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Centre of Clinical Laboratories
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L 1554

TEST REPORT N. 50/DP/21_F

Quantitative suspension test for the evaluation of fungicidal or antifungal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Customer:
RETECH, s.r.o.
Vackova 1541/4
155 00 Praha 5 - Stodůlky

The order number: not provided
Date of order: 21. 10. 2021
Reference number: ZU/30184/2021

Identification of disinfectant- sample:

Name of the product [i]:	**ULTRASONIC CLEANING SOLUTION**
Batch number [i]:	not provided
Expiry date [i]:	24 months from manufacturing date
Manufacturing date [i]:	not provided
Storage condition [i]:	5–30 °C
Product diluent recommended by the manufacturer [i]:	ready to use
Active substance(s) and concentration(s) [i]:	ethanol: 0,558g Quaternary ammonium compounds, benzyl-C12-16-alkyldimethyl, chlorides: 0,5 g Didecyl(dimethyl)ammonium-chloride: 0,125 g
Other substance (s) [i]:	
Purpose of product [i]:	PT 2 - surface disinfection outside medical area and professional use
Appearance of the product:	clear colourless liquid
Date of delivery:	21. 10. 2021
Date(s) of tests (period of analysis):	8. 12. - 13. 12. 2021

[i] - data provided by the customer

Results (for more details see the annexes to the protocol):

The disinfectant **ULTRASONIC CLEANING SOLUTION** intended for surface disinfection was tested according to ČSN EN 1650 on test organisms *Candida albicans* and *Aspergillus brasiliensis.*

The required concentration was 100 %, at contact time 45 minutes, at temperature 20 °C±1 °C, under dirty conditions.

The reduction for *Candida albicans CCM 8215* was at a concentration of 100 % >5.32 lg, at 50 % > 5.32 lg and at 0.5 % <2.95 lg.

The reduction for *Aspergillus brasiliensis* CCM 8222 was at a concentration of 100 % >4.33 lg, at 50 % >4.33 lg and at 0.5% <3.26 lg.
The average reduction (R) in logarithmic orders with the test organism *Aspergillus brasiliensis* CCM 8222 was at a concentration of 100 % (V/V) R> 4.33 ± 0.000 lg *.

All controls and validations were within basic limits. At least one concentration of the product demonstrated a reduction of less than 4 lg.

Conclusion:

The product **ULTRASONIC CLEANING SOLUTION** demonstrated bactericidal activity according to the standard ČSN EN 1650 under dirty conditions (bovine albumin 3.0 g/l) and a contact time of 45 minutes at a concentration of 100 and 50 %.

The average reduction (R) in logarithmic orders with the test organism *Aspergillus brasiliensis* CCM 8222 was at a concentration of 100 % (V/V) R> 4.33 ± 0.000 lg *.

* standard deviation of reproducibility

In Ostrava: 22. 12. 2021

Authorized by: MUDr. Linda Stryjová

Annex to the protocol n. 1: 50/DP/21_F

According to procedure SOP 3037 – ČSN EN 1650 - Quantitative suspension test for the evaluation of fungicidal or antifungal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Name of the product [i]: **ULTRASONIC CLEANING SOLUTION**

Storage condition [i]:	5–30 °C
Diluent:	water
Number of inoculated plates:	2 x 1 ml
Test method:	Neutralization- dilution method
Neutralizer:	Polysorbate 80 30 g/l + lecithin 3 g/l + sodium thiosulphate 15 g/l
Testing concentration(s) [i]:	100 %
Other testing concentration:	50 %, 0,5 %
Contact times [i]:	45 minutes
Stability and appearance of the product during tests:	clear colourless liquid
Testing temperature [i]:	20 ± 2 °C
Interfering substance(s) [i]:	Bovine albumin 3 g/l
Test organism:	***Candida albicans*** **CCM 8215**
Incubation temperature and time:	30 ± 1 °C, 48 h
Date(s) of tests (period of analysis):	8. 12. 2021

[i] - data provided by the customer

Processed by: Mgr. Kateřina Podjuklová
Checked by: MUDr. Linda Stryjová Signature:

Preparation of bacterial test suspension

Dilution of primary suspension	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Number of colonies per plate 1	>330	>330	>330	>330	>330	300	23
Number of colonies per plate 2	>330	>330	>330	>330	>330	299	25

