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Centre of Clinical Laboratories
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TEST REPORT N. 11/DP/16

Quantitative suspension test for the evaluation of fungicidal activity in the medical area – Test method and requirements (phase 2, step 1)

Client:

Schulke CZ s. r. o.
Lidická 326
735 81 Bohumín

The order number:

Date of order: 16. 5. 2016
Reference number: ZU/15093/2016

Identification of disinfectant- sample:

Name of the product:
Lot number:
Expiration date:
Manufacturer:
Storage condition (temp. and other):
Product diluent recommended by the manufacturer for use:
Appearance of the product:
Active substance (s):

Desam Effekt +
018A160323

Schulke s. r. o.
-10 °C to +30 °C
water
liquid
N-(3-aminopropyl)-N
dodecylpropan-1,3-diamin: 7,2 %
didecyldimethylamonium-
chloride: 3 %
Benzyl-C12-16-alkyldimethyl,
chlorides: 19 %,
2-fenoxyethanol:10 %

Other substance (s):

Use of the product:

disinfection and cleaning of all
washable surfaces of medical
resources

Date of delivery of the product:

Date of tests:

17. 5. 2016

25. 5. 2016, 2. 6. 2016, 13. 6. 2016

The protocol can not be reproduced without written consent of laboratory other than whole. The results relates only with tested samples. The centre of clinical laboratories - testing laboratory n. 1554 accredited ČIA according to ČSN EN ISO/IEC 17025. The list of accredited methods is shown in www.zuova.cz. The sample was tested according to SOP n. 3039.

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

The product Desam Effekt + used to surface disinfection was tested with use test organisms *Aspergillus brasiliensis* and *Candida albicans*. Desired test concentrations were 0,5 % and 15 minutes contact time for *Aspergillus brasiliensis*, 0,25 % and 5 minutes contact time for *Candida albicans*.

Determined fungicidal concentration according to ČSN EN 13624 under dirty conditions (bovine albumin 3 g/l+ sheep erythrocytes 3 ml/l), temperature 24 °C, contact time 15 minutes is 1 % with use test organisms *Aspergillus brasiliensis* CCM 8222, and after contact time 5 minutes concentration is 0,25 % with use test organism *Candida albicans* CCM 8215.

Reductions in logarithmic orders are with use test organism *Aspergillus brasiliensis* CCM 8222 R >4,06 and > 4,07 in 1 % concentration.

Reductions in logarithmic orders are with use test organism *Candida albicans* CCM 8215 R >4,31 and > 4,11 in 0,25 % concentration.

Note:

All the validations and the controls were in limits. At least one concentration of product shown reduce less than 4 logarithmic orders. There were observed precipitates of interfering substance in the test mixture (0,5 % and above percentage concentrations) and validations C.

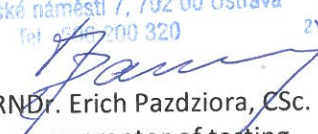
Conclusion:

Determined fungicidal concentration of the product Desam Effekt + (lot number 018A160323) according to EN 13624, with the contact time 15 minutes, under dirty conditions with use test organism *Aspergillus brasiliensis* CCM 8222 is after repeated examination 1 %.

Determined yeasticidal (levurocidal) concentration according to EN 13624, with the contact time 5 minutes, under dirty conditions with use test organism *Candida albicans* CCM 8215 is after repeated examination 0,25 %.

In Ostrava: 22. 6. 2016

Diagnostický ústav se sídlem v Ostravě
Centrum klinických laboratoří
Centrum bakteriologie a mykologie
Laboratoř antibiotické středisko
Pražská náměstí 7, 702 00 Ostrava
Tel. 59 600 320



RNDr. Erich Pazdziora, CSc.
guarantor of testing

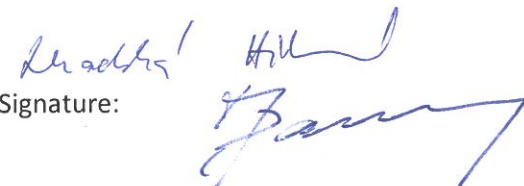
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Annex to the protocol n. 1: 11/DP/16

