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# Testing of the direct antimicrobial efficiency of Bacoban® (water-based, 3 %) on glazed tiles after evaporation by an evaporator

Yr. Order from: 10.12.2010

IBFE-Test-No.: 1241210

Start of testing: 23.12.2010

End of testing: 28.12.2010







Geschäftsführung: Holger Graf
Registergericht: HR Saarbrücken B 14445 | USt-Id. Nr.: DE 232594386 | Steuer-Nr.: 075/111/00571
Bankverbindung: Kreissparkasse Saarpfalz | BLZ: 594 500 10 | Konto: 1011036447

Deutscher Akkreditierungs Rat
DAC-PL-0361-05-00

## *I. Materials*

The experiments were carried out in a room with a volume of about 57 $m^3$. Bacoban® (water-based, 3 %) was dispersed by help of an evaporator from Adexano following the instructions of the manufacturer.

Materials to be tested:

Adexano GmbH, Neunkirchen, provided the evaporator and Bacoban® (water-based, 3 %). IBFE GmbH provided glazed tiles as test surfaces.

Test organism:

*Staphylococcus aureus* ATCC 6538 from the DSMZ (German Collection of Microorganisms and Cell cultures), Braunschweig, was used for the tests.

## *II. Experiments and Results*

In order to determine the direct antimicrobial efficiency of evaporated Bacoban® (water-based, 3 %) on *Staphylococcus aureus*, glazed tiles (3 x 3 cm) were disinfected with 70 % ethanol. The glazed tile surfaces were artificially contaminated in a parallel each with 50 µl of a cell suspension of *Staphylococcus aureus*; table 1 shows the used living cell count. After surface-drying of the cell suspension the contaminated tiles were positioned at different places in the room, in a distance of approximately 1.0 m from the evaporator:

- Floor
- Table (height approximately 90 cm)
- At a height of approximately 200 cm
- Rack in an open cupboard (height approximately 70 cm)

The tiles determined as control tiles were not positioned in the examination room.





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Due to the volume of the room of 57 $m^3$ the evaporator was set to a treatable room volume of 60 $m^3$ and was then started following the instructions of the manufacturer. After the evaporation of Bacoban® (water-based, 3 %) by the help of an evaporator and a waiting period of 10 minutes, the so treated tiles were brought into sterile beakers. The viable cells were recovered from the tiles by shaking the tiles several times in 20 ml of peptone water. Afterwards, the still viable cells were determined by help of dilutions between $10^0$ und $10^{-4}$ of which 0.1 ml were applied on casein-soy-agar plates and then incubated for 24 to 48 hours at 37°C.

**Table 1:** Cell suspension used for the experiments

| Test organism | Living cell count / ml |
|---|---|
| *Staphylococcus aureus* | $1.35 \times 10^9$ |

Table 2 and 3 show the recovered viable cells from the differently positioned tiles after treatment with Bacoban® (water-based, 3 %) by the help of an evaporator.

Table 4 shows the recovered viable cells from the differently positioned tiles after treatment with Bacoban® (water-based, 3 %) by the help of an evaporator in relation to the applied microorganisms ($6.75 \times 10^7$) or respectively in relation to the control tiles. So it becomes evident that the evaporation of Bacoban® (water-based, 3 %) leads to an inactivation of microorganisms of > 99.58 %.





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# Testing of the antimicrobial long-term efficiency of Bacoban® (water-based, 3 %) on glazed tiles after evaporation by an evaporator according ASTM E 2180

| | |
|---|---|
| Yr. Order of: | 10.12.2010 |
| IBFE-Test-No.: | 1241210 a |
| Start of testing: | 23.12.2010 |
| End of testing: | 31.12.2010 |







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Deutscher Akkreditierungs Rat
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## I. *Materials*

The experiments were carried out in a room with a volume of about 57 $m^3$. Bacoban® (water-based, 3 %) was dispersed by the help of an evaporator from Adexano following the instructions of the manufacturer.

