

TEST REPORT N°

68/2020

TEST METHOD:

JIS Z 2801:2000

Antimicrobial products -Test for antimicrobial activity and efficacy

LABORATORY

M&R LAB

Microbiology & Research

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Address:

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Producer / Supplier:

X HAUS stavby a.s.

Dělnická 213/12

170 00 170 00 Praha 7 – Holešovice

NIP: CZ036 49 997

Type of method:

assessment of antimicrobial effectiveness by determining the reduction of bacteria on the surface tested after 24 hours contact at the optimum temperature for growth and survival

Test conditions

paint coating samples were tested randomly out of 40 submitted by the customer

Test strains used in the research:

Escherichia coli ATCC 8739

Staphylococcus aureus ATCC 6538P

DATE OF DELIVERY OF SAMPLES FOR TESTING:

7.07.2020.

SAMPLING BY:

Costumer

SUPPLIER OF SAMPLES:

Princípal

DATE OF START TEST:

10.07.2020.

DATE OF END TESTS:

26.08.2020.

TYPE OF MATERIAL ACCORDING TO THE ORDERING PARTY'S DECLARATION:

multi-component material intended for thermal insulation, thermo-reflective, wood-dilutable, based on acrylic dispersion and glass microspheres. The declaration of conformity of materials provided by the ordering party applies to the following products: PSC 250T ECI Inside, PSC 250T BUILD, PSC 250T ECF Floor, PSC 250 Eco Outside, PSC 250T ECE Elastic

SAMPLE IDENTIFICATION

Sample ID

251/2020

Test subject:

dry coatings of acrylic paint PSC 250T / ECI interior

Description of test samples:

a multi-layer coating in the form of plates 50mm x 50mm x 3mm with smooth surfaces (40 pcs) according to photo 1

Antibacterial additives present in the samples according to the information of the Ordering Party:

5-chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC No 220-239-6] post-reaction mixture (3: 1)

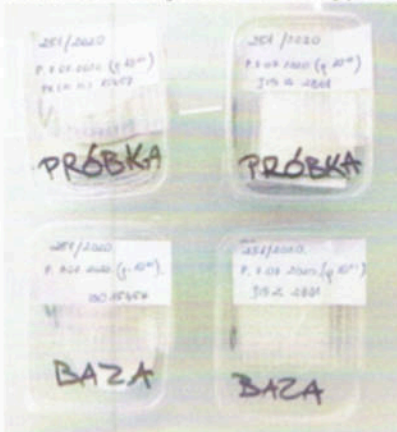


Photo 1. Samples intended for testing (dry coatings of acrylic paint PSC 250T / ECI interior)

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Photo 2. Set of samples tested for each strain

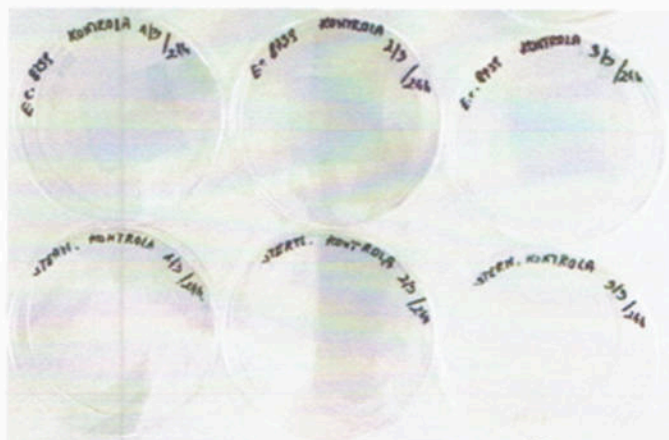


Photo 3. Set of additional control samples for each strain

Control samples provided by Client:

Control samples used in the study:

1) positive controls at the start of the test:

2) positive control samples after 24h:

3) positive control samples after 24h:

4) negative control samples after 24h:

"Base" - paint coatings without biocide

Base - without additives - test samples - time "To" with aseptically covering film with the tested strain (B1-B3 / "To")

Base - no additives - test samples after "24h" (B1-B3 / 24h)

sterile auxiliary materials: Petri dishes with aseptically covering film with the tested strain (D1-D3 / 24h)

sterile auxiliary materials: Petri dishes with aseptically covering film (C1-C3 / 24h)

TEST METHOD: Determination of the antibacterial effectiveness of the material according to JIS Z 2801: 2000