According to procedure SOP 3039 - ČSN EN 13624 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)

Name of the product: **Desam effekt +**
Manufacturer: Schulke s. r. o.
Storage conditions: -10° C to +30°C
Solvent: hard water
Number plates: 2 x 1 ml
Neutralizer: Polysorbate 80 30 g/l+ lecithin 3 g/l+
Sodium thiosulphate 5 g/l + L-histidin 1g/l
Tested concentrations of product: 0,5 %, 0,25 %, 0,1 %, 0,01 %
Contact time: 5 minutes
Stability and appearance of the product during tests: clear to milky solution, formation of precipitates in 0,5 % test mixture and validation C
Test temperature: 24°C
Interfering substance: bovinne albumin 3 g/l + sheep erythrocytes 3ml/ l in diluent
Test organism: **Candida albicans CCM 8215**
Temperature of incubation, time of incubation: 30 °C, 48 h
Date of test: 25. 5. 2016

Processed by: Mgr. Pavlína Hradská, Irena Willerthová
Checked by: RNDr. Erich Pazdziora, CSc.

Signature: 

Preparation of the fungal test suspension

Dilution of primary suspension	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Number of colonies on plate 1	>330	>330	>330	>330	>330	145	24	-	-
Number of colonies on plate 2	>330	>330	>330	>330	>330	210	28	-	-

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The sample was tested according to SOP n. 3039.

Validation and controls:

Validation suspension N _{vo} (N _v)				Control of the experimental conditions (A)				Neutralizer toxicity control (B)				Dilution- neutralization control (C):			
Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2
99	80	99	80	70	106	70	106	100	95	100	95	110	90	110	90
Arithmetic mean Vc1+Vc2: $\bar{x} = 89,5$				Arithmetic mean Vc1+Vc2 $\bar{x} = 88$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 97,5$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 100$			
Is $30 \leq \bar{x} z N_{vo} \leq 160$? <u>yes</u> - no				Is $\bar{x} z A \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no				Je $\bar{x} z B \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no				Je $\bar{x} z C \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no			
Validation suspension N _{vB} (validation suspension for the control neutralizer B)								89	80	85	80				
								Arithmetic mean Vc1+ Vc2 $\bar{x} = 82,5$							
								Is $30 \leq \bar{x} z N_{vB} / 1000 \leq 160$?				<u>yes</u> - no			

Test suspension

Test suspension N	Dilution	Number of colonies on the plate		Vc1	Vc2	Weighted mean $\bar{x}_{wm} = \frac{C (\text{sum of values } Vc)}{(n1 + 0,1 n2) \times 10^{-5} (2+0,2) \times 10^{-5}} = \frac{407}{(2+0,2) \times 10^{-5}}$
						$\bar{x}_{wm} = \text{sum of values } (Vc) 407 : 2,2 \times 10^{-5} = 1,85 \times 10^7$
	10 ⁻⁵	145	210	145	210	lg N = 7,26
	10 ⁻⁶	24	28	24	28	Je $7,17 \leq \lg N \leq 7,70$? <u>yes</u> - no
Test suspension No = N/10	Dilution	Number of colonies on the plate		Vc1	Vc2	Weighted mean $\bar{x}_{wm} = \frac{C (\text{sum of values } Vc)}{(n1 + 0,1 n2) \times 10^{-5} (2+0,2) \times 10^{-5}} = \frac{407}{(2+0,2) \times 10^{-5}}$
						$\bar{x}_{wm} = \text{sum of values } (Vc) 407 : 2,2 \times 10^{-5} = 1,85 \times 10^7$
	10 ⁻⁵	145	210	145	210	lg No = 6,26
	10 ⁻⁶	24	28	24	28	Je $6,17 \leq \lg No \leq 6,70$? <u>yes</u> - no

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Test

Concentration of product (%)	Dilution	Number of cfu on 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 - lg Na lg N ₀ = 6,26	Contact time (min)
0,5	10 ⁰	0	1	<14	<14	<140	<2,15	>4,11	5
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,25	10 ⁰	1	0	<14	<14	<140	<2,15	>4,11	5
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,1	10 ⁰	0	0	<14	<14	<140	<2,15	>4,11	5
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,01	10 ⁰	>330	>330	>330	>330	>33000	>4,52	<1,74	5
	10 ⁻¹	>330	>330	>330	>330				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								

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; N₀ = N/10 = number of cells on 1 ml in test suspension at the beginning of contact time (time=0);

N_{vo} = N_v/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 N₀ - lg N_a).

