SPECJALISTYCZNE LABORATORIUM BADAWCZE ITA-TEST S.C.
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REPORT FROM A MICROBIOLOGICAL TEST
B- 43232/24375/15
THE TESTS OF ANTI-MICROORGANISM PROPERTIES

Copy No. 3

NAME OF THE TESTED PRODUCT: AIR-FLEX TUBE

The Client: Z.P.T.S. INGREMIO-PESZEL ul. Laskowska 93, 32-329 Bolesław

Order No.: B-43232 **of: 24.03.2015**

The procedure of obtaining and/or delivering the product's sample for the test: The samples for the test were delivered by the Client. The Client is responsible for the appropriate taking of samples supplied for the test.

Characteristics of the product: Paper envelope bag with a label with a name: "Próbki z dodatkiem Ag" containing 50x50 mm plastic plates. Additionally, the Client supplied control samples of the same size.

Condition of the sample at the moment of delivering to the Laboratory: good

Sample No.: 24375
Date of starting the test: 07.04.2015
Date of finishing the test: 24.04.2015
Date of preparing the report: 11.06.2015

1. THE AIM/SCOPE OF THE TEST

The aim of the test was to determine, whether the tested samples are characterized by anti-microorganism effect with reference to the bacteria: *Escherichia coli* and *Staphylococcus aureus*.

2. DESCRIPTION OF THE TEST

The tests were carried out in accordance with the norm:

- **ISO 22196:2011** *Plastics – Measurement of antibacterial activity on plastics surfaces*

According to the Client's declaration, the supplied material should have anti-microorganism properties. The test aimed at verification and confirmation of potential cidal properties with reference to *Escherichia coli*, *Staphylococcus aureus* bacteria.

2.1 Methodology of the performance

a) The principle of the method according to ISO 22196:2011

Defining the anti-microorganism effect of the samples. The samples supplied by the Client, not containing the antibacterial admixture were applied as the control samples.

b) Characteristics of the tested samples

50x50 mm plastic plates covered with paint. Additionally, the Client supplied control samples of the same size.

c) Characteristics of the covering layer „*cover film*"

Sterile Stomacher bags were used in the test, made of 0,07 mm thick polyethylene, from which were carved out 40mm x 40mm squares.

d) Test organisms purchased at the Biomedica company

a) *Staphylococcus aureus* (ATCC 6538P)
b) *Escherichia coli* (ATCC 8739)

e) Volume of the inoculum

The volume of the microorganism inoculum was 0,4 ml

f) General number of living microorganism cells in the inoculum:

- *Escherichia coli* (ATCC 8739) – 2,5 x 10^5 cfu/ml
- *Staphylococcus aureus* (ATCC 6538) – 8,8 x 10^5 cfu/ml

3. RESULTS

a) Calculations of the anti-microorganism activity (R)

Table 1. Anti-microorganism activity (R) of the samples: **AIR-FLEX TUBE** towards the ***Escherichia coli* (ATCC 8739)*** strain.

The results obtained for the tested and control sample									
Determined parameter	**Control sample directly after inoculation**			**Control sample after 24 h**			**tested sample after 24 h**		
The number of living bacteria cells units/cm^2	$1,7x10^4$	$1,6x10^4$	$1,7x10^4$	$5,4x10^7$	$4,6x10^7$	$4,7x10^7$	$1,9x10^7$	$1,8x10^7$	$2,1x10^7$
Decimal logarithm of the number of living microorganism cells	4,23	4,20	4,23	7,73	7,66	7,67	7,28	7,26	7,32
The average	**U_o = 4,22**			**U_t = 7,69**			**A_t =7,29**		

*** photographs and graph in the appendix No.1 (photo. A) and 2**

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$$

$$\underline{R = 7,69 - 7,29 = 0,40}$$

Table 2. Anti-microorganism activity (R) of the samples **AIR-FLEX TUBE** towards the ***Staphylococcus aureus* (ATCC 6538P)* strain.**

