

DETERMINATION OF THE MICROBIOCIDAL ACTIVITY ACCORDING TO STANDARD ISO 22196:2011*

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a) Identification of the test laboratory	"Control Microbiológico Bialcon, S.L.U."
b) Client	PINTURAS BLATEM, S.L.
c) Identification of the sample	
- Product name	BIOTEM WITH ANTIBACTERIA AND ANTI-MOLD PRESERVATIVE
- Batch number	ELB-190386 WITH ELB-190386 SIN
- Manufacturer	PINTURAS BLATEM, S.L.
- Delivery date	07/08/2019
- Storage conditions	Not included
- Product appearance	50mm x 50mm plates
- Active substances and their concentration (information provided by client)	Not included
d) Experimental conditions	
- Period of analysis	From 26/08/2019 to 03/09/2019
- Sample washing	SCDLP broth
- Diluent of the product used during the test	Physiological saline phosphate buffer
- Contact times	24 hours
- Incubation temperature of the pieces	35±1°C
- Relative humidity during incubation of the pieces	90%
- Temperature of incubation	30±1°C fungal 37±1°C bacteria
- Identification of test strains	<i>Escherichia coli</i> CECT 405 <i>Staphylococcus aureus</i> CECT 239 <i>Salmonella typhimurium</i> CECT 4594 <i>Legionella pneumophila</i> CECT 7109 <i>Aspergillus brasiliensis</i> CECT 2574
f) Test results. See table C1, C2, C3, C4, C5.	
g) Test effectiveness conditions	
1- In order to evaluate the bactericidal activity of the pieces, you must meet the following standards - $(L_{max} - L_{min}) / (L_{media}) \leq 0.2$ • L_{max} is the maximum log. of bacteria count of the non-incubated control parts • L_{min} is the minimum log. of bacteria count of the non-incubated control parts • L_{media} is the log. the average bacterial count of the non-incubated control parts - The average number of bacteria counted in the non-incubated control pieces must be between $6,2 \times 10^3$ and $2,5 \times 10^4$ cells/cm² - The number of viable bacteria in the untreated test piece after 24 h shall not be less than 62 cells/cm² for the three test pieces. When a film is used for the untreated test piece; however, the number of viable bacterial cells after 24 h shall not be less than $6,3 \times 10^2$ cells/cm² for the three test pieces. 2- Calculate the biocidal bactericidal activity according to the following formula $R = (U_1 - U_0) - (A_1 - U_0) = U_1 - A_1$	
h) Conclusion	
The analyzed sample of the product "BIOTEM WITH PRESERVATIVE ANTIBACTERIA AND ANTI-MOLD", complies with the conditions of effectiveness of the test according to ISO 22196: 2011, after 24 hours of contact and with a relative humidity of not less than 90%, against strains of <i>Escherichia coli</i>, <i>Staphylococcus aureus</i>, <i>Salmonella typhimurium</i>, <i>Legionella pneumophila</i> and <i>Aspergillus brasiliensis</i> reference	

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Table C.1 Test results

Tests organisms	CONTROL WITHOUT INCUBATION		
	CONTROL 1	CONTROL 2	CONTROL 3
<i>Escherichia coli</i> CECT 405	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 117; 102 10 ⁻³ : 18; 14 N: 2,3x10 ⁴ Log: 4,36	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 132; 136 10 ⁻³ : 13; 12 N: 2.7x10 ⁴ Log: 4,42	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 86; 99 10 ⁻³ : 9; 10. N: 1,8x10 ⁴ Log: 4,27
	U ₀ = 2,3x10 ⁴ Log = 4.36		
	The average number of bacteria counted in the non-incubated control parts must be between 6.2x10 ³ and 2.5x10 ⁴ cells/cm ²		