Table I. TEST CONDITIONS		
Sample ID:	examined	251/2020
	control	according to Table II
The strains used in the study		<i>Escherichia coli</i> ATCC 8739
Date of start the test		10.07.2020.
Date of end the test		15.07.2020.
Tested area		outer surface
The surface of the prepared samples		50 x 50 mm ²
The size of the tested surfaces		40 x 40 mm ²
The size and type of the covering film used in the research		40 x 40 mm ² , film prepared aseptically
Thickness of the test samples		do 10 mm
Type of material disinfection prior to testing		wipe with ethanol (75%) and evaporation in a laminar chamber for 2 h
Amount of inoculum normally used in the test		0,4 ± 0,1 ml
Conditions for preparing test samples		temperature 20 ± 2 ° C
Incubation temperature		temperature 35 ± 2 ° C
Humidity		min. 90% (24h)
Strain control		positive
Incubation time		16- 24 h
Type of diluent		Bulion NB 1/500
Type of neutralizer		bulion SCDLP
Volume of neutralizer used to recover the strain		10 ml
Medium for determining the number of recovered bacteria		Nutrient agar
Incubation time after recovery		24-48h

Table II. Types of samples intended for testing

Table 11. Types of samples intended for testing			
Types of tested samples	Symbol	Type of samples - description	
	Nw	stock suspension of the strain	
	B1 "0"	strain control before incubation	Control 251/2020
	B2 "0"		
	B3 "0"		
	B1 "24"	"Base" control with test strain after incubation	
	B2 "24"		
	B3 "24"		
	D1 "24"	the test inoculum covered with an aseptically prepared film on sterile petri dishes	
	D2 "24"		
	D3 "24"		
	C1 "24"	controls (negative) after incubation	
	C2 "24"		
	C3 "24"		
	251-1	samples with the test strain after incubation	251/2020
	251-2		
	251-3		

Table III. The results of the antibacterial effectiveness of the tested material against *Escherichia coli* ATCC 8739

Table III. The results of the antibacterial effectiveness of the tested material against Escherichia coli ATCC 8739																
Symbols of the test samples as described in Table II		The results of the tested samples					Number of CFU / samples tested		symbol	Number of CFUs recovered from the samples			Indicator		Control of test conditions	Antibacterial activity factor [R]
		[cfu /10ml]	10 ⁵	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁴		[cfu/1ml]	log 10	log 10	symbol/ value	log 10		
NW	N-1	-	>300	>300	>300	80	6	0	NW	695 455	-	-	1.	Nw=2,5-10x10 ⁵ cfu/cm ³ fulfilled	R= Log(B/A)-log(C/A) [log 10]	
	N-2	-	>300	>300	>300	60	7	0								
B1 "0"	K _{B1.1}	>300	>300	>300	>300	30	-	-	A	277000	Lmax 5,53	L4r	2.	(Lmax - Lmin)/(L4r) ≤ 0,2 fulfilled		
	K _{B1.2}	>300	>300	>300	>300	38	-									
B2 "0"	K _{B2.1}	>300	>300	>300	>300	40	-	-	A	277000	5,69	5,60	3.	U ₀ = 1 - 4 × 10 ⁵ cfu/cm ³ fulfilled		
	K _{B2.2}	>300	>300	>300	>300	58	-									
B3 "0"	K _{B3.1}	>300	>300	>300	>300	35	-	-	A	277000	Lmin 5,57	5,60	3.	U ₀ = 1 - 4 × 10 ⁵ cfu/cm ³ fulfilled		
	K _{B3.2}	>300	>300	>300	>300	40	-									
B1 "24"	K _{B1.1}	<10	<1	<1	<1	<1	-	-	B	10	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{B1.2}	<10	<1	<1	<1	<1	-									
B2 "24"	K _{B2.1}	<10	<1	<1	<1	<1	-	-	B	10	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{B2.2}	<10	<1	<1	<1	<1	-									
B3 "24"	K _{B3.1}	<10	<1	<1	<1	<1	-	-	B	10	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{B3.2}	<10	<1	<1	<1	<1	-									
D1 "24"	K _{D1.1}	>300	>300	>300	>300	40	3	-	B	20000	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{D1.2}	>300	>300	>300	>300	35	2	-								
D2 "24"	K _{D2.1}	>300	>300	>300	>300	33	4	-	B	20000	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{D2.2}	>300	>300	>300	>300	48	5	-								
D3 "24"	K _{D3.1}	>300	>300	>300	>300	56	4	-	B	20000	-	-	4.	Ut = min. 1× 10 ³ cfu/cm ³ fulfilled		
	K _{D3.2}	>300	>300	>300	>300	40	5	-								
251-1	N _{B251.1}	<10	<1	<1	<1	<1	-	-	C	23,33	-	-	-	-		
	N _{B251.2}	<10	<1	<1	<1	<1	-									
251-2	N _{B251.1}	5	<1	<1	<1	<1	-	-	C	23,33	-	-	-	-		
	N _{B251.2}	3	<1	<1	<1	<1	-									
251-3	N _{B251.1}	3	<1	<1	<1	<1	-	-	C	23,33	-	-	-	-		
	N _{B251.2}	1	<1	<1	<1	<1	-									
C1 "24"	K _{C1.1}	<1	<1	<1	<1	<1	-	-	D	-	-	-	5.	fulfilled		
	K _{C1.2}	<1	<1	<1	<1	<1	-									
C2 "24"	K _{C2.1}	<1	<1	<1	<1	<1	-	-	D	-	-	-	5.	fulfilled		
	K _{C2.2}	<1	<1	<1	<1	<1	-									
C3 "24"	K _{C3.1}	<1	<1	<1	<1	<1	-	-	D	-	-	-	5.	fulfilled		
	K _{C3.2}	<1	<1	<1	<1	<1	-									