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Calculations:

Reduction of *Candida albicans* CCM 8215 is after the contact time 5 minutes in logarithmic order:

For 0,5 % : $\lg R = \lg N_0 - \lg N_a = 6,26 - 2,15 = >4,11$

For 0,25 % : $\lg R = \lg N_0 - \lg N_a = 6,26 - 2,15 = >4,11$

For 0,1 % : $\lg R = \lg N_0 - \lg N_a = 6,26 - 2,15 = >4,11$

For 0,01 % : $\lg R = \lg N_0 - \lg N_a = 6,26 - 4,52 = <1,74$

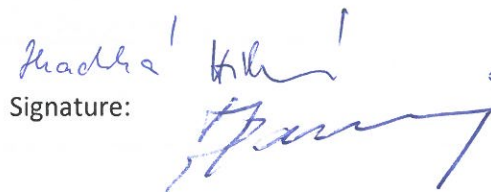
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Annex to the protocol n. 2: 11/DP/16

According to procedure SOP 3039 - ČSN EN 13624 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)

Name of the product: **Desam effekt +**
Manufacturer: Schulke s. r. o.
Storage conditions: -10° C to +30°C
Solvent: hard water
Number plates: 2 x 1 ml
Neutralizer: Polysorbate 80 30 g/l+ lecithin 3 g/l+
Sodium thiosulphate 5 g/l + L-histidin 1g/l
Tested concentrations of product: 1 %, 0,5 %, 0,25 %, 0,1 %
Contact time: 15 minutes
Stability and appearance of the product during tests: clear to milky solution, formation of precipitates in 1 % and 0,5 % test mixture and validation C
Test temperature: 24°C
Interfering substance: bovine albumin 3 g/l + sheep erythrocytes 3ml/ l in diluent
Test organism: ***Aspergillus brasiliensis* CCM 8222**
Temperature of incubation, time of incubation: 30 °C, 48 h
Date of test: 25. 5. 2016

Processed by: Mgr. Pavlína Hradská, Irena Willerthová
Checked by: RNDr. Erich Pazdziora, CSc.

Signature: 

Preparation of the fungal test suspension

Dilution of primary suspension	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Number of colonies on plate 1	>330	>330	>330	>330	>330	176	30	-	-
Number of colonies on plate 2	>330	>330	>330	>330	>330	232	24	-	-

The presence of mature conidiospores of *Aspergillus brasiliensis* was more than 75 % in conidiospore suspension.

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Validation and controls:

Validation suspension N _v (N _v)				Control of the experimental conditions (A)				Neutralizer toxicity control (B)				Dilution- neutralization control (C):			
Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2
90	75	90	75	55	43	55	43	80	78	80	78	70	67	70	67
Arithmetic mean Vc1+Vc2: $\bar{x} = 82,5$				Arithmetic mean Vc1+Vc2 $\bar{x} = 49$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 79$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 68,5$			
Is $30 \leq \bar{x} \leq N_{v0} \leq 160$? <u>yes</u> - no				Is $\bar{x} \geq 0,5 \times \bar{x} \leq N_{v0}$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq N_{v0}$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq N_{v0}$? <u>yes</u> - no			
Validation suspension N _{vB} (validation suspension for the control neutralizer B)								65	75	65	75				
								Arithmetic mean Vc1+ Vc2 $\bar{x} = 70$							
								Is $30 \leq \bar{x} \leq N_{vB}/1000 \leq 160$?				<u>yes</u> - no			

