



Sieć Badawcza Łukasiewicz - Institute of Biopolymers & Chemical Fibres

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Microbiological Laboratory

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Lodz, date: 10/06/2020

Copy No: 1

Report of microbiological tests
No 34/2020

ISO 22196:2011

Measurement of antibacterial activity
on plastics and other non-porous surfaces.

Notes:

1. The examinations results refer only to the examined sample.
2. Test report belongs to the orderer and can be used only upon his agreement.
3. Without agreement of our laboratory this test report can be reprinted only as a whole.
4. The owner of the test report when utilizes the results is obliged to inform that the results were obtained in Microbiological Laboratory of Sieć Badawcza Łukasiewicz-Institute of Biopolymers and Chemical Fibres in Łódź which is accredited by PCA.
5. The orderer has the right to complaint in the sequence 30 days from the date of receiving the test report.
6. Test report comprises the results of properties not included in accreditation scope PCA.



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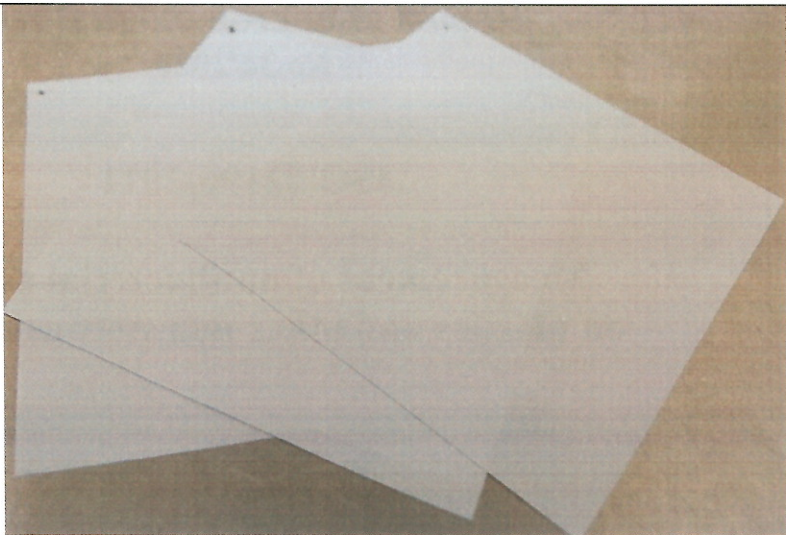
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OBJECTIVE:	Determination of antibacterial effect of film samples
CUSTOMER:	Media Ikonos Sp. z o.o., ul. Gosławicka 2d, 45-446 Opole
TEST REPORT REFERES TO:	Order of 28/05/2020
OBJEDT OF TESTING:	Film
SAMPLES MARKED BY CUSTOMER:	Film Profiflex Pro GPT FX100+ Antibacterial
RECIVED SAMPLES:	
TESTING METHOD:	ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces.
SAMPLING:	The customer
SAMPLE RECEIVED:	29/05/2020
DATE OF TEST:	01-04/06/2020
PRINCIPLE:	A suspension of <i>E. coli</i> i. <i>S. aureus</i> with known cell density is applied to the test sample. After 24 hours, the bacteria are washed away and the number of cells that survived contact with the sample is determined. This number is compared to the number of bacteria on the control sample and on this basis the antibacterial activity of the test sample is calculated.
MICROORGANISMS:	<i>Escherichia coli</i> ATCC 11229, <i>Staphylococcus aureus</i> ATCC 6538

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SAMPLES SIZE:	Samples prepared by the client. Three replicate test specimens were used for each bacterial strain. The test surface of each sample was 50x50mm. In addition, squares of 0.07 mm thick sterile polyethylene film were prepared. 40 x 40 mm squares were prepared to cover the test samples and 50x50 mm squares as a control film without antibacterial effect.
STERILIZATION:	Samples were disinfected by wiping with 70% ethanol. The samples were then dried at room temperature under sterile conditions.
PREPARATION OF BACTERIAL SUSPENSION:	<i>E. coli</i> and <i>S. aureus</i> bacteria were reduced-seeded in Petri dishes with NBA medium before the start of the test and incubated 20h at 35 ° C. Then the culture was taken with a loop and inoculated with NB medium diluted 1/500 in distilled water. In this way, a bacterial suspension was made to inoculate the samples (inoculum). The density of the suspension was determined densitometrically on the McFarland scale, the number of cells was read from the standard curve and diluted to obtain an inoculum with a density corresponding to the number of bacteria from 2.5×10^5 cfu / ml to 10×10^5 cfu / ml.
INOCULATION OF THE SAMPLES:	The prepared bacterial suspension was inoculated with test samples in a ratio of 0.4 ml per sample and covered with polyethylene film so that the suspensions were evenly distributed on the surfaces of the test samples.
INCUBATION CONDITIONS:	Samples were incubated 24h at 35°C in a humid atmosphere.

