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

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Test report No. D256/2016

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Date: 2022.12.26 15:57:50 EET
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Location: Moldova

DETERMINATION OF BACTERICIDAL (ČSN EN 13727+A2) AND YEASTICIDAL (ČSN EN 13624) ACTIVITY OF THE PRODUCT **CHIROSAN PLUS**

Sample ID: D256/2016
Sample name: **CHIROSAN PLUS**
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín
Producer: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín
Sampling point: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Incoming date:
5.10.2016

Delivery date:
6.2.2017

Hodonín, 6.2.2017

Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
Testing date: 1.11. – 4.11.2016
Delivered amount: 0.5 kg
Batch No: 064A160623

Subject of testing:
Determination of bactericidal and yeasticidal activity of the product.

Identification of the sample:

Name of the product:	**CHIROSAN PLUS**
Batch number:	064A160623
Date of manufacture:	23.6.2016
Expiry date:	23.6.2017
Manufacturer:	SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín
Incoming date:	5.10.2016
Storage conditions:	room temperature
Active compounds and concentrations:	CAS 15630-89-4 sodium percarbonate ≤ 50%

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method
SOP-M-19-00 (ČSN EN 13727+A2)

Period of analysis:	2.11. – 3.11.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	white powder
Product diluent:	hard water
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
Incubation conditions:	37 °C ± 1 °C, 24 hours

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:
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).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

The standard:
ČSN EN 13727+A2 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) June 2016

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

Sample ID: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
Testing date: 1.11. – 4.11.2016
Delivered amount: 0.5 kg
Batch No: 064A160623

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

1. Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** on *Pseudomonas aeruginosa* ATCC 15442

Tab No. 1.1 Verification of methodology, temperature 20°C, 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	$\Phi_{Nv0} = 52.5$	V_{c1}	49	$\Phi_A = 47$	V_{c1}	53	$\Phi_B = 47.5$	V_{c1}	51	$\Phi_C = 48.5$
V_{c2}	50		V_{c2}	45		V_{c2}	42		V_{c2}	46	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	43	V_{c2}	50	Φ_{NVB}	46.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 1.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 50 \times 10^7 = \lg 8.70$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.70$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	57	43	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** 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 ($\lg N_0 = \lg 7.70$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.55**

2. Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** on *Staphylococcus aureus* ATCC 6538

Tab No. 2.1 Verification of methodology, temperature 20°C, 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}	28	$\Phi_{Nv0} = 30.5$	V_{c1}	29	$\Phi_A = 26$	V_{c1}	32	$\Phi_B = 28.5$	V_{c1}	29	$\Phi_C = 27$
V_{c2}	33		V_{c2}	23		V_{c2}	25		V_{c2}	25	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	26	V_{c2}	30	Φ_{NVB}	28	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) ≤ 160		
							x	yes	no

Tab No. 2.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0
$\Phi = 34 \times 10^7 = \lg 8.53$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 7.53$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	33	35	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 2.3 Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** 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 ($\lg N_0 = \lg 7.53$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 5.38**

Notes: V_c = the number of cfu per ml, Φ = average of V_{c1} and V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml in the test suspension, N_0 = the number of cfu/ml in the test mixture at the beginning of the contact time (time "zero"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before neutralization, N_v = the number of cfu/ml in the bacterial test suspension for validation, N_{v0} = the number of cfu/ml in the mixtures A,B and C at the beginning of the contact time (time "zero"), N_{VB} = the number of cfu/ml in the suspension for neutralizer control, A, B, C = the number of survivors per ml in the control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation) R = N_0/N_a = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

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

Sample ID: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
Testing date: 1.11. – 4.11.2016
Delivered amount: 0.5 kg
Batch No: 064A160623

3. Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** on *Enterococcus hirae* ATCC 10541

Tab No. 3.1 Verification of methodology, temperature 20°C, 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}	54	$\Phi_{Nv0} = 49.5$	V_{c1}	51	$\Phi_A = 48.5$	V_{c1}	52	$\Phi_B = 48$	V_{c1}	56	$\Phi_C = 49$
V_{c2}	45		V_{c2}	46		V_{c2}	44		V_{c2}	42	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	49	V_{c2}	46	Φ_{NVB}	47.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$		
							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}	57	42	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 3.3 Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** 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. Evaluation of bactericidal activity of the product **CHIROSAN PLUS**