Test suspension

Test suspension N	Dilution	Number of colonies on the plate		Vc1	Vc2	C (sum of values Vc) 362 Weighted mean $\bar{x}_{wm} = \frac{362}{(n1 + 0,1 n2) \times 10^{-5} + (2+0,2) \times 10^{-5}}$ $\bar{x}_{wm} = \text{sum of values (Vc)} 362 : 2,2 \times 10^{-5} = 1,64 \times 10^7$
	10 ⁻⁵	176	232	176	232	lg N = 7,21
	10 ⁻⁶	30	24	30	24	Je $7,17 \leq \lg N \leq 7,70$? <u>yes</u> - no
Test suspension No = N/10	Dilution	Number of colonies on the plate		Vc1	Vc2	C (sum of values Vc) 362 Weighted mean $\bar{x}_{wm} = \frac{362}{(n1 + 0,1 n2) \times 10^{-5} + (2+0,2) \times 10^{-5}}$ $\bar{x}_{wm} = \text{sum of values (Vc)} 362 : 2,2 \times 10^{-5} = 1,64 \times 10^7$
	10 ⁻⁵	176	232	176	232	lg No = 6,21
	10 ⁻⁶	30	24	30	24	Je $6,17 \leq \lg No \leq 6,70$? <u>yes</u> - no

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Test

Concentration of product (%)	Dilution	Number of cfu on plate		Vc1	Vc2	Na = mean \bar{x} or weighted mean $\bar{x}_{wm} \times 10$	lg Na = lg (\bar{x} or \bar{x}_{wm}) x 10	lg R = lg No - lg Na lg No = 6,21	Contact time (min)
1	10 ⁰	2	0	<14	<14	<140	<2,15	>4,06	15
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻								
	10 ⁻								
	10 ⁻								
0,5	10 ⁰	46	40	46	40	430	2,63	3,58	15
	10 ⁻¹	4	2	<14	<14				
	10 ⁻²								
	10 ⁻								
	10 ⁻								
	10 ⁻								
0,05	10 ⁰	>165	>165	>165	>165	3800	3,58	2,63	15
	10 ⁻¹	26	50	26	50				
	10 ⁻²								
	10 ⁻								
	10 ⁻								
	10 ⁻								
0,05	10 ⁰	>165	>165	>165	>165	>16500	>4,22	<1,99	15
	10 ⁻¹	>165	>165	>165	>165				
	10 ⁻²								
	10 ⁻								
	10 ⁻								
	10 ⁻								

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} = N_v/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_o - lg N_a).

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Calculations:

Reduction of *Aspergillus brasiliensis* CCM 8222 is after the contact time 15 minutes in logarithmic orders:

$$\text{Pro } 1 \quad \% : \quad \lg R = \lg N_0 - \lg N_a = 6,21 - 2,15 = >4,06$$

$$\text{Pro } 0,5 \quad \% : \quad \lg R = \lg N_0 - \lg N_a = 6,21 - 2,63 = 3,58$$

$$\text{Pro } 0,25 \quad \% : \quad \lg R = \lg N_0 - \lg N_a = 6,21 - 3,58 = 2,63$$

$$\text{Pro } 0,1 \quad \% : \quad \lg R = \lg N_0 - \lg N_a = 6,21 - 4,22 = <1,99$$

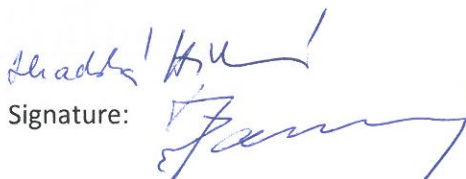
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Annex to the protocol n. 3: 11/DP/16

According to procedure SOP 3039 - ČSN EN 13624 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)