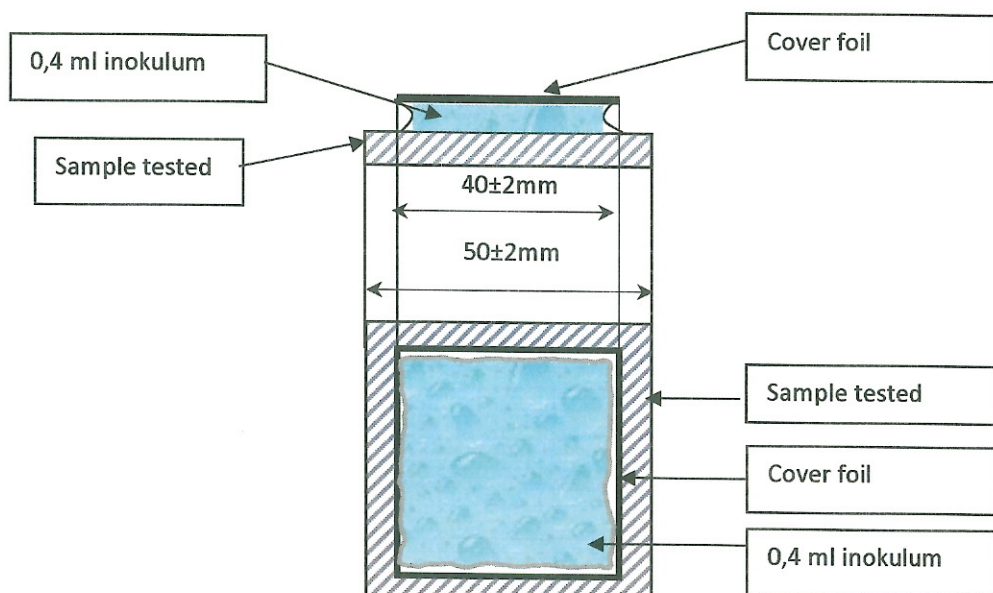


Fig. 1 Sample inoculation scheme according to ISO 22196: 2011



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**EVALUATION OF
BACTERIA GROWTH:**

After incubation, the bacteria were washed out from the samples by shaking using 10 ml of SCDLP neutralizing fluid. Appropriate decimal dilutions were made from the obtained suspension. For each dilution, inoculation on agar plates (PCA medium) was performed and incubated 24h at 35°C. After incubation, colonies were counted on each plate and the number of bacteria was calculated using the formula:

$$N = (100 \times C \times V \times D) / A$$

where:

N - number of bacteria per cm²

C - arithmetic mean of all colonies from two plates from one dilution, where the plate must contain at least 30 colonies

D - dilution factor corresponding to the first calculated dilution (e.g. 10²)

V - the number of milliliters of SDCLP used to wash the bacteria from the sample (10 ml)

A - surface of the cover foil in mm²

Antibacterial activity R for the test sample was calculated as:

$$R = (U_t - U_0) - (A_t - A_0) = U_t - A_t$$

where:

A_t - decimal logarithm of the average number of bacteria on the active sample (in triplicate) after 24 hours

U_t - decimal logarithm of the average number of bacteria on the active control foil (in triplicate) after 24 hours

U₀ - decimal logarithm of the average number of bacteria on the active control foil (in triplicate) at time 0h.