Signs:

Nw - number of bacteria in the initial suspension

A - average cfu number recovered from the sample without addition during the time To

B - average number of bacteria recovered from samples without addition after 24h, expressed in cfu/ml

B' - average number of bacteria recovered from control samples after 24h, expressed in cfu/ml

C - average number of bacteria recovered from samples with bactericidal additive after 24h, expressed in cfu/ml

C' - the average number of bacteria obtained from the sterile materials used in the test

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TEST METHOD: Determination of the antibacterial effectiveness of the material according to JIS Z 2801: 2000

Table IV. TEST CONDITIONS

The strains used in the study	251/2020
Date of start the test	according to table V
Date of end the test	<i>Staphylococcus aureus</i> ATCC 6538P
Tested area	21.08.2020.
The surface of the prepared samples	26.08.2020.
The size of the tested surfaces	outer surface
The size and type of the covering film used in the research	50 x 50 mm ²
Thickness of the test samples	40 x 40 mm ²
Type of material disinfection prior to testing	40 x 40 mm ² , film prepared aseptically
Amount of inoculum normally used in the test	do 10 mm
Conditions for preparing test samples	wipe with ethanol (75%) and evaporation in a laminar chamber for 2 h
Incubation temperature	0,4 ± 0,1 ml
Humidity	temperature 20 ± 2 °C
Strain control	temperature 35 ± 2 °C
Incubation time	positive
Type of diluent	24±1 h
Type of neutralizer	Bullion NB 1/500
Volume of neutralizer used to recover the strain	bullion SCDLP
Medium for determining the number of recovered bacteria	10 ml
Incubation time after recovery	Nutrient agar
Incubation time after recovery	24-48h

Table V. Types of samples intended for testing

Table 1. Types of samples included for testing			
Types of tested samples	Symbol	Type of samples - description	
	Nw	stock suspension of the strain	
	B1 "0"	strain control before incubation	Control 251/2020
	B2 "0"		
	B3 "0"		
	B1 "24"	"Base" control with test strain after incubation	
	B2 "24"		
	B3 "24"		
	D1 "24"	the test inoculum covered with an aseptically prepared film on sterile petri dishes	
	D2 "24"		
	D3 "24"		
	C1 "24"	controls (negative) after incubation	
	C2 "24"		
	C3 "24"		
	251-1	samples with the test strain after incubation	251/2020
	251-2		
251-3			

Table VI. The results of the antibacterial effectiveness of the tested material against *Staphylococcus aureus* ATCC 6538P