Name of the product: **Desam effekt +**
Manufacturer: Schulke s. r. o.
Storage conditions: -10° C to +30°C
Solvent: hard water
Number plates: 2 x 1 ml
Neutralizer: Polysorbate 80 30 g/l+ lecithin 3 g/l+
Sodium thiosulphate 5 g/l + L-histidin 1g/l
Tested concentrations of product: 0,75 %, 0,5 %
Contact time: 15 minutes
Stability and appearance of the product during tests: clear to milky solution, formation of precipitates in 1 % and 0,5 % test mixture and validation C
Test temperature: 24°C
Interfering substance: bovinne albumin 3 g/l + sheep erythrocytes 3ml/ l in diluent
Test organism: ***Aspergillus brasiliensis* CCM 8222**
Temperature of incubation, time of incubation: 30 °C, 48 h
Date of test: 2. 6. 2016

Processed by: Mgr. Pavlína Hradská, Irena Willerthová
Checked by: RNDr. Erich Pazdziora, CSc.

Signature: 

Preparation of the fungal test suspension

Dilution of primary suspension	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Number of colonies on plate 1	>330	>330	>330	>330	>330	204	35	-	-
Number of colonies on plate 2	>330	>330	>330	>330	>330	200	37	-	-

The presence of mature conidiospores of *Aspergillus brasiliensis* was more than 75 % in conidiospore suspension.

Validation and controls:

Validation suspension Nvo (Nv)				Control of the experimental conditions (A)				Neutralizer toxicity control (B)				Dilution- neutralization control (C):			
Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2
90	120	90	120	70	62	70	62	130	160	130	160	80	115	80	115
Arithmetic mean Vc1+Vc2: $\bar{x} = 105$				Arithmetic mean Vc1+Vc2 $\bar{x} = 66$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 145$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 97,5$			
Is $30 \leq \bar{x} \leq Nvo \leq 160$? <u>yes</u> - no				Is $\bar{x} \geq 0,5 \times \bar{x} \leq Nvo$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq Nvo$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq Nvo$? <u>yes</u> - no			
Validation suspension N _{VB} (validation suspension for the control neutralizer B)								156	120	156	120				
								Arithmetic mean Vc1+ Vc2 $\bar{x} = 138$ Is $30 \leq \bar{x} \leq N_{VB}/1000 \leq 160$? <u>yes</u> - no							

Test suspension

Test suspension N	Dilution	Number of colonies on the plate		Vc1	Vc2	C (sum of values Vc) 476 Weighted mean $\bar{x}_{wm} = \frac{476}{(n1 + 0,1 n2) \times 10^{-5} + (2+0,2) \times 10^{-5}}$ $\bar{x}_{wm} = \text{sum of values (Vc) } 476 : 2,2 \times 10^{-5} = 2,16 \times 10^7$
	10 ⁻⁵	204	200	204	200	lg N = 7,33
	10 ⁻⁶	35	37	35	37	Je $7,17 \leq \lg N \leq 7,70$? <u>yes</u> - no
Test suspension No =N/10	Dilution	Number of colonies on the plate		Vc1	Vc2	C (sum of values Vc) 476 Weighted mean $\bar{x}_{wm} = \frac{476}{(n1 + 0,1 n2) \times 10^{-5} + (2+0,2) \times 10^{-5}}$ $\bar{x}_{wm} = \text{sum of values (Vc) } 476 : 2,2 \times 10^{-5} = 2,16 \times 10^7$
	10 ⁻⁵	204	200	204	200	lg No =6,33
	10 ⁻⁶	35	37	35	37	Je $6,17 \leq \lg No \leq 6,70$? <u>yes</u> - no

Test

Concentration of product (%)	Dilution	Number of cfu on plate		Vc1	Vc2	Na = mean \bar{x} or weighted mean $\bar{x}_{wm} \times 10$	lg Na = lg (\bar{x} or \bar{x}_{wm}) x 10	lg R = lg No - lg Na lg No = 6,33	Contact time (min)
0,75	10 ⁰	148	136	148	136	1420	3,15	3,18	15
	10 ⁻¹	12	9	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,5	10 ⁰	>165	140	>165	140	4600	3,66	2,67	15
	10 ⁻¹	16	30	16	30				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								

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; N₀ = N/10 = number of cells on 1 ml in test suspension at the beginning of contact time (time=0);

N_{vo} = N_v/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 N₀ - lg N_a).

