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Your notice of	Your reference	Date
14-07-2016		15-09-2016

Analysis Report 16.03900.01

Required tests :

ISO 22196 (2011) **Assessment of the antibacterial activity using the "Film contact" method**

Identification number	Information given by the client	Date of receipt
T1614625	Marburger Hygiene Wall Covering article no. 1301 batch 5	14-07-2016
T1614631	Internal Centexbel control	14-07-2016

Yvette Rogister

Order responsible

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Reference: **T1614625 - Marburger Hygiene Wall Covering article no. 1301 batch 5**
T1614631 - Internal Centexbel control

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

Date of ending the test 19-08-2016
Standard used ISO 22196 (2011)

1. Method

ISO 22196 : 2011 : Measurement of antibacterial activity on plastic surfaces or other non-porous surfaces.

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 ± 2.5-10 10^5 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 present 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. during 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 ATCC 8739
  Staphylococcus aureus ATCC 6538P

- **Conditions for a valid test**

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

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

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

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

2. 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.
3. 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 : Hygiene wall covering
  Dimensions : 5 cm x 5 cm
  Thickness : 3 mm
- 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.
- 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
- Tested strains : *Staphylococcus aureus ATCC 6538*
- Microbiological technique used for the determination of the viable cells: count of number of 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 evaluated in parallel in order to validate the test conditions.
It is the same PE as the PE used as cover film.

Staphylococcus aureus ATCC 6538

- **Conditions for a valid test**

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

Inoculum concentration : 5.8 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	1.6 10^4	4.21	6.9 10^2	2.84
	2	1.4 10^4	4.14	4.3 10^2	2.63
	3	1.8 10^4	4.24	6.9 10^2	2.84
Averages		***1.6 10^4***	***4.20***	***6.0 10^2***	***2.77***
(L_{Max} - L_{Min}) / L_{Mean}			***0.024***		

Cover film surface : 16 cm^2

Validity of the test :

1. At 0 contact time : **(L_{Max} - L_{Min}) / L_{Mean} ≤ 0.2 → OK**
2. At 0 contact time : **The average of CFU within the range 6.2 10^3 cells/cm^2 to 2.5 10^4 cells/cm^2 → OK**
3. After 24 hours : CFU number on the untreated samples ≥ **6.2 10^1 cells/cm^2**

Internal Centexbel control → OK

Conclusion :

- The Internal Centexbel control has a normal behaviour. The test is validated.

- **Evaluation of the antibacterial activity**

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

Inoculum concentration : 5.8 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***	
Internal Centexbel control PE	1	1.6 10^4	4.21	6.9 10^2	2.84	
	2	1.4 10^4	4.14	4.3 10^2	2.63	
	3	1.8 10^4	4.24	6.9 10^2	2.84	
Averages		***1.6 10^4***	***4.20 = U_0***	***6.0 10^2***	***2.77 = Ut***	
Treated Sample T1614625	1			< 1	0	
	2			< 1	0	
	3			< 1	0	
Averages				***< 1***	***0 = At***	***2.77***

< 1 signifies that no Staphylococcus aureus has been counted on the extraction liquid.

Performed in the microbiological lab under the responsibility of Yvette Rogister