10^{-6}	17	22	$6,17 \leq N_0 \leq 6,70 ?$ x yes no		

Table 3

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)							
organism: <i>Clostridium difficile</i> spores (NCTC 13366)							
Non accredited SOP-NRL/DS-01, method R							
Laboratory sample No. 2819: Chirosan® plus							
Test							
Conc. of the Product %	Dilution	V _{C1}	V _{C2}	N _a = (\bar{x} or $\bar{x}_{wm} \times 10$)	lg N _a	lg R (N ₀ = 6,29)	Contact time (min)
0,5	10 ⁰	3	2	< 140	< 2,15	> 4,14	60
	10 ⁻¹	0	0				
1	10 ⁰	1	1	< 140	< 2,15	> 4,14	30
	10 ⁻¹	0	0				
2	10 ⁰	16	14	150	2,18	4,11	10
	10 ⁻¹	3	1				
2	10 ⁰	5	2	< 140	< 2,15	> 4,14	15
	10 ⁻¹	1	0				

Counting per plate for (2 plates on 1 ml):

N: 10⁻⁵ = 91+97, 104+99, 10⁻⁶ = 7+10, 12+10

N_a: 0,5 % (60 min) 10⁰ = 1+2, 0+2; 1 % (30 min) 10⁰ = 0+1, 1+0; 2 % (10 min) 10⁰ = 9+7, 8+6, 10⁻¹ = 2+1, 0+1; 2 % (15 min) 10⁰ = 1+4, 1+1, 10⁻¹ = 0+1, 0+0

Explanations:

V_C = count per ml (one plate or more)

R = reduction (lg R = lg N₀ - lg N_a)

\bar{x} = average of V_{C1} and V_{C2} (1. + 2. duplicate)

\bar{x}_{wm} = weighted mean of \bar{x}

Table 4

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)											
organism: <i>Bacillus subtilis</i> spores (ATCC 6633)											
Non accredited SOP-NRL/DS-01, method R											
Laboratory sample No. 2819: ChiroSan® plus											
Validation and Controls											
Validation suspension (N_{V0})			Experimental Conditions Control (A)			Neutralizer control (B)			Method validation (C) Product conc.: 10 ml/l		
V_{C1}	53 (25+28)	$\bar{x} = 54$	V_{C1}	43 (23+20)	$\bar{x} = 47$	V_{C1}	51 (27+24)	$\bar{x} = 46$	V_{C1}	35 (17+18)	$\bar{x} = 37,5$
V_{C2}	55 (29+26)		V_{C2}	51 (25+26)		V_{C2}	41 (19+22)		V_{C2}	40 (21+19)	
$30 \leq \bar{x} \text{ of } N_{V0} \leq 160?$			$\bar{x} \text{ of A is } \geq 0,5 \times \bar{x} \text{ of } N_{V0}?$			$\bar{x} \text{ of B is } \geq 0,5 \times \bar{x} \text{ of } N_{V0} \text{ or } \bar{x} \text{ of } N_{VB}/1000?$			$\bar{x} \text{ of C is } \geq 0,5 \times \bar{x} \text{ of } N_{V0}?$		
x yes no			x yes no			x yes no			x yes no		
Validation suspension (N_{VB})			$V_{C1} = 49$ (27+22)	$V_{C2} = 54$ (28+26)	$\bar{x} = 51,5$		$30 \leq \bar{x} \text{ of } N_{VB}/1000 \leq 160?$ x yes no				

Table 5

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)					
organism: <i>Bacillus subtilis</i> spores ATCC 6633)					
Non accredited SOP-NRL/DS-01, method R					
Laboratory sample No. 2819: ChiroSan® plus					
Test suspension					
Test-suspension (N and N_0):	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 168,18 \times 10^5 = \lg 7,23$	
	10^{-5}	172	164	$N_0 = N / 10 = \lg 6,23$	
	10^{-6}	18	16	$6,17 \leq N_0 \leq 6,70 ?$ x yes no	

Table 6

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)							
organism: <i>Bacillus subtilis</i> spores (ATCC 6633)							
Non accredited SOP-NRL/DS-01, method R							
Laboratory sample No. 2819: ChiroSan® plus							
Test							
Conc. of the Product %	Dilution	V _{C1}	V _{C2}	N _a = (\bar{x} or $\bar{x}_{wm} \times 10$)	lg N _a	lg R (N ₀ = 6,23)	Contact time (min)
0,5	10 ⁰	2	1	< 140	< 2,15	> 4,08	60
	10 ⁻¹	0	0				
1	10 ⁰	0	0	< 140	< 2,15	> 4,08	30
	10 ⁻¹	0	0				
2	10 ⁰	0	0	< 140	< 2,15	> 4,08	10
	10 ⁻¹	0	0				
2	10 ⁰	0	0	< 140	< 2,15	> 4,08	15
	10 ⁻¹	0	0				

Counting per plate for (2 plates on 1 ml):

N: 10⁻⁵ = 89+83, 88+76, 10⁻⁶ = 10+8, 8+8

N_a: 2 % (15 min) 10⁰ = 2+0, 0+1

Explanations:

V_C = count per ml (one plate or more)

R = reduction (lg R = lg N₀ - lg N_a)

\bar{x} = average of V_{C1} and V_{C2} (1. + 2. duplicate)

\bar{x}_{wm} = weighted mean of \bar{x}

Table 7

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)											
organism: <i>Bacillus cereus</i> spores (CIP 105151)											
Non accredited SOP-NRL/DS-01, method R											
Laboratory sample No. 2819: Chirosan® plus											
Validation and Controls											
Validation suspension (N _{v0})			Experimental Conditions Control (A)			Neutralizer control (B)			Method validation (C) Product conc.: 10 ml/l		
V _{C1}	67 (32+35)	x̄ = 71	V _{C1}	61 (33+28)	x̄ = 63,5	V _{C1}	64 (35+29)	x̄ = 61	V _{C1}	47 (22+25)	x̄ = 49,5
V _{C2}	75 (41+34)		V _{C2}	66 (34+32)		V _{C2}	58 (27+31)		V _{C2}	52 (28+24)	
30 ≤ x̄ of N _{v0} ≤ 160?			x̄ of A is ≥ 0,5 × x̄ of N _{v0} ?			x̄ of B is ≥ 0,5 × x̄ of N _{v0} or x̄ of N _{vB} /1000?			x̄ of C is ≥ 0,5 × x̄ of N _{v0} ?		
x yes no			x yes no			x yes no			x yes no		
Validation suspension (N _{vB})			V _{C1} = 65 (33+32)	V _{C2} = 76 (41+35)	x̄ = 70,5		30 ≤ x̄ of N _{vB} /1000 ≤ 160? x yes no				

Table 8

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)				
organism: <i>Bacillus cereus</i> spores (CIP 105151)				
Non accredited SOP-NRL/DS-01, method R				
Laboratory sample No. 2819: Chirosan® plus				
Test suspension				
Test-suspension (N and N ₀):	N	V _{C1}	V _{C2}	x̄ _{wm} = 190,45 × 10 ⁵ = lg 7,28 N ₀ = N / 10 = lg 6,28 6,17 ≤ N ₀ ≤ 6,70 ? x yes no
	10 ⁻⁵	193	187	
	10 ⁻⁶	21	18	

Table 9

Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectant and antiseptics (EN 17126)							
organism: <i>Bacillus cereus</i> spores (CIP 105151)							
Non accredited SOP-NRL/DS-01, method R							
Laboratory sample No. 2819: Chirosan® plus							
Test							
Conc. of the Product %	Dilution	V _{C1}	V _{C2}	N _a = (\bar{x} or $\bar{x}_{wm} \times 10$)	lg N _a	lg R (N ₀ = 6,28)	Contact time (min)
0,5	10 ⁰	0	0	< 140	< 2,15	> 4,13	60
	10 ⁻¹	0	0				
1	10 ⁰	0	0	< 140	< 2,15	> 4,13	30
	10 ⁻¹	0	0				
2	10 ⁰	0	0	< 140	< 2,15	> 4,13	10
	10 ⁻¹	0	0				
2	10 ⁰	0	0	< 140	< 2,15	> 4,13	15
	10 ⁻¹	0	0				

Counting per plate for (2 plates on 1 ml):

N: 10⁻⁵ = 98+95, 89+98, 10⁻⁶ = 12+9, 8+10

N_a:

Explanations:

V_C = count per ml (one plate or more)

R = reduction (lg R = lg N₀ - lg N_a)

\bar{x} = average of V_{C1} and V_{C2} (1. + 2. duplicate)

\bar{x}_{wm} = weighted mean of \bar{x}

Conclusion (test result, table 1-9):

The sporicidal activity against *Clostridium difficile* for the product ChiroSan® plus (Laboratory sample No. 2819), determined for general purposes according to the EN 17126 standard (obligatory conditions) under clean conditions is:

According to the EN 17126, the product ChiroSan® plus (Laboratory sample No. 2819), demonstrates sporicidal activity against *Clostridium difficile* in concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l).

A reduction of spores of 4 log was noticed. This reduction guarantees according to the EN 17126 sporicidal activity against *Clostridium difficile* for products used in the Medical area.

The sporicidal activity against *Clostridium difficile* was tested on test organism *Clostridium difficile* spores.

The sporicidal activity for the product ChiroSan® plus (Laboratory sample No. 2819), determined for general purposes according to the EN 17126 standard (obligatory conditions) under clean conditions is:

According to the EN 17126, the product ChiroSan® plus (Laboratory sample No. 2819), demonstrates sporicidal activity in concentration 0,5 % for 60 minutes, in concentration 1 % for 30 minutes and in concentration 2 % for 10 and 15 minutes at 20 °C under clean conditions (bovine albumin 0,3 g/l).

A reduction of spores of 4 log was noticed. This reduction guarantees according to the EN 17126 sporicidal activity for products used in the Medical area.

The sporicidal activity was tested on test organisms *Bacillus subtilis* spores and *Bacillus cereus* spores.