Calculations:

Reduction of *Aspergillus brasiliensis* CCM 8222 is after the contact time 15 minutes in logarithmic orders:

$$\text{For } 0,75 \% : \lg R = \lg N_0 - \lg N_a = 6,33 - 3,15 = 3,18$$

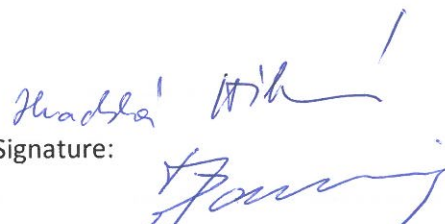
$$\text{For } 0,5 \% : \lg R = \lg N_0 - \lg N_a = 6,33 - 3,66 = 2,67$$

Annex to the protocol n. 4: 11/DP/16

According to procedure SOP 3039 - ČSN EN 13624 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)

Name of the product: **Desam effekt +**
Manufacturer: Schulke s. r. o.
Storage conditions: -10° C to +30°C
Solvent: hard water
Number plates: 2 x 1 ml
Neutralizer: Polysorbate 80 30 g/l+ lecithin 3 g/l+
Sodium thiosulphate 5 g/l + L-histidin 1g/l
Tested concentrations of product: 2 %, 1 %, 0,75 %, 0,5 %, 0,1 %
Contact time: 15 minutes
Stability and appearance of the product during tests: clear to milky solution, formation of precipitates in 1 % and 0,5 % test mixture and validation C
Test temperature: 24°C
Interfering substance: bovinne albumin 3 g/l + sheep erythrocytes 3ml/ l in diluent
Test organism: ***Aspergillus brasiliensis* CCM 8222**
Temperature of incubation, time of incubation: 30 °C, 48 h
Date of test: 13. 6. 2016

Processed by: Mgr. Pavlína Hradská, Irena Willerthová
Checked by: RNDr. Erich Pazdziora, CSc.

Signature: 

Preparation of the fungal test suspension

Dilution of primary suspension	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Number of colonies on plate 1	>330	>330	>330	>330	>330	144	200	-	-
Number of colonies on plate 2	>330	>330	>330	>330	>330	11	15	-	-

The presence of mature conidiospores of *Aspergillus brasiliensis* was more than 75 % in conidiospore suspension.

Validation and controls:

Validation suspension N _{vo} (N _v)				Control of the experimental conditions (A)				Neutralizer toxicity control (B)				Dilution- neutralization control (C):			
Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2
27	48	27	48	33	43	33	43	44	56	44	56	40	53	40	53
Arithmetic mean Vc1+Vc2: $\bar{x} = 37,5$				Arithmetic mean Vc1+Vc2 $\bar{x} = 38$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 50$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 46,5$			
Is $30 \leq \bar{x} z N_{vo} \leq 160$? <u>yes</u> - no				Is $\bar{x} z A \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no				Je $\bar{x} z B \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no				Je $\bar{x} z C \geq 0,5 x \bar{x} z N_{vo}$? <u>yes</u> - no			
Validation suspension N _{VB} (validation suspension for the control neutralizer B)								43	52	43	52				
								Arithmetic mean Vc1+ Vc2 $\bar{x} = 47,5$							
								Is $30 \leq \bar{x} z N_{VB}/1000 \leq 160$?				<u>yes</u> - no			