The results obtained for the tested and control sample									
Determined parameter	**Control sample directly after inoculation**			**Control sample after 24 h**			**tested sample after 24 h**		
The number of living bacteria cells units/cm^2	$1,8x10^4$	$1,7x10^4$	$1,7x10^4$	$3,0x10^6$	$2,4x10^6$	$2,3x10^6$	$2,1x10^5$	$2,2x10^5$	$2,3x10^5$
Decimal logarithm of the number of living microorganism cells	4,26	4,20	4,23	6,48	6,38	6,36	5,32	5,34	5,36
The average	**U_o = 4,24**			**U_t = 6,41**			**A_t =5,34**		

*** photographs and graph in the appendix No.1 (photo. B) and 2**

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t$$

$$\underline{R = 6,41\text{-}5,34\text{=}1,07}$$

Explanation:

R – anti-microorganism activity

U_o – the average from the decimal logarithm of the number of living microorganisms (units/cm^2) covering the control working sample directly after inoculation;

U_t – the average from the decimal logarithm of the number of living microorganisms (units/cm2) covering the control working sample after 24 hours;

A_t – the average from the decimal logarithm of the number of living microorganisms (units/cm2) covering the tested working sample after 24 hours;.

b) validation conditions

1. The value of logarithm of the number of living microorganism cells determined directly after inoculation on the control working sample should meet the following requirements:

$$\underline{(L_{max} - L_{min}) / L_{mean} \leq 0{,}2}$$

Explanation:

L_{max} – decimal logarithm of the highest number of living microorganism cells found on the control working sample;

L_{min} – decimal logarithm of the lowest number of living microorganism cells found on the control working sample;

L_{mean} – decimal logarithm of the average number of living microorganism cells found on the control working sample.

Obtained results:

(4,23-4,20) / 4,22 = 0,01 → value for *Escherichia coli* meets the ≤ 0,2 condition

(4,26-4,23) / 4,24 = 0,01→ value for *Staphylococcus aureus* meets the ≤ 0,2 condition

2. The average number of living microorganism cells determined directly after inoculation on the control working sample should fall within the range **from 6,2 x 10^3 units/cm^2 to 2,5 x 10^4 units/cm^2.**

Obtained results:

average N = 1,7 x 10^4 cfu/ cm^2 → value for *Escherichia coli*

average N = 1,7 x 10^3 cfu/cm^2 → value for *Staphylococcus aureus*

3. The number of living microorganism cells determined from each control working sample after 24h from incubation should be **not lower than 6,2 x 10^1 units/cm^2.**

Obtained results

5,7x10^7 cfu/ cm^2; 4,6x10^7 cfu/ cm^2; 4,7x10^7 cfu/ cm^2→ values for *Escherichia coli* (Tab.1)

3,0x10^6 cfu/ cm^2; 2,4x10^6 cfu/ cm^2; 2,3x10^6 cfu / cm^2→ values for *Staphylococcus aureus* (Tab.2)

All validation conditions of the performed test have been fulfilled.

4. TEST CONCLUSIONS

Anti-microorganism activity (R) of the samples submitted and delivered for the tests by the Client: **AIR-FLEX TUBE** amounts to:

for *Escherichia coli* (ATCC 8739) - 0,40

for *Staphylococcus aureus* (ATCC 6538P) - 1,07

Appendix No. 1. Exemplary photographs illustrating the anti-microorganism activity of the tested sample compared to the effect of the control sample (without the anti-microorganism agent) after 24 h of contact with the strain.

Photo. A – the photograph of reduction of cells of the Escherichia coli strain (ATCC 8739) on the MacConkey Agar substrate

Control sample
(10^{-5} dilution)

Tested sample
(10^{-5} dilution)