Table VI. The results of the antibacterial effectiveness of the tested material against Staphylococcus aureus ATCC 6538P																																				
Symbols of test samples as described in Table V		The results of the tested samples						Number of ctf/ samples tested		symbol	Number of CFUs recovered from the samples			Indicator		Control of test conditions		Antibacterial activity factor [R]																		
		[cfu/10ml]		[cfu/1ml]				log 10	[cfu/1ml]		log 10	symbol / log 10	symbol/ value	log 10																						
		10 ⁰	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵																													
NW	N-1	-	>300	>300	50	4	0	4,18E+05	5,62	NW	418 182	-	-	1.	Nw=2,5-10×10 ⁵ cfu/cm ³	R= Log(B/A)-log(C/A)	[log 10]																			
B1 "0"	N-2	-	>300	>300	35	3	0	2,4E+05	5,37	A	152000	Lmax 5,37 Lmin 5,32	5,18	2.	(Lmax - Lmin)/(L&r) ≤ 0,2																					
	K _{6.1-1}	>300	>300	>300	22	-	-	2,2E+05	5,34					3.	U ₀ = 1 - 4 × 10 ⁵ cfu/cm ³																					
B2 "0"	K _{6.1-2}	>300	>300	>300	25	-	-	1,8E+05	5,26					fulfilled																						
K _{6.2-1}	>300	>300	>300	19	-	-	1,0E+00	0,00	10					not fulfilled																						
K _{6.2-2}	>300	>300	>300	23	-	-	1,9E+04	4,27	B'					14000	0,09	-1,04	4.	Ut = min. 1× 10 ³ CFU/cm ³																		
K _{6.3-1}	>300	>300	>300	18	1	-	2,3E+04	4,36		C	38,33	C/A	-3,60						5.	fulfilled																
K _{6.3-2}	>300	>300	>300	13	1	-	1,6E+04	4,20													D	-	0,000252	-<1	-<1	-										
N _{6.1-1}	<10	<1	<1	<1	<1	-	1,0E+01	1,00																			-	-	-	-	-	-				
N _{6.1-2}	<10	<1	<1	<1	<1	-	8,0E+01	1,90																									-	-	-	-
N _{6.2-1}	3	<1	<1	<1	<1	-	2,5E+01	1,40	-					-	-	-	-	-																		
N _{6.2-2}	2	<1	<1	<1	<1	-	0,0E+00	0,00		-	-	-	-						-	-																
N _{6.3-1}	<1	<1	<1	<1	<1	-	0,0E+00	0,00													-	-	-	-	-	-										
K _{6.1-1}	<1	<1	<1	<1	<1	-	0,0E+00	0,00																			-	-	-	-	-	-				
K _{6.1-2}	<1	<1	<1	<1	<1	-	0,0E+00	0,00																									-	-	-	-
K _{6.2-1}	<1	<1	<1	<1	<1	-	0,0E+00	0,00	-					-	-	-	-	-																		
K _{6.2-2}	<1	<1	<1	<1	<1	-	0,0E+00	0,00		-	-	-	-						-	-																
K _{6.3-1}	<1	<1	<1	<1	<1	-	0,0E+00	0,00													-	-	-	-	-	-										
K _{6.3-2}	<1	<1	<1	<1	<1	-	0,0E+00	0,00																			-	-	-	-	-	-				
D1 "24"	K _{6.1-1}	>300	>300	18	1	-	1,9E+04	4,27																									-	-	-	-
D2 "24"	K _{6.1-2}	>300	>300	20	2	-	2,3E+04	4,36	-					-	-	-	-	-																		
D3 "24"	K _{6.2-1}	>300	>300	22	2	-	1,6E+04	4,20		-	-	-	-						-	-																
251-1	K _{6.2-2}	>300	>300	19	2	-	1,0E+01	1,00													-	-	-	-	-	-										
251-2	N _{6.1-1}	<10	<1	<1	<1	<1	8,0E+01	1,90																			-	-	-	-	-	-				
251-3	N _{6.1-2}	13	<1	<1	<1	<1	2,5E+01	1,40																									-	-	-	-
C1 "24"	N _{6.2-1}	2	<1	<1	<1	<1	0,0E+00	0,00	-					-	-	-	-	-																		
C2 "24"	N _{6.2-2}	3	<1	<1	<1	<1	0,0E+00	0,00		-	-	-	-						-	-																
C3 "24"	N _{6.3-1}	<1	<1	<1	<1	<1	0,0E+00	0,00													-	-	-	-	-	-										
	K _{6.3-2}	<1	<1	<1	<1	<1	0,0E+00	0,00																			-	-	-	-	-	-				

Signs:

Nw - number of bacteria in the initial suspension

A - average cfu number recovered from the sample without addition during the time To

B - average number of bacteria recovered from samples without addition after 24h, expressed in cfu/ml

B' - average number of bacteria recovered from control samples after 24h, expressed in cfu/ml

C - average number of bacteria recovered from samples with bactericidal additive after 24h, expressed in cfu/ml

C' - the average number of bacteria obtained from the sterile materials used in the test



SUMMARY RESEARCH

Special notes on the results:

No adequate recovery was obtained from test samples - without additives - after "24h" (B1-B3 / 24h)

In order to establish the survival of the strain under the conditions of the test after 24h, positive control samples were introduced, containing the test strain inoculum covered with an aseptically prepared foil in sterile Petri dishes (D1-D3).

The remaining checks and validations were within the basic limits.

Method criterion

The value of antibacterial activity obtained by the test methods specified in this standard may not be less than 2.0 for the declaration of the antibacterial efficacy of the products. Values below 2.0 may apply subject to agreement between the parties.

In tests carried out with the method according to JIS Z 2801: 2000 for samples: **dry coatings of acrylic paint PSC 250T / ECI interior** after 24h contact with the tested bacteria at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ showed antibacterial activity against the test strains in accordance with the following data:

Antibacterial activity of samples	Type of strain tested
log 10	
3,05	<i>E.coli</i> ATCC 8739
2,56	<i>Staphylococcus aureus</i> ATCC 6538P

Dyrektor ds. badań M&R LAB

dr Renata Szczygłowska

Place, date: Lipnik, 30 .09.2020.
Name, Surname and function: Scientific Director: Ph.D. Renata Szczygłowska

The results are exclusively referred to the test sample. In the case of samples provided by the Ordering Party, the Laboratory is not responsible for the description of the samples, origin, representativeness, and the method of collection and delivery.

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END OF REPORT