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

Sample ID:	S215/2023	Sampling date:	6.11.2023
Sample name:	Calcium hypochlorite	Sample delivered:	22.11.2023
Sampled:	by client	Testing date:	4.1. - 5.1.2024
Sampling point:	"B-KONTAKT" Ltd Bulgaria	Delivered amount:	2 x 750 g
Client:	"B-KONTAKT" Ltd Bulgaria	Page:	2

Subject of testing:

Determination of bactericidal activity of the product.

Information supplied by the client:

Name of the product:	Calcium hypochlorite
Batch number (Lot):	18
Date of manufacture:	6.11.2023
Expiry date:	6.11.2024
Manufacturer:	"B-KONTAKT" Ltd Bulgaria, 23 "Nikola Petkov" Str., 7100 Byala, Rousse, Bulgaria
Incoming date:	22.11.2023
Storage conditions:	temperature up to 25 °C, dry and airy
Active ingredients:	CAS:7778-54-3, Calcium hypochlorite, 31%

Experimental conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers

SOP:	SOP-M-22-12 (EN 13697:2015+A1:2019)
Period of analysis:	4. 1. 2024 - 5.1.2024
Test temperature:	18-25°C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 5344
Appearance of the product:	white powder
Product diluent:	hard water
Test concentration:	1 % active chlorine (3,111 g/100 ml)
Contact time:	10 min
Interfering substances:	3 g/l BSA (dirty conditions)
Test organisms:	<i>Escherichia coli</i> ATCC 10536 <i>Pseudomonas aeruginosa</i> ATCC 15442 <i>Staphylococcus aureus</i> ATCC 6538 <i>Enterococcus hirae</i> 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 carrier 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 on carriers under defined conditions by at least a 4 lg reduction (10^4).

The drying time: 25 - 45 min

The standard:

EN 13697:2015+A1:2019 Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2, step 2) July 2019

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

Testing the efficacy of chemical disinfectant **Calcium hypochlorite** on *Escherichia coli* ATCC 10536

Test suspension:

Dilution	Vc1	Vc2	N
10^{-6}	330	326	
10^{-7}	34	30	6,91
$\Phi = 8,18 \times 10^6$			$6,57 \leq \lg N \leq 7,1$

Method validation NC

Testing conditions	Dilution	Vc1	Vc2	NC
10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-3}	182	240	
10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-4}	18	24	NC: 6,32 NC-Nc $\geq \pm 0,3 \lg$

Method validation NT

Testing conditions	Dilution	Vc1	Vc2	NT
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-3}	244	228	
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-4}	25	20	NT: 6,37 NT-Nc $\geq \pm 0,3 \lg$

Method validation Nc

Testing conditions	Dilution	Vc1	Vc2	Nc
10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-3}	296	234	
10 min, 3 g/l BSA (dirty conditions), 20°C	10^{-4}	27	23	Nc: 6,42
				NTS > 100

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	Nd	R
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10^0	<14	<14	<2,15	$\geq 4,27$ NTS 0

$N = \log_{10} [\{0.025 \cdot (x + x')\} / 2 \cdot d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$NC \text{ or } NT = \log_{10} [\{10 \cdot (y + y')\} / 2 \cdot d]$ where y and y' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$Nc \text{ or } Nd = \log_{10} [\{10 \cdot (a + a')\} / 2 \cdot d]$ where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Reduction $R = Nc - Nd$

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

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Testing the efficacy of chemical disinfectant **Calcium hypochlorite** on *Pseudomonas aeruginosa* ATCC 15442

Test suspension:

Dilution	Vc1	Vc2	N
10 ⁻⁶	324	302	
10 ⁻⁷	33	30	6,89
$\Phi = 7,83 \times 10^6$			$6,57 \leq \lg N \leq 7,1$

Method validation NC

Testing conditions	Dilution	Vc1	Vc2	NC
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	146	216	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	16	20	NC: 6,26 NC-Nc $\geq \pm 0,3 \lg$

Method validation NT

Testing conditions	Dilution	Vc1	Vc2	NT
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	199	196	
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	21	16	NT: 6,29 NT-Nc $\geq \pm 0,3 \lg$

Method validation Nc

Testing conditions	Dilution	Vc1	Vc2	Nc
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	182	211	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	23	18	Nc: 6,29
				NTS > 100

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	Nd	R
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁰	<14	<14	<2,15	$\geq 4,14$ NTS 0

$N = \log_{10} [\{0,025 \cdot (x + x')\} / 2 \cdot d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$NC \text{ or } NT = \log_{10} [\{10 \cdot (y + y')\} / 2 \cdot d]$ where y and y' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$Nc \text{ or } Nd = \log_{10} [\{10 \cdot (a + a')\} / 2 \cdot d]$ where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Reduction $R = Nc - Nd$