24 HOUR INCUBATED CONTROL			PRODUCT INCUBATED 24 HOURS		
CONTROL 1	CONTROL 2	CONTROL 3	PROBLEM 1	PROBLEM 2	PROBLEM 3
10 ⁻⁰ : >330; >330 10 ⁻¹ : 99; 92 10 ⁻² : 9; 9 10 ⁻³ : 0; 0 N: 1.9x10 ³ Log: 3,28	10 ⁻⁰ : >330; >330 10 ⁻¹ : 96; 96 10 ⁻² : 9; 9 10 ⁻³ : 0; 0 N: 1,9x10 ³ Log: 3,28	10 ⁻⁰ : >330; >330 10 ⁻¹ : 102; 106 10 ⁻² : 10; 11 10 ⁻³ : 1; 0 N: 2,1x10 ³ Log: 3,32	10 ⁻⁰ : 69; 75 10 ⁻¹ : 7; 7 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,4x10 ² Log: 2,16	10 ⁻⁰ : 52; 59 10 ⁻¹ : 7; 5 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,1x10 ² Log: 2,04	10 ⁻⁰ : 60; 66 10 ⁻¹ : 6; 6 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,3x10 ² Log: 2,10
U ₁ = 2x10 ³ Log = 3.29			A ₁ = 1,3x10 ² Log = 2.10		
- The average number of bacteria counted in control parts incubated without film >62 cells/cm² - The average number of bacteria counted in the control parts incubated with film >6,3x10² cells/cm²			EVALUATION OF BACTERICIDE ACTIVITY: R = (U ₁ - U ₀) - (A ₁ - U ₀) = U ₁ - A ₁		
			R = 1,19		
			R: bactericidal activity value U ₀ : mean bacteria count of the non-incubated control parts U ₁ : mean bacterial count in control parts after 24 hours incubation A ₁ : mean bacterial count of test pieces after 24 hours incubation		

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Table C.2 Test results

Tests organisms	CONTROL WITHOUT INCUBATION		
	CONTROL 1	CONTROL 2	CONTROL 3
<i>Staphylococcus aureus</i> CECT 239	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 96; 99 10 ⁻³ : 11; 10 N: 2x10 ⁴ Log: 4,29	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 86; 97 10 ⁻³ : 10; 12 N: 1.9x10 ⁴ Log: 4,27	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 106; 108 10 ⁻³ : 11; 10. N: 2,1x10 ⁴ Log: 4,33
	U ₀ = 2x10 ⁴ Log = 4.30		
	The average number of bacteria counted in the non-incubated control parts must be between 6.2x10 ³ and 2.5x10 ⁴ cells/cm ²		

24 HOUR INCUBATED CONTROL			PRODUCT INCUBATED 24 HOURS		
CONTROL 1	CONTROL 2	CONTROL 3	PROBLEM 1	PROBLEM 2	PROBLEM 3
10 ⁻⁰ : >330; >330 10 ⁻¹ : 42; 47 10 ⁻² : 4; 4 10 ⁻³ : 0; 0 N: 8.9x10 ² Log: 2,95	10 ⁻⁰ : >330; >330 10 ⁻¹ : 31; 39 10 ⁻² : 3; 4 10 ⁻³ : 0; 0 N: 7x10 ² Log: 2,84	10 ⁻⁰ : >330; >330 10 ⁻¹ : 61; 52 10 ⁻² : 7; 7 10 ⁻³ : 0; 0 N: 1,1x10 ³ Log: 3,05	10 ⁻⁰ : 94; 95 10 ⁻¹ : 8; 8 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,9x10 ² Log: 2,28	10 ⁻⁰ : 92; 102 10 ⁻¹ : 9; 9 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,9x10 ² Log: 2,29	10 ⁻⁰ : 89; 94 10 ⁻¹ : 9; 6 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,8x10 ² Log: 2,26
U ₁ = 9x10 ² Log = 2.95			A ₁ = 1,9x10 ² Log = 2.27		
- The average number of bacteria counted in control parts incubated without film >62 cells/cm ² - The average number of bacteria counted in the control parts incubated with film >6,3x10 ² cells/cm ²			EVALUATION OF BACTERICIDE ACTIVITY: R = (U ₁ - U ₀) - (A ₁ - U ₀) = U ₁ - A ₁		
			R = 0,68		
			R: bactericidal activity value U ₀ : mean bacteria count of the non-incubated control parts U ₁ : mean bacterial count in control parts after 24 hours incubation A ₁ : mean bacterial count of test pieces after 24 hours incubation		