Tab No. 4.1 The efficacy of chemical disinfectant **CHIROSAN PLUS** on test strains – bactericidal activity

Bactericidal activity of the product (ČSN EN 13727+A2)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R ČSN EN 13727+A2	**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**

Notes: V_c = the number of cfu per ml, Φ = average of V_{c1} and V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml in the test suspension, N_0 = the number of cfu/ml in the test mixture at the beginning of the contact time (time "zero"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before neutralization, N_v= the number of cfu/ml in the bacterial test suspension for validation, N_{v0} = the number of cfu/ml in the mixtures A,B and C at the beginning of the contact time (time "zero"), N_{VB} = the number of cfu/ml in the suspension for neutralizer control, A, B, C = the number of survivors per ml in the 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: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
Testing date: 1.11. – 4.11.2016
Delivered amount: 0.5 kg
Batch No: 064A160623

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (ČSN EN 13624)
Period of analysis:	1.11. – 4.11.2016
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	white powder
Product diluent:	hard water
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 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:
ČSN 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) May 2014

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

Sample ID: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
Testing date: 1.11. – 4.11.2016
Delivered amount: 0.5 kg
Batch No: 064A160623

5. Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** on *Candida albicans* ATCC 10231

Tab No. 5.1 Verification of methodology, temperature 20°C, 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}	33	$\Phi_{Nvo} = 31$	V_{c1}	35	$\Phi_A = 31$	V_{c1}	36	$\Phi_B = 30.5$	V_{c1}	36	$\Phi_C = 29$
V_{c2}	29		V_{c2}	27		V_{c2}	25		V_{c2}	22	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	30	V_{c2}	23	Φ_{NVB}	26.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$			
							x	yes		no

Tab No. 5.2 Test suspension

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)
$\Phi = 37.5 \times 10^6 = \lg 7.57$	10^{-5}	> 330	> 330	$\lg N_0 = \lg N/10 = \lg 6.57$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	37	38	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **CHIROSAN PLUS** on *Candida albicans* ATCC 10231

Test concentration (%) /contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.57$)**
0.5/5/dirty	10^0	<14	<14	< 2.15	**≥ 4.42**

6. Evaluation of yeasticidal activity of the product **CHIROSAN PLUS**

Tab No. 6.1 The efficacy of chemical disinfectant **CHIROSAN PLUS** on test strains – yeasticidal activity

Yeasticidal activity of the product (ČSN EN 13624)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	lg R ČSN EN 13624	**lg R**
Candida albicans ATCC 10231	20	5	0.5	dirty	≥ 4	**> 4**

Notes: V_c = the number of cfu per ml, Φ = average of V_{c1} and V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml in the test suspension, N_0 = the number of cfu/ml in the test mixture at the beginning of the contact time (time "zero"), N_a = the number of survivors per ml in the test mixture at the end of the contact time and before neutralization, N_v= the number of cfu/ml in the bacterial test suspension for validation, N_{v0} = the number of cfu/ml in the mixtures A,B and C at the beginning of the contact time (time "zero"), N_{VB} = the number of cfu/ml in the suspension for neutralizer control, A, B, C = the number of survivors per ml in the 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: D256/2016
Rep No: 238
Sample name: **CHIROSAN PLUS**
Sampled: by client
Sampling point: SCHULKE CZ s.r.o., Lidická 445, Bohumín
Client: SCHULKE CZ s.r.o., Lidická 445, 735 81 Bohumín

Sampling date: 23.6.2016
Sample delivered: 5.10.2016
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Delivered amount: 0.5 kg
Batch No: 064A160623
Page: 7

Interpretation:
Results of the tests are in Tabs.
According to ČSN EN 13727+A2 the tested product **CHIROSAN PLUS**, batch No. 064A160623, in the concentration 0.5%, diluted in hard water, 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 by at least 5 (lg) orders.

According to ČSN EN 13624 the tested product **CHIROSAN PLUS**, batch No. 064A160623, in the concentration 0.5%, diluted in hard water, 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.

Conclusion:
The product **CHIROSAN PLUS** is capable of reducing the number of viable bacterial and vegetative yeast cells of the relevant organisms in the suspension under defined conditions to the declared values, and consequently, may be called bactericidal and yeasticidal.

6.2.2017, Hodonín

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Ing. Eva Kremlová, Leader of Study

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