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### **Test for antimicrobial effect of the BioGuardian seal from Garlock GmbH (Neuss)**

The "BioGuardian" sealing profiles are active materials which are intended to achieve a biocidal effect against microorganisms through the incorporation of silver ions (Ag<sup>2+</sup>). In the case of silver ions, the biocidal effectiveness can refer to bacterial pathogens (antibacterial) and fungi (antifungal). Here it must be distinguished whether inhibition with significant germ killing (for example, bactericidal effect) or with a growth-inhibiting (for example, bacteriostatic) effect is present. In general for products which are produced for low germ count environments in the food or pharmaceutical industry, biostatic growth inhibition is desired and sufficient. For plastic surfaces in contact with food for a longer periods, i.e. several days, measurable reductions of the microbial counts of 3 log<sub>10</sub> levels (=99.9%) are required with reference to a contact time of 24 hours.

The test took place in suspension and/or as a challenge test. This enabled quantitative statements on the reduction of the applied gram negative and gram positive bacteria or fungi. In the procedure, suspensions of specific microbes at defined concentrations were applied to the materials to be tested, and the microbial count was determined after a dwell time of 1 minute and after 24 hours incubation in the incubator at 37°C (or 30 °C for fungi). The difference constitutes the log<sub>10</sub> reduction rate.

In this test the procedure from the Japanese standard JIS L 1902:1998 and JIS L 1902:2002 was used. Tests according to this standard permit a specific statement regarding the antimicrobial effect of the material. Since the quantity of nutrient reserves present enables the microorganisms to resist the antimicrobial effect, in collaboration with several European institutes a modification to the method was made in which a mineral-based solution with less available substrate is used as a test medium. Furthermore, it was recommended from the other side to extend the contact time to 24 hours. The test here was carried out accordingly. In addition, a measurement was also made after 4 hours' contact time, in order to observe any short-term effects. All tests were repeated 5 times.

The results showed that the BioGuardian seal has different effects against the bacterial colonies and yeast *Candida albicans* tested. The values are shown in Table 1.

Table 1: Bio Guardian seal, microbial count determination by modified JIS L 1902:1998, 2002

Species	24 hr control	CFU/ml 60 sec.	CFU/ml 4 hr	CFU/ml 24 hr
Gram-positive cocci				
<i>Enterococcus faecalis</i>	1 x 10 <sup>4</sup>	0.7 x 10 <sup>4</sup>	2 x 10 <sup>2</sup>	1 x 10 <sup>1</sup>
<i>Enterococcus faecium</i> (VRE)	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	0.1 x 10 <sup>2</sup>	0
<i>Staphylococcus aureus</i> (MRSA)	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	0.7 x 10 <sup>4</sup>	0
Gram-negative enterobacteria				
<i>Escherichia coli</i>	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	1 x 10 <sup>3</sup>	0
<i>Klebsiella pneumoniae</i>	1 x 10 <sup>4</sup>	1.3 x 10 <sup>4</sup>	0.8 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>
<i>Citrobacter freundii</i>	8x 10 <sup>4</sup>	4 x 10 <sup>4</sup>	1.5 x 10 <sup>4</sup>	1.2 x 10 <sup>4</sup>
Gram-negative non-fermenters				
<i>Pseudomonas aeruginosa</i>	1 x 10 <sup>4</sup>	1 x 10 <sup>3</sup>	0.5 x 10 <sup>3</sup>	0.5 x 10 <sup>3</sup>
<i>Acinetobacter baumannii</i>	5x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	0.6 x 10 <sup>4</sup>	2.5 x 10 <sup>2</sup>
Yeasts				
<i>Candida albicans</i>	5x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	2.3 x 10 <sup>3</sup>	0

This results in the following reduction rates:

Table 2: BioGuardian reduction rates

Reduction in log10 levels	
Gram-positive cocci	
<i>Enterococcus faecalis</i>	3
<i>Enterococcus faecium</i> (VRE)	4
<i>Staphylococcus aureus</i> (MRSA)	4
Gram-negative enterobacteria	
<i>Escherichia coli</i>	4
<i>Klebsiella pneumoniae</i>	0
<i>Citrobacter freundii</i>	0
Gram-negative non-fermenters	
<i>Pseudomonas aeruginosa</i>	0
<i>Acinetobacter baumannii</i>	2
Yeasts	
<i>Candida albicans</i>	4

From the data it can be concluded that the sealing material meets the requirements profile for the food industry very well with reduction rates of three to four log10 levels for the tested species *Enterococcus faecalis* (including VRE), *Staphylococcus aureus* (MRSA here), *Escherichia coli* and the yeast *Candida albicans*. A lesser, but still acceptable effect was observed versus *Acinetobacter baumannii* and no effect with the two enterobacteria *Citrobacter friends* and *Klebsiella pneumoniae*.

The values from the tests with four hour incubation show that the fast effect on the two enterococci types (wild type and VRE) and on *Candida albicans* are attributable to a direct biocidal effect of the silver on these strains. With the other strains, the die-off kinetics took effect later.

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