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Table C.3 Test results

Tests organisms	CONTROL WITHOUT INCUBATION		
	CONTROL 1	CONTROL 2	CONTROL 3
<i>Salmonella typhimurium</i> CECT 4594	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 43; 35 10 ⁻³ : 5; 3 N: 7.8x10 ³ Log: 3,89	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 44; 38 10 ⁻³ : 4; 6 N: 8.2x10 ³ Log: 3,91	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 38; 31 10 ⁻³ : 3; 2. N: 6,9x10 ³ Log: 3,84
	U ₀ = 7.6x10 ³ Log = 3.88		
	The average number of bacteria counted in the non-incubated control parts must be between 6.2x10 ³ and 2.5x10 ⁴ cells/cm ²		

24 HOUR INCUBATED CONTROL			PRODUCT INCUBATED 24 HOURS		
CONTROL 1	CONTROL 2	CONTROL 3	PROBLEM 1	PROBLEM 2	PROBLEM 3
10 ⁻⁰ : >330; >330 10 ⁻¹ : 34; 30 10 ⁻² : 3; 4 10 ⁻³ : 0; 0 N: 6.4x10 ² Log: 2,81	10 ⁻⁰ : >330; >330 10 ⁻¹ : 46; 42 10 ⁻² : 3; 4 10 ⁻³ : 0; 0 N: 8.8x10 ² Log: 2,94	10 ⁻⁰ : >330; >330 10 ⁻¹ : 39; 30 10 ⁻² : 3; 3 10 ⁻³ : 0; 0 N: 6,9x10 ² Log: 2,84	10 ⁻⁰ : 51; 64 10 ⁻¹ : 6; 7 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,2x10 ² Log: 2,06	10 ⁻⁰ : 66; 55 10 ⁻¹ : 7; 5 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,2x10 ² Log: 2,08	10 ⁻⁰ : 50; 50 10 ⁻¹ : 5; 5 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1x10 ² Log: 2,0
U ₁ = 7.3x10 ² Log = 2.87			A ₁ = 1,1x10 ² Log = 2.05		
- The average number of bacteria counted in control parts incubated without film >62 cells/cm² - The average number of bacteria counted in the control parts incubated with film >6,3x10² cells/cm²			EVALUATION OF BACTERICIDE ACTIVITY: R = (U ₁ - U ₀) - (A ₁ - U ₀) = U ₁ - A ₁		
			R = 0,82		
			R: bactericidal activity value U ₀ : mean bacteria count of the non-incubated control parts U ₁ : mean bacterial count in control parts after 24 hours incubation A ₁ : mean bacterial count of test pieces after 24 hours incubation		

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Table C.4 Test results

Tests organisms	CONTROL WITHOUT INCUBATION		
	CONTROL 1	CONTROL 2	CONTROL 3
<i>Legionella pneumophila</i> CECT 7109	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 40; 42 10 ⁻³ : 4; 2 N: 8.2x10 ³ Log: 3,91	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 41; 40 10 ⁻³ : 4; 4 N: 8.1x10 ³ Log: 3,91	10 ⁻⁰ : >330; >330 10 ⁻¹ : >330; >330 10 ⁻² : 38; 34 10 ⁻³ : 3; 3. N: 7,2x10 ³ Log: 3,86
	U ₀ = 7.8x10 ³ Log = 3.89		
	The average number of bacteria counted in the non-incubated control parts must be between 6.2x10 ³ and 2.5x10 ⁴ cells/cm ²		