Test suspension

Test suspension N	Dilution	Number of colonies on the plate		Vc1	Vc2	Weighted mean $\bar{x}_{wm} = \frac{C (\text{sum of values } Vc) \quad 370}{(n1 + 0,1 n2) \times 10^{-5} \quad (2+0,2) \times 10^{-5}} = \dots$
						$\bar{x}_{wm} = \text{sum of values } (Vc) \quad 370 : 2,2 \times 10^{-5} = 1,68 \times 10^7$
	10 ⁻⁵	144	200	144	200	lg N = 7,22
	10 ⁻⁶	11	15	11	15	Je $7,17 \leq \lg N \leq 7,70$? <u>yes</u> - no
Test suspension No = N/10	Dilution	Number of colonies on the plate		Vc1	Vc2	Weighted mean $\bar{x}_{wm} = \frac{C (\text{sum of values } Vc) \quad 370}{(n1 + 0,1 n2) \times 10^{-5} \quad (2+0,2) \times 10^{-5}} = \dots$
						$\bar{x}_{wm} = \text{sum of values } (Vc) \quad 370 : 2,2 \times 10^{-5} = 1,68 \times 10^7$
	10 ⁻⁵	144	200	144	200	lg No = 6,22
	10 ⁻⁶	11	15	11	15	Je $6,17 \leq \lg No \leq 6,70$? <u>yes</u> - no

Test

Concentration of product (%)	Dilution	Number of cfu on plate		Vc1	Vc2	Na = mean \bar{x} or weighted mean $\bar{x}_{wm} \times 10$	lg Na = lg (\bar{x} or \bar{x}_{wm}) x 10	lg R = lg No - lg Na lg No = 6,22	Contact time (min)
2	10 ⁰	0	0	<14	<14	<140	<2,15	>4,07	15
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
1	10 ⁰	0	2	<14	<14	<140	<2,15	>4,07	15
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,75	10 ⁰	20	9	20	<14	<170	<2,23	>3,99	15
	10 ⁻¹	7	10	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,5	10 ⁰	43	50	43	50	465	2,67	3,55	15
	10 ⁻¹	12	10	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,1	10 ⁰	>165	>165	>165	>165	>16500	4,22	2	15
	10 ⁻¹	>165	>165	>165	>165				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								

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; N₀ = N/10 = number of cells on 1 ml in test suspension at the beginning of contact time (time=0);

N_{vo} = N_v/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 N₀ - lg N_a).

Calculations:

Reduction of *Aspergillus brasiliensis* CCM 8222 is after the contact time 15 minutes in logarithmic orders:

For 2 % : $\lg R = \lg N_0 - \lg N_a = 6,22 - 2,15 = >4,07$

For 1 % : $\lg R = \lg N_0 - \lg N_a = 6,22 - 2,15 = >4,07$

For 0,75 % : $\lg R = \lg N_0 - \lg N_a = 6,22 - 2,23 = >3,99$

For 0,5 % : $\lg R = \lg N_0 - \lg N_a = 6,22 - 2,67 = 3,55$

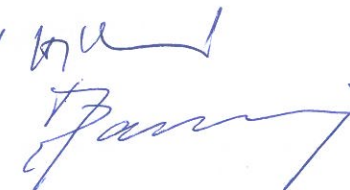
For 0,1 % : $\lg R = \lg N_0 - \lg N_a = 6,22 - 4,22 = 2$

Annex to the protocol n. 5: 11/DP/16

According to procedure SOP 3039 - ČSN EN 13624 - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)

Name of the product: **Desam effekt +**
Manufacturer: Schulke s. r. o.
Storage conditions: -10° C to +30°C
Solvent: hard water
Number plates: 2 x 1 ml
Neutralizer: Polysorbate 80 30 g/l+ lecithin 3 g/l+
Sodium thiosulphate 5 g/l + L-histidin 1g/l
Tested concentrations of product: 0,5 %, 0,25 %, 0,1%, 0,05%, 0,01 %
Contact time: 5 minutes
Stability and appearance of the product during tests: clear to milky solution, formation of precipitates in 0,5 % test mixture and validation C
Test temperature: 24°C
Interfering substance: bovine albumin 3 g/l + sheep erythrocytes 3ml/ l in diluent
Test organism: **Candida albicans CCM 8215**
Temperature of incubation, time of incubation: 30 °C, 48 h
Date of test: 13. 6. 2016

Processed by: Mgr. Pavlína Hradská, Irena Willerthová
Checked by: RNDr. Erich Pazdziora, CSc.