Test suspension

Test suspension N	Dilution	Number of colonies per plate		Weighted mean $\bar{x}_{wm} = \frac{C \text{ (sum of values Vc)}}{(n1 + 0{,}1\ n2) \times 10^{-5}} = \frac{647}{(2+0{,}2) \times 10^{-5}}$
		Vc1	Vc2	$\bar{x}_{wm}$= součet hodnot (Vc) 647 : 2,2 x 10^5 = 2,94 x 10^7
	10^{-5}	300	299	lg N = 7,47
	10^{-6}	23	25	Is 8,17 ≤ lg N ≤ 8,70 ? <u>yes</u> - no
Test suspension No	N_o = N/10; lg N_o = 6,47			Is 7,17 ≤ lg N ≤ 7,70 ? <u>yes</u> - no

Validation and controls:

Validation suspension (Nv)			**Experimental conditions control (A)**			**Neutralizer toxicity control (B)**			**Dilution- neutralization control (C):**		
Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2
	62	60		54	51		42	45		39	40
Arithmetic mean Vc1+Vc2: $\bar{x}$ = 61			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 52,5			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 43,5			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 39,5		
Is 30 ≤ $\bar{x}$ of Nvo ≤ 160 ? yes - no			Is $\bar{x}$ of A ≥ 0,5 x $\bar{x}$ of Nvo ? yes - no			Is $\bar{x}$ of B ≥ 0,5 x $\bar{x}$ of NvB ? yes - no			Is $\bar{x}$ of C ≥ 0,5 x $\bar{x}$ of Nvo ? yes - no		

Test

Product concentrations (%)	Dilution	Number of colonies per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weighted mean $\bar{x}_{wm}$ x 10	lg Na = lg ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg R = lg N_0 - lgNa lg No = 6,47	Contact time (min)
100	10^0	0	0	<14	<14	<140	<2,15	>4,32	45
50	10^0	0	0	<14	<14	<140	<2,15	>4,32	45
0,5	10^0	>330	>330	>330	>330	>3 300	>3,52	<2,95	45

Explanations:

V_c = number of cells on 1 ml (one or more plates), $\bar{x}$= mean V_{c1} a V_{c2} (1. + 2. duplicate determination);
N_a =number of viable cells on 1 ml at the end of the contact time
N = test suspension; N_o = N/10 =number of cells on 1 ml in test suspension at the beginning of contact time (time=0);
N_{vo} = Nv/10 = number of cells on 1 ml in validation suspension at the beginning of the contact time(time= 0);
N_{vb}= number of cells on 1 ml in validation suspension for the control neutralizer (B);
$\bar{x}_{wm}$ = weighted mean $\bar{x}$; R = reduction (lg R = lg N_0 - lg Na).

Annex to the protocol n. 2: 50/DP/21_F

According to procedure SOP 3037 – ČSN EN 1650 - Quantitative suspension test for the evaluation of fungicidal or antifungal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Name of the product [i]:	**ULTRASONIC CLEANING SOLUTION**
Storage condition [i]:	5–30 °C
Diluent:	water
Number of inoculated plates:	2 x 1 ml
Test method:	Neutralization- dilution method
Neutralizer:	Polysorbate 80 30 g/l + lecithin 3 g/l + sodium thiosulphate 15 g/l
Testing concentration(s) [i]:	100 %
Other testing concentration:	50 %, 0,5 %
Contact times [i]:	45 minutes
Stability and appearance of the product during tests:	clear colourless liquid
Testing temperature [i]:	20 ± 2 °C
Interfering substance(s) [i]:	Bovine albumin 3 g/l
Test organism:	***Aspergillus brasiliensis*** **CCM 8222**
Incubation temperature and time:	30 ± 1 °C, 48 h
Date(s) of tests (period of analysis):	8. 12. 2021

[i] - data provided by the customer

Processed by: Mgr. Kateřina Podjuklová
Checked by: MUDr. Linda Stryjová Signature:

Preparation of bacterial test suspension

Dilution of primary suspension	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Number of colonies per plate 1	>165	>165	>165	>165	>165	>165	>165
Number of colonies per plate 2	>165	>165	>165	>165	>165	>165	>165

Test suspension

Test suspension N	Dilution	Number of colonies per plate		C (sum of values Vc) 660 Weighted mean $\bar{x}wm$= ---------- = ---------- (n1 + 0,1 n2)x10^{-5} (2+0,2)x10^{-5}
		Vc1	Vc2	$\bar{x}_{wm}$= součet hodnot (Vc) 660 : 2,2 x 10^5 = 3,00 x 10^7
	10^{-5}	>165	>165	lg N = 7,48
	10^{-6}	>165	>165	Is 8,17 ≤ lg N ≤ 8,70 ? yes - no
Test suspension No	N_o = N/10; lg N_o = 6,48			Is 7,17 ≤ lg N ≤ 7,70 ? yes - no

Validation and controls:

Validation suspension (Nv)			**Experimental conditions control (A)**			**Neutralizer toxicity control (B)**			**Dilution- neutralization control (C):**		
Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2	Number of colonies per plate	Vc1	Vc2
	112	130		100	94		62	64		71	65
Arithmetic mean Vc1+Vc2: $\bar{x}$ = 121			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 97			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 63			Arithmetic mean Vc1+Vc2: $\bar{x}$ = 68		
Is 30 ≤ $\bar{x}$ of Nvo ≤ 160 ? yes - no			Is $\bar{x}$ of A ≥ 0,5 x $\bar{x}$ of Nvo ? yes - no			Is $\bar{x}$ of B ≥ 0,5 x $\bar{x}$ of NvB ? yes - no			Is $\bar{x}$ of C ≥ 0,5 x $\bar{x}$ of Nvo ? yes - no		

Test

Product concentrations (%)	Dilution	Number of colonies per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weighted mean $\bar{x}_{wm}$ x 10	lg Na = lg ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg R = lg N_0 - lgNa lg No = 6,48	Contact time (min)
100	10^0	0	0	<14	<14	<140	<2,15	>4,33	45
50	10^0	8	11	<14	<14	<140	<2,15	>4,33	45
0,5	10^0	>165	>165	>165	>165	>1 650	>3,22	>3,26	45

Explanations:

V_c = number of cells on 1 ml (one or more plates), $\bar{x}$= mean V_{c1} a V_{c2} (1. + 2. duplicate determination);
N_a =number of viable cells on 1 ml at the end of the contact time
N = test suspension; N_o = N/10 =number of cells on 1 ml in test suspension at the beginning of contact time (time=0);
N_{vo} = Nv/10 = number of cells on 1 ml in validation suspension at the beginning of the contact time(time= 0);
N_{vb}= number of cells on 1 ml in validation suspension for the control neutralizer (B);
$\bar{x}_{wm}$ = weighted mean $\bar{x}$; R = reduction (lg R = lg N_0 - lg Na).

Annex to the protocol n. 3: 50/DP/21_F

According to procedure SOP 3037 – ČSN EN 1650 - Quantitative suspension test for the evaluation of fungicidal or antifungal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

Repetitions for the test organism *Aspergillus brasiliensis*:
A separate bacterial suspension and a tested product concentration of 100 % were prepared for each replicate.

Name of the product [i]**:**	**ULTRASONIC CLEANING SOLUTION**
Storage condition [i]:	5–30 °C
Diluent:	water
Number of inoculated plates:	2 x 1 ml
Test method:	Neutralization- dilution method
Neutralizer:	Polysorbate 80 30 g/l + lecithin 3 g/l + sodium thiosulphate 15 g/l
Testing concentration(s) [i]:	100 %
Other testing concentration:	-
Contact times [i]:	45 minutes
Stability and appearance of the product during tests:	clear colourless liquid
Testing temperature [i]:	20 ± 2 °C
Interfering substance(s) [i]:	Bovine albumin 3 g/l
Test organism:	***Aspergillus brasiliensis* CCM 8222**
Incubation temperature and time:	30 ± 1 °C, 48 h
Date(s) of tests (period of analysis):	10. 12. 2021

[i] - data provided by the customer

Processed by: Mgr. Kateřina Podjuklová
Checked by: MUDr. Linda Stryjová Signature:

Test suspension

N. of repetition	Dilution	Number of colonies per plate		$N = \bar{x}_{wm}$= sum of values (Vc) C : 2,2 x 10^5 No = N/10
		Vc1	Vc2	
1 (8. 12. 2021)	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48
2	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48
3	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48
4	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48
5	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48
6	10^{-5}	>165	>165	N = 3,00 x 10^7 lg N = 7,48
	10^{-6}	>165	>165	No = 3,00 x 10^6 lg No = 6,48

Test

N. of repetition (concentration 100 %)	Dilution	Number of colonies per plate		Vc1	Vc2	Na = mean $\bar{x}$ or weighted mean $\bar{x}_{wm}$ x 10	lg Na = lg ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg R = lg N_0 - lgNa	Contact time (min)
1 (8. 12. 2021)	10^0	0	0	<14	<14	<140	<2,15	>4,33	45
2	10^0	2	1	<14	<14	<140	<2,15	>4,33	45
3	10^0	1	0	<14	<14	<140	<2,15	>4,33	45
4	10^0	3	2	<14	<14	<140	<2,15	>4,33	45
5	10^0	2	0	<14	<14	<140	<2,15	>4,33	45
6	10^0	0	0	<14	<14	<140	<2,15	>4,33	45
Average reduction:								**>4,33 lg**	
Standard deviation:								**0,000 ± lg**	

End of the protocol