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Your notice of	Your reference	Date
07-05-2020	SSIBCC 2003641 ANTI BACTERIA	18-06-2020

Analysis Report 20.02720.01

Required tests :

	JIS Z 2801 - amendment 1 (2012)	Assessment of the antibacterial activity using the "Film contact" method

Identification number	Information given by the client	Date of receipt
T2009902	J3	07-05-2020
T2009903	G0	07-05-2020
T2009904	X51	07-05-2020

Sylvie Niessen
Order responsible

Reference: **T2009902 - J3**
                T2009903 - G0
                T2009904 - X51

Assessment of the antibacterial activity using the "Film contact" method

Date of ending the test   13-06-2020
Standard used   JIS Z 2801 - amendment 1 (2012)

1. Method

JIZ Z2801 : Antibacterial products – Test for antibacterial activity and efficacy.

Method and principle of the test:

Treated samples and untreated samples are cut into squares and placed in a Petri dish after being "cleaned".
Six untreated samples in individual Petri dish plus three test samples constitute one test.
Each sample is inoculated with 400 µl of a bacterial suspension adjusted to $\pm\,2.5\ 10^5 - 1.0\ 10^6$ CFU /ml with a 500 times diluted Nutrient Broth.
Then. a cover "film" is put on the surface of the inoculated sample. That film takes in "sandwich" the bacteria and ensures the continuous contact of the bacteria with the sample throughout the incubation
Directly after inoculation (0 contact time). an extraction of the bacteria presents on 3 of the 6 untreated samples is done by using a neutralizing solution. A counting on that solution is then carried out by plate count method.
Other samples are incubated at 37 °C and ≥ 90% R.H. for 24 hours.
After incubation the extraction and the measurement of the number of viable cells still present on the remaining samples (3 treated and 3 untreated) are made in the same way as it is done at 0 contact time.

Strains mentioned in the standard:
Escherichia coli ATCC 8739
Staphylococcus aureus ATCC 6538

Conditions for a valid test

The test is considered as valid if the 3 following conditions are satisfied:

The logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement:

$$(L_{Max} - L_{Min})\,/\,L_{Mean} \le 0.2$$

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where. L_{Max} : is the common logarithm of the maximum number of viable bacteria found on a specimen

L_{Min} : is the common logarithm of the minimum number of viable bacteria found on a specimen

L_{Mean} : is the common logarithm of the mean number of viable bacteria found on a specimen

The average number of viable bacteria recovered immediately after inoculation from the untreated test specimen shall be within the range $6.2 \; 10^3$ cells/cm^2 to $2.5 \; 10^4$ cells/cm^2.

The number of viable bacteria recovered from each untreated test specimen after incubation for 24 h shall not be less than $6.2 \; 10^1$ cells/cm^2.

The calculation of the activity values is obtained according to the following formula :

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

Where.   R: is the antibacterial activity

U_0: is the average of the common logarithm of the number of viable bacteria. in cells/cm^2. recovered from the untreated test specimens immediately after inoculation

U_t: is the average of the common logarithm of the number of viable bacteria. in cells/cm^2. recovered from the untreated test specimens after 24 h

A_t: is the average of the common logarithm of the number of viable bacteria. in cells/cm^2. recovered from the treated test specimens after 24 h

2. Results

Technical data:
- Sample: Dimensions: 5 cm x 5 cm
- Side in contact with the bacteria : **side without the adhesive**
- Cover « Film »:   Sterile PE film (cut in a "stomacher" bag)
                 Dimensions: 4 cm x 4 cm
                 Thickness: 0.07 mm
- Cleaning of the samples: Samples have been rubbed gently. 2 to 3 times with cotton wool soaked in an ethanol-water mixture. in the proportion. by mass. of 70:30 and dried.
- Tested strains :   *Staphylococcus aureus ATCC 6538*
                 Escherichia coli ATCC 8739
- Bacterial suspension volume put down on the samples: 400 µl
- Neutralizing solution volume used: 10 ml *(SCDLP broth)*
- Contact time used: 24 hours at 37°C and ≥ 90% relative humidity
- Microbiological technique used for the determination of the viable cells: count of number of

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colonies on Petri dishes of dilution series

Important remark :
The customer has not supplied an untreated control sample. An internal Centexbel control (of known behaviour) has been assessed in parallel in order to validate the test conditions.
It is the same PE as the PE used as cover film.