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Testing the efficacy of chemical disinfectant **Calcium hypochlorite** on *Staphylococcus aureus* ATCC 6538

Test suspension:

Dilution	Vc1	Vc2	N
10 ⁻⁶	154	199	
10 ⁻⁷	16	21	6,65
$\Phi = 4,43 \times 10^6$			$6,57 \leq \lg N \leq 7,1$

Method validation NC

Testing conditions	Dilution	Vc1	Vc2	NC
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	298	292	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	30	28	NC: 6,47 NC-Nc $\geq \pm 0,3 \lg$

Method validation NT

Testing conditions	Dilution	Vc1	Vc2	NT
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	252	228	
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	27	23	NT: 6,38 NT-Nc $\geq \pm 0,3 \lg$

Method validation Nc

Testing conditions	Dilution	Vc1	Vc2	Nc
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	284	276	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	31	26	Nc: 6,45
				NTS > 100

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	Nd	R
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁰	<14	<14	<2,15	$\geq 4,30$ NTS 0

$N = \log_{10} [\{0,025 \cdot (x + x')\} / 2 \cdot d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$NC \text{ or } NT = \log_{10} [\{10 \cdot (y + y')\} / 2 \cdot d]$ where y and y' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$Nc \text{ or } Nd = \log_{10} [\{10 \cdot (a + a')\} / 2 \cdot d]$ where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Reduction $R = Nc - Nd$

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Testing the efficacy of chemical disinfectant **Calcium hypochlorite** on *Enterococcus hirae* ATCC 10541

Test suspension:

Dilution	Vc1	Vc2	N
10 ⁻⁶	218	224	
10 ⁻⁷	24	22	6,74
$\Phi = 5,55 \times 10^6$			$6,57 \leq \lg N \leq 7,1$

Method validation NC

Testing conditions	Dilution	Vc1	Vc2	NC
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	270	266	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	24	28	NC: 6,43 NC-Nc $\geq \pm 0,3 \lg$

Method validation NT

Testing conditions	Dilution	Vc1	Vc2	NT
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	261	304	
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	28	30	NT: 6,45 NT-Nc $\geq \pm 0,3 \lg$

Method validation Nc

Testing conditions	Dilution	Vc1	Vc2	Nc
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻³	250	278	
10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁻⁴	34	23	Nc: 6,42
				NTS > 100

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	Nd	R
1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	10 ⁰	<14	<14	<2,15	$\geq 4,27$ NTS 0

$N = \log_{10} [\{0.025 \cdot (x + x')\} / 2 \cdot d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$NC \text{ or } NT = \log_{10} [\{10 \cdot (y + y')\} / 2 \cdot d]$ where y and y' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

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Evaluation of BACTERICIDAL (EN 13697:2015+A1:2019) activity of the product Calcium hypochlorite

Strain	Test conditions	lgR	lgR
<i>Escherichia coli</i> ATCC 10536	1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	≥4	> 4
<i>Pseudomonas aeruginosa</i> ATCC 15442	1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	≥4	> 4
<i>Staphylococcus aureus</i> ATCC 6538	1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	≥4	> 4
<i>Enterococcus hirae</i> ATCC 10541	1 %, 10 min, 3 g/l BSA (dirty conditions), 20°C	≥4	> 4

$N = \log_{10} \left[\frac{0.025 \cdot (x + x')}{2 \cdot d} \right]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$NC \text{ or } NT = \log_{10} \left[\frac{10 \cdot (y + y')}{2 \cdot d} \right]$ where y and y' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

$Nc \text{ or } Nd = \log_{10} \left[\frac{10 \cdot (a + a')}{2 \cdot d} \right]$ where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Reduction R = Nc – Nd

Prepared by: Hana Konevalíková, Lab Technician, Bc. Šárka Vašíčková Dohnalová, Lab Technican

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Conclusion:

The tested product: **Calcium hypochlorite**
Batch number: 18
Standard: EN 13697:2015+A1:2019
Test method: dilution neutralization method

For conditions: 1 % active chlorine (3,111 g/100 ml), 10 min, 3 g/l BSA (dirty conditions), 20°C
Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*
the efficacy is confirmed.

The tested product is capable of reducing the number of viable cells of the relevant organisms under defined conditions to the declared values, and consequently, can be called bactericidal on carriers.

Approved by: Ing. Barbora Stoklásková, Leader of Study

Hodonín, 16.1.2024

Ing. Jana Šitrová, Head of Laboratory