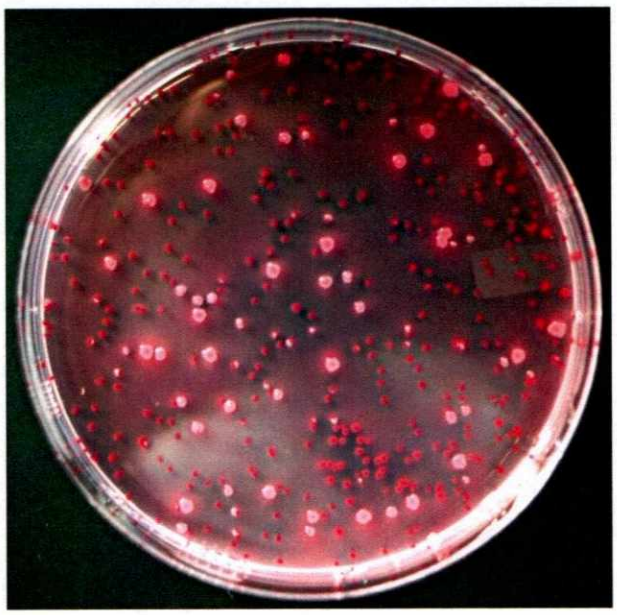
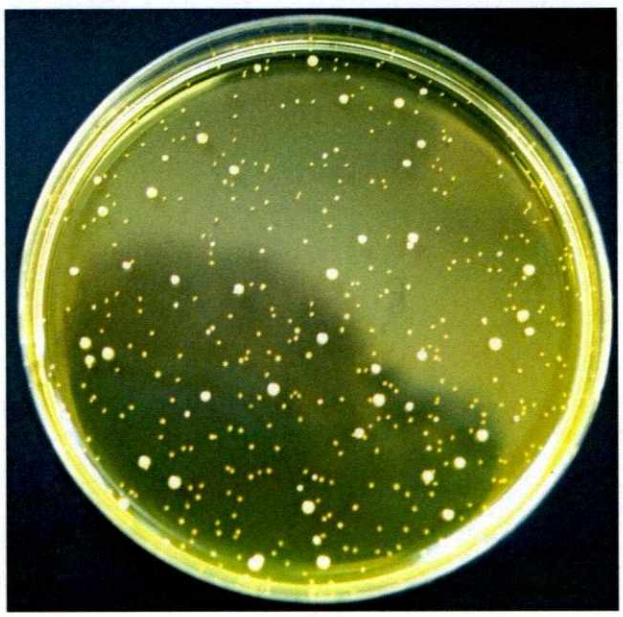
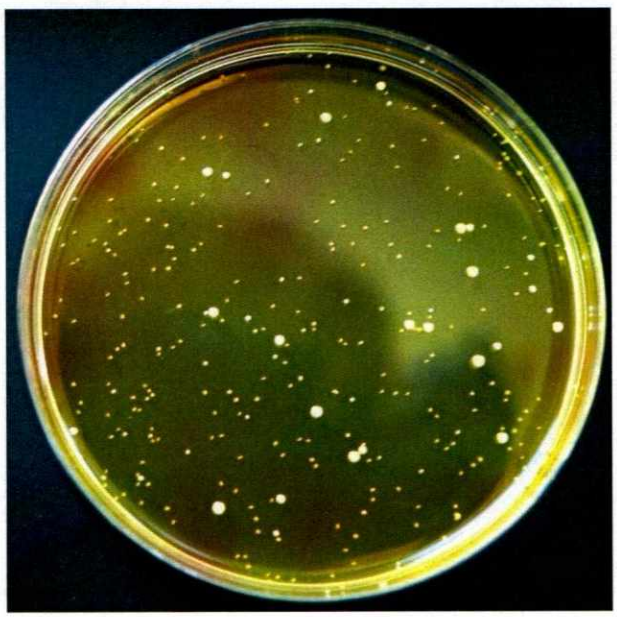


Photo. B – the photograph of reduction of cells of the Staphylococcus aureus (ATCC 6538) strain on the Mannitol Salt Agar substrate.