2.1 Results obtained with Staphylococcus aureus ATCC 6538

Conditions for a valid test

Table 1: Control of the behaviour of the strain on the Internal Centexbel control

Inoculum concentration: 6.5 10^5 CFU/ml

Sample identification	Trial	0 contact time Number of viable cells/ cm^2		24 hours contact time Number of viable cells/ cm^2	
		CFU/cm^2	Log CFU/cm^2	CFU/cm^2	Log CFU/cm^2
Internal Centexbel control *PE*	1	2.0 10^4	4.30	4.8 10^2	2.68
	2	2.3 10^4	4.36	1.1 10^3	3.05
	3	2.0 10^4	4.30	/*	/*
Averages		*2.1 10^4*	*4.32 = U_0*	*8.0 10^2*	*2.87 = U_t*
(L_{Max} - L_{Min}) / L_{Mean}			**0.01**		

*Result non available due to the presence of contaminations

Cover film surface : 16 cm^2

Validity of the test:
The test can be considered as valid. All the conditions are fulfilled.

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Evaluation of the antibacterial activity

Table 2: Antibacterial activity (R) of the samples with *Staphylococcus aureus* compared to the Internal Centexbel control

Inoculum concentration: 6.5 10^5 CFU/ml

Sample identification	Trial	0 contact time Number of viable cells/cm²		24 hours contact time Number of viable cells/cm²		Final evaluation $R = U_t - A_t$
		CFU/cm²	Log CFU/cm²	CFU/cm²	Log CFU/cm²	
T2009902	1			1.2 10^3	3.07	
	2			5.0 10^2	2.70	
	3			8.8 10^2	2.94	
Averages				*8.5 10^2*	*2.90 = A_t*	***-0.03***
T2009903	1			1.1 10^3	3.05	
	2			1.9 10^2	2.29	
	3			2.3 10^2	2.36	
Averages				*5.2 10^2*	*2.57 = A_t*	***0.30***
T2009904	1			4.4 10^2	2.65	
	2			6.3 10^1	1.80	
	3			8.8 10^2	2.94	
Averages				*4.6 10^2*	*2.46 = A_t*	***0.41***

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2.2 Results obtained with Escherichia coli ATCC 8739

Conditions for a valid test

<u>Table 3</u>: Control of the behaviour of the strain on the Internal Centexbel control

Inoculum concentration: 4.1 10^5 CFU/ml

Sample identification	Trial	0 contact time Number of viable cells/ cm^2		24 hours contact time Number of viable cells/ cm^2	
		CFU/cm^2	Log CFU/cm^2	CFU/cm^2	Log CFU/cm^2
Internal Centexbel control PE	1	1.1 10^4	4.03	6.9 10^4	4.84
	2	1.2 10^4	4.07	2.0 10^5	5.30
	3	9.4 10^3	3.97	1.9 10^5	5.29
Averages		*1.1 10^4*	*4.02 = U$_0$*	*1.5 10^5*	*5.14 = U$_t$*
(L$_{Max}$ - L$_{Min}$) / L$_{Mean}$			**0.02**		

Cover film surface : 16 cm^2

Validity of the test:
The test can be considered as valid. All the conditions are fulfilled.

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INRICHTING ERKEND BIJ TOEPASSING VAN DE BESLUITWET VAN 30 JANUARI 1947 / ETABLISSEMENT RECONNU PAR APPLICATION DE L'ARRÊTÉ-LOI DU 30 JANVIER 1947

Evaluation of the antibacterial activity

<u>Table 4</u>: Antibacterial activity (R) of the samples with *Escherichia coli* <u>compared to the Internal Centexbel control</u>

Inoculum concentration: 4.1 10^5 CFU/ml

Sample identification	Trial	0 contact time Number of viable cells/cm²		24 hours contact time Number of viable cells/cm²		Final evaluation $R = U_t - A_t$
		CFU/cm²	*Log CFU/cm²*	*CFU/cm²*	*Log CFU/cm²*	
T2009902	1			$1.5\ 10^2$	2.18	
	2			$3.6\ 10^2$	2.56	
	3			$1.9\ 10^2$	2.27	
Averages				*$2.3\ 10^2$*	*2.34 = A_t*	**2.80**
T2009903	1			$1.8\ 10^4$	4.24	
	2			$1.5\ 10^4$	4.18	
	3			$1.7\ 10^4$	4.23	
Averages				*$1.6\ 10^4$*	*4.22 = A_t*	**0.92**
T2009904	1			$1.1\ 10^3$	3.05	
	2			$4.6\ 10^4$	4.66	
	3			$1.8\ 10^3$	3.24	
Averages				*$1.6\ 10^4$*	*3.65 = A_t*	**1.49**

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