24 HOUR INCUBATED CONTROL			PRODUCT INCUBATED 24 HOURS		
CONTROL 1	CONTROL 2	CONTROL 3	PROBLEM 1	PROBLEM 2	PROBLEM 3
10 ⁻⁰ : >330; >330 10 ⁻¹ : 95; 99 10 ⁻² : 10; 10 10 ⁻³ : 0; 0 N: 1.9x10 ³ Log: 3,29	10 ⁻⁰ : >330; >330 10 ⁻¹ : 101; 104 10 ⁻² : 10; 10 10 ⁻³ : 0; 1 N: 2x10 ³ Log: 3,31	10 ⁻⁰ : >330; >330 10 ⁻¹ : 103; 111 10 ⁻² : 12; 12 10 ⁻³ : 1; 1 N: 2,1x10 ³ Log: 3,33	10 ⁻⁰ : 43; 52 10 ⁻¹ : 5; 5 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 95 Log: 1,98	10 ⁻⁰ : 61; 66 10 ⁻¹ : 6; 6 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,3x10 ² Log: 2,10	10 ⁻⁰ : 55; 69 10 ⁻¹ : 7; 6 10 ⁻² : 0; 0 10 ⁻³ : 0; 0 N: 1,2x10 ² Log: 2,09
U ₁ = 2x10 ³ Log = 3.30			A ₁ = 1,2x10 ² Log = 2.10		
- The average number of bacteria counted in control parts incubated without film >62 cells/cm ² - The average number of bacteria counted in the control parts incubated with film >6,3x10 ² cells/cm ²			EVALUATION OF BACTERICIDE ACTIVITY: R = (U ₁ - U ₀) - (A ₁ - U ₀) = U ₁ - A ₁		
			R = 1,2		
			R: bactericidal activity value U ₀ : mean bacteria count of the non-incubated control parts U ₁ : mean bacterial count in control parts after 24 hours incubation A ₁ : mean bacterial count of test pieces after 24 hours incubation		

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Table C.5 Test results

Tests organisms	CONTROL WITHOUT INCUBATION		
	CONTROL 1	CONTROL 2	CONTROL 3
<i>Aspergillus brasiliensis</i> CECT 2574	10 ⁻⁰ : >165; >165 10 ⁻¹ : >165; >165 10 ⁻² : 35; 30 10 ⁻³ : 3; 2 N: 6.5x10 ³ Log: 3,81	10 ⁻⁰ : >165; >165 10 ⁻¹ : >165; >165 10 ⁻² : 37; 28 10 ⁻³ : 2; 3 N: 6.5x10 ³ Log: 3,81	10 ⁻⁰ : >165; >165 10 ⁻¹ : >165; >165 10 ⁻² : 31; 31 10 ⁻³ : 3; 2. N: 6,2x10 ³ Log: 3,79
	U ₀ = 6.4x10 ³ Log = 3.81		
	The average number of bacteria counted in the non-incubated control parts must be between 6.2x10 ³ and 2.5x10 ⁴ cells/cm ²		

24 HOUR INCUBATED CONTROL			PRODUCT INCUBATED 24 HOURS		
CONTROL 1	CONTROL 2	CONTROL 3	PROBLEM 1	PROBLEM 2	PROBLEM 3
10 ⁻⁰ : >165; >165 10 ⁻¹ : 32; 49 10 ⁻² : 4; 4 10 ⁻³ : 0; 0 N: 8.1x10 ² Log: 2,91	10 ⁻⁰ : >165; >165 10 ⁻¹ : 26; 29 10 ⁻² : 1; 4 10 ⁻³ : 0; 0 N: 5.5x10 ² Log: 2,74	10 ⁻⁰ : >165; >165 10 ⁻¹ : 27; 27 10 ⁻² : 2; 2 10 ⁻³ : 0; 0 N: 5,4x10 ² Log: 2,73	10 ⁻⁰ : >165; >165 10 ⁻¹ : 19; 18 10 ⁻² : 1; 1 10 ⁻³ : 0; 0 N: 3,7x10 ² Log: 2,57	10 ⁻⁰ : >165; >165 10 ⁻¹ : 16; 14 10 ⁻² : 1; 1 10 ⁻³ : 0; 0 N: 3x10 ² Log: 2,48	10 ⁻⁰ : >165; >165 10 ⁻¹ : 16; 18 10 ⁻² : 1; 1 10 ⁻³ : 0; 0 N: 3,4x10 ² Log: 2,53
U ₁ = 6.3x10 ² Log = 2.80			A ₁ = 3,4x10 ² Log = 2.53		
- The average number of bacteria counted in control parts incubated without film >62 cells/cm² - The average number of bacteria counted in the control parts incubated with film >6,3x10² cells/cm²			EVALUATION OF BACTERICIDE ACTIVITY: R = (U ₁ - U ₀) - (A ₁ - U ₀) = U ₁ - A ₁		
			R = 0,27		
			R: bactericidal activity value U ₀ : mean bacteria count of the non-incubated control parts U ₁ : mean bacterial count in control parts after 24 hours incubation A ₁ : mean bacterial count of test pieces after 24 hours incubation		