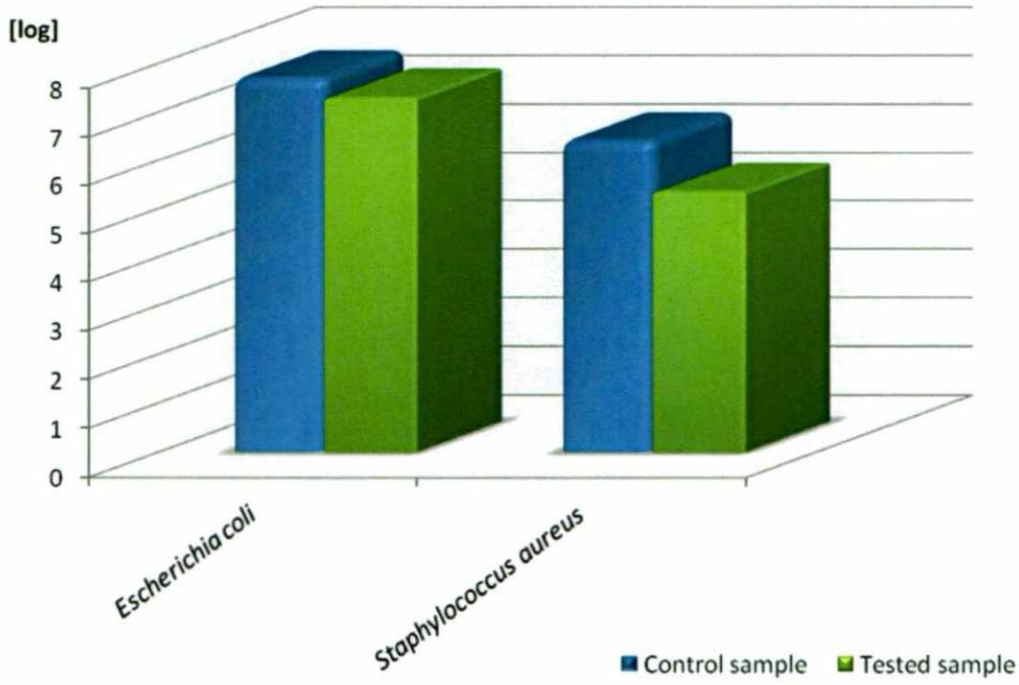
Control sample
(10^{-4} dilution)



Tested sample
(10^{-4} dilution)



Appendix No. 2. The graph illustrating the mean logarithm of the number of living microorganism cells on the control sample and sample tested after 24 hour contact.



Surname and signature of the person/ persons performing the test

Specjalistyczne Laboratorium Badawcze
ita-test
mgr Karolina ZIMAKOWSKA
mikrobiolog

Declaration of the test's supervisor:

I declare, that the tests were performed in accordance with the rules of Good Laboratory Practice and the finishing report is consistent with the source data.

Date and signature of the Test's Supervisor

Specjalistyczne Laboratorium Badawcze
ita-test
dr inż. Joanna ROMAN
Kierownik Pracowni Badań mikrobiologicznych

11.06.2015

The report has been drawn up in 3 identical copies.
The report receive :
Copy No. 1 and No. 2 – The Client
Copy No. 3 – Archives of the Specialized Testing Laboratory „ITA – TEST".

The samples for microbiological test and water samples are not archived, and are liquidated after 7 days from finishing the test.

The test results apply exclusively to the tested sample.
END OF REPORT

ita-test laboratorium
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Warsaw, 11.06.2015

Z.P.T.S. INGREMIO-PESZEL

ul. Laskowska 93, 32-329 Bolesław

We present the summary of results of percentage reduction of living microorganism cells obtained for the plates **AIR-FLEX TUBE**

At the same time we inform, that, the values presented below were calculated from the results obtained on the basis of the 22196:2011 norm *"Plastics – Measurement of antibacterial activity on plastics surfaces"*. The calculated reduction in the number of living microorganisms refers to the results obtained after 24h in the case of control plates, which constituted 100% of the number of bacteria.

Percentage reduction in the number of living microorganism cells [%]		
Tested plate	***Escherichia coli*** **(ATCC 8739)**	***Staphylococcus aureus*** **(ATCC 6538P)**
AIR-FLEX TUBE	**61%**	**92%**

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A SHORTENED TEST CERTIFICATE

No. B- 43232/24375/15

On the basis of the tests carried out at the SLB ITA-TEST in Warsaw ul. Obozowa 82a between 07.04.2015 – 24.04.2015, it has been stated, that:

AIR-FLEX TUBE shows antibacterial properties, defined on the basis of the norm:
ISO 22196 *Plastics – Measurement of antibacterial activity on plastics surfaces.*

Antibacterial activity (R) amounts to:

for *Escherichia coli* (ATCC 8739) - 0,40

for *Staphylococcus aureus* (ATCC 6538P) - 1,07

The test was performed by

Specjalistyczne Laboratorium Badawcze
ita-test
mgr Karolina ZIMAKOWSKA
mikrobiolog

Test supervisor

Specjalistyczne Laboratorium Badawcze
ita-test
dr inż. Joanna ROMAN
Kierownik Pracowni badań mikrobiologicznych

Warsaw, June 11, 2015