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IAC-R08-0468

STUDY REPORT

Study Reference	2008-0468
Date	08/27/2008
Testing Facilities	Laboratoire Intertek, Route de Demigny, 71102 Chalon sur Saône cedex
Project Leader	Soria Hamdaoui
Study Supervisor	Christian Gimenez
Starting Date of Study	08/18/2008
Ending Date of Study	08/20/2008

STUDY PROTOCOL

The aim is to evaluate the antimicrobial activity of a silver composed product
For that, a quantification of this activity was done with the ability of this support to reduce a concentration of bacteria.
This protocol is made according to the Japanese Method JIS Z 2801: 2000. For all samples, including the negative control, three replicate were used.

References of the samples are written in tables of results

1. Materials and Methods

The method deals with antimicrobial testing of samples according the quantitative Japanese method JIS Z 2801 :2000, used in the Intertek laboratory under the reference MICRBIO-MO-016.

1.1. Preparation of test pieces

The negative samples and the antimicrobial samples of 1 cm x 1 cm coupons were deposit on sterile 90mm-diameter Petri dishes.
The sample used is:

- Fresha Tank

1.2. Preparation of the suspension of bacteria

A suspension of *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 were cultivated 1 to 2 days before in Trypticase Soya medium (Biomérieux, France). After this incubation, a concentration of 7 10^7 to 10^8 UFC/ml is prepared for the suspension test in a physiological buffer. These suspensions were also controlled by culture to determinate the exact concentration.

1.3. Inoculation of samples

Sterile samples were aseptically inoculated with 100 µl of the suspension test. Droplets were covered by an aluminium film. All the samples were deposit in a box containing water to maintain the humidity and incubated 37°C +/- 1°C during each time point: 6h and 24h for *Pseudomonas aeruginosa* and 24h for *eMRSA* and *E.coli*.

1.4. Washing out the test bacteria inoculated

Samples were placed in sterilized tubes and vortexed during 1 minute in 10 ml of peptone-phosphate-buffered solution (Fischer Bioblock, France) supplemented with 1% of polysorbate 80, to remove bacteria from the surfaces.
An additional step of sonication during 20 seconds was done for Pseudomonas aeruginosa due to a hign adherence of bacteria on the support.
Serial 10-fold dilutions were made in a physiological buffer to determine the total viable count after each time point.

1.5. Plate culture method

100 µl of each washings and 10-fold serial dilutions (10^{-1} to 10^{-3}) were inoculated on TSA medium (Biomérieux, France) in Petri dishes and spread with sterile spreaders.
All dishes are incubated at 30°C+/- 2°C during 48-72 hours and the number of CFU (Colony Formant Unit) per coupon was counted and the means were calculated.

2. Results and statistical analysis

According to the JIS Z 2801 regulatory, the value of antimicrobial efficacy obtained by the testing method shall not be less than 2.0 log of reduction of the bacterial number.
Each test was carried out independently three times to be statistically relevant. Data are expressed as the means +/- SEM (standard error of the means).

Applied formula: R = Log (N / Na)

N: Concentration of bacteria inoculated with the sample
Na: Concentration of removed bacteria after the time contact at 30°C, 40°C
R: Log Reduction obtained in the assay conditions

3. Results

Strains	Bacterial Suspension UFC/ml	Product: Fresha Tank Bacteria after 24h contact
S.aureus **UFC/ml**	(10^{-5}): =44; =40 **N: 4,2 x 10^{7}**	(10^{0}): =0 =0 = 0 **Na: 1,0 10^{1}** **R: 6,62**
E.coli **UFC/ml**	(10^{-5}): =54; =58 **N: 6,0 x 10^{7}**	(10^{0}): =0 =0 = 0 **Na: 1,0 10^{1}** **R: 6,62**

4. Conclusion

Data from this study demonstrate that the viability of bacteria could be affected by different parameters such as a high stress, the porosity of the material, the natural desiccation of suspensions, the antimicrobial disposition at the surface, and the presence of surfactants.
All these parameters have to be controlled as seriously as possible to determine the real antimicrobial activity of each sample.
These results would show important decrease of all bacteria tested in contact with the product at 24h time contact.
To conclude, the inhibitory effects are an intrinsic property of the material.

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