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Test results for *Escherichia coli* ATCC 11229

Date of test: 01-04/06/2020

Number of replicates: three replicates of each sample

Incubation temperature: 35°C

E. coli inoculum concentration: 7.9×10^5 cfu / ml,

Inoculum volume: 0.4ml

Conditions for a valid test		
Requirements	Obtained results	Assessment of the study
The logarithmic value of the number of bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement: $(L_{max}-L_{min}) / (L_{mean}) < 0.2$ where: L_{max} , L_{min} - decimal logarithm of the largest and smallest number of bacteria on the sample immediately after inoculation of bacteria L_{mean} - decimal logarithm of the average number of bacteria on the sample immediately after inoculation of bacteria	$L_{min} = 4.17$ $L_{max} = 4.22$ $L_{mean} = 4.20$ $(4.22-4.17)/(4.20) = 0.01$	Meets the criteria
The average number of bacteria determined immediately after inoculation on the control sample should be within the range of 6.2×10^3 to 2.5×10^4 cfu/cm ²	1.6×10^4 cfu/cm ²	Meets the criteria
The number of bacteria on each control sample after 24 hours of incubation should be not less than 6.1×10^1 cfu/cm ²	7.9×10^5 cfu/cm ² 9.3×10^5 cfu/cm ² 9.0×10^5 cfu/cm ²	Meets the criteria

Table 1. Antibacterial activity results of film samples against *E. coli* - results are given as the average of three replicates.

Sample symbol	Time [h]	<i>Escherichia coli</i> ATCC 11229		
		Number of bacteria average value N_{mean} [cfu/cm ²]	$\log N_{mean}$	Antibacterial activity R
control	0	1.6×10^4	$U_0 = 4.20$	-
control	24	8.8×10^5	$U_t = 5.94$	-
Film Profiflex Pro GPT FX100+ Antibacterial	24	5.5×10^3	$A_t = 3.72$	2.22



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Test results for *Staphylococcus aureus* ATCC 6538

Date of test: 01-04/06/2020

Number of replicates: three replicates of each sample

Incubation temperature: 35°C

S. aureus inoculum concentration: 4.2×10^5 cfu / ml,

Inoculum volume: 0.4ml

Conditions for a valid test		
Requirements	Obtained results	Assessment of the study
The logarithmic value of the number of bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement: $(L_{max}-L_{min}) / (L_{mean}) < 0.2$ where: L_{max} , L_{min} - decimal logarithm of the largest and smallest number of bacteria on the sample immediately after inoculation of bacteria L_{mean} - decimal logarithm of the average number of bacteria on the sample immediately after inoculation of bacteria	$L_{min}=4.08$ $L_{max}=4.12$ $L_{mean}=4.10$ $(4.12-4.08)/(4.10)= 0.01$	Meets the criteria
The average number of bacteria determined immediately after inoculation on the control sample should be within the range of 6.2×10^3 to 2.5×10^4 cfu/cm ²	1.3×10^4 cfu/cm ²	Meets the criteria
The number of bacteria on each control sample after 24 hours of incubation should be not less than 6.1×10^1 cfu/cm ²	5.2×10^4 cfu/cm ² 3.8×10^4 cfu/cm ² 4.5×10^4 cfu/cm ²	Meets the criteria

Table 2. Antibacterial activity results of film samples against *S. aureus* - results are given as the average of three replicates.

Sample symbol	Time [h]	<i>Staphylococcus aureus</i> ATCC 6538		
		Number of bacteria average value N_{mean} [cfu/cm ²]	log N_{mean}	Antibacterial activity R
control foil	0	1.3×10^4	$U_0 = 4.10$	-
control foil	24	4.5×10^4	$U_t = 4.65$	-
Film Profiflex Pro GPT FX100+ Antibacterial	24	1.3×10^0	$A_t = 0.10$	4.55

Conclusions:

The tested sample labeled **Film Profiflex Pro GPT FX100+ Antibacterial** showed **antibacterial activity** against tested strains of *Escherichia coli* ATCC 11229 and **strong antibacterial activity** with reference to *Staphylococcus aureus* ATCC 6538.

After 24 hours of incubation the number of *E. coli* bacteria in the control sample was 8.8×10^5 cfu/cm², while on the test sample was 5.5×10^3 cfu/cm². Antibacterial activity **R** was **2.22** with reference to *E. coli*.

After 24 hours of incubation the number of *S. aureus* bacteria in the control sample was 4.5×10^4 cfu/cm², while on the test sample it was 1.3×10^0 cfu/cm². Antibacterial activity **R** was **4.55** with reference to *S. aureus*.

The sample has antibacterial effect.

THE TEST PERFORMER: M. Sc. Eng Krystyna Guzińska

The date of report: 10/06/2020

The report drawn up in two identical copies:

copy 1 - the client receives

copy 2 - remains at the contractor's

AUTHORIZED:

Head of Laboratory

PhD Dorota Kaźmierczak

Dorota Kaźmierczak

The end of the report