Signature: 

Preparation of the fungal test suspension

Dilution of primary suspension	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Number of colonies on plate 1	>330	>330	>330	>330	>330	300	40	-	-
Number of colonies on plate 2	>330	>330	>330	>330	>330	260	45	-	-

Validation and controls:

Validation suspension N _{vo} (N _v)				Control of the experimental conditions (A)				Neutralizer toxicity control (B)				Dilution- neutralization control (C):			
Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2	Number of colonies on the plate		Vc1	Vc2
128	176	128	176	90	100	90	100	115	112	115	112	124	132	124	132
Arithmetic mean Vc1+Vc2: $\bar{x} = 152$				Arithmetic mean Vc1+Vc2 $\bar{x} = 95$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 113,5$				Arithmetic mean Vc1+ Vc2 $\bar{x} = 128$			
Is $30 \leq \bar{x} \leq N_{vo} \leq 160$? <u>yes</u> - no				Is $\bar{x} \geq 0,5 \times \bar{x} \leq N_{vo}$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq N_{vo}$? <u>yes</u> - no				Je $\bar{x} \geq 0,5 \times \bar{x} \leq N_{vo}$? <u>yes</u> - no			
Validation suspension N _{vb} (validation suspension for the control neutralizer B)								140	148	140	148	Arithmetic mean Vc1+ Vc2 $\bar{x} = 144$ Is $30 \leq \bar{x} \leq N_{vb}/1000 \leq 160$? <u>yes</u> - no			

Test suspension

Test suspension N	Dilution	Number of colonies on the plate		Vc1	Vc2	$\bar{x}_{wm} = \frac{C \text{ (sum of values Vc)} \quad 645}{(n1 + 0,1 n2) \times 10^{-5} \quad (2+0,2) \times 10^{-5}} = \dots$
						$\bar{x}_{wm} = \text{sum of values (Vc)} \quad 645 : 2,2 \times 10^{-5} = 2,93 \times 10^7$
	10 ⁻⁵	300	260	300	260	lg N = 7,46
	10 ⁻⁶	40	45	40	45	Je $7,17 \leq \lg N \leq 7,70$? <u>yes</u> - no
Test suspension No = N/10	Dilution	Number of colonies on the plate		Vc1	Vc2	$\bar{x}_{wm} = \frac{C \text{ (sum of values Vc)} \quad 645}{(n1 + 0,1 n2) \times 10^{-5} \quad (2+0,2) \times 10^{-5}} = \dots$
						$\bar{x}_{wm} = \text{sum of values (Vc)} \quad 645 : 2,2 \times 10^{-5} = 2,93 \times 10^7$
	10 ⁻⁵	300	260	300	260	lg No = 6,46
	10 ⁻⁶	40	45	40	45	Je $6,17 \leq \lg No \leq 6,70$? <u>yes</u> - no

Test

Concentration of product (%)	Dilution	Number of cfu on plate		Vc1	Vc2	Na = mean \bar{x} or weighted mean $\bar{x}_{wm} \times 10$	lg Na = lg (\bar{x} or \bar{x}_{wm}) $\times 10$	lg R = lg N ₀ - lg Na lg N ₀ = 6,46	Contact time (min)
0,5	10 ⁰	0	0	<14	<14	<140	<2,15	>4,31	5
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,25	10 ⁰	0	1	<14	<14	<140	<2,15	>4,31	5
	10 ⁻¹	0	0	<14	<14				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,1	10 ⁰	160	136	160	136	1570	3,20	3,26	5
	10 ⁻¹	19	32	19	32				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,05	10 ⁰	>330	>330	>330	>330	>33000	>4,52	<1,94	5
	10 ⁻¹	>330	>330	>330	>330				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								
0,01	10 ⁰	>330	>330	>330	>330	>33000	>4,52	<1,94	5
	10 ⁻¹	>330	>330	>330	>330				
	10 ⁻²								
	10 ⁻³								
	10 ⁻⁴								
	10 ⁻⁵								

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; N₀ = N/10 = number of cells on 1 ml in test suspension at the beginning of contact time (time=0);

N_{vo} = N_v/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 N₀ - lg Na).