Materials to be tested:

Adexano GmbH, Neunkirchen, provided the evaporator and Bacoban® (water-based, 3 %). IBFE GmbH provided glazed tiles as test surfaces.

Test organism:

*Staphylococcus aureus* ATCC 6538 from the DSMZ (German Collection of Microorganisms and Cell cultures), Braunschweig, was used for the tests.

## II. *Experiments and Results*

In order to determine the antimicrobial long-term efficiency of evaporated Bacoban® (water-based, 3 %) on *Staphylococcus aureus,* glazed tiles (3 x 3 cm) were disinfected with 70 % ethanol an then positioned at different places in the room, in a distance of approximately 1.0 m from the evaporator:

- Floor
- Table (height approximately 90 cm)
- At a height of approximately 200 cm
- Rack in an open cupboard (height approximately 70 cm)

The tiles determined as control tiles were not positioned in the examination room.

Due to the volume of the room of 57 $m^3$ the evaporator was set to a treatable room volume of 60 $m^3$ and was then started following the instructions of the manufacturer. After the evaporation of Bacoban® (water-based, 3 %) by the help of an evaporator and a waiting period of 10 minutes, the so treated tiles were brought into sterile beakers and were, subsequently, stored for 5 days at room temperature.





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After this storing time, the tiles were contaminated on their glazed surface in three parallels with 100 µl of a cell suspension of *Staphylococcus aureus* as an agar film on each tile; table 1 shows the used living cell count. After surface-drying of the agar film (about 30 minutes at room temperature) the viable cells were recovered from the tiles by shaking the tiles several times in 20 ml of peptone water. Afterwards, the still viable cells were determined by help of dilutions between $10^0$ und $10^{-4}$ of which 0.1 ml were applied on casein-soy-agar plates and then incubated for 24 to 48 hours at 37°C.

**Table 1:** Cell suspension as agar film used for the experiments

| Test organism | Living cell count / ml |
|---|---|
| *Staphylococcus aureus* | $3.8 \times 10^8$ |

Table 2 and 3 show the recovered viable cells from the differently positioned tiles after treatment with evaporated Bacoban® (water-based, 3 %) by the help of an evaporator and a storing time of 5 days.

Table 4 shows the recovered viable cells from the differently positioned tiles after treatment with evaporated Bacoban® (water-based, 3 %) by the help of an evaporator and a 5 days storage in relation to the applied microorganisms ($3.8 \times 10^7$) or respectively in relation to the control tiles. So it becomes evident that the evaporation of Bacoban® (water-based, 3 %) and a following 5 days storage led to an inactivation of microorganisms of > 96.65 %.




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**Table 2:** Antimicrobial long-term efficiency of evaporated Bacoban® (water-based, 3 %) on *Staphylococcus aureus* after treatment of differently positioned tiles and a 5 days storage at room temperature and control tiles without any treatment;
cfu* of the used suspension: $3.8 \times 10^8$ / ml

| Position of tiles in the room with 5 days storage at room temperature | Dilution | Recovered microorganisms (cfu* / 100 µl) |
|---|---|---|
| Control (not stored in the room) | $10^{-4}$ | 21 / 32 / 23 |
| Floor | $10^{0}$ | 49 / 68 / 55 |
| Table | $10^{-2}$ | 23 / 32 / 41 |
| At a height of approximately 200 cm | $10^{-2}$ | 19 / 17 / 21 |
| Rack in an open cupboard | $10^{-2}$ | 94 / 81 / 77 |

*cfu: colony forming unit

**Tabelle 3:** Antimicrobial long-term efficiency of evaporated Bacoban® (water-based, 3 %) on *Staphylococcus aureus* after treatment of differently positioned tiles and a 5 days storage at room temperature and control tiles without any treatment; (applied microorganisms: $3.8 \times 10^7$); average values from the parallels

| Position of tiles in the room with 5 days storage at room temperature | Recovered cfu* |
|---|---|
| Control (not stored in the room) | $5.01 \times 10^7$ |
| Floor | $1.15 \times 10^4$ |
| Table | $6.40 \times 10^5$ |
| At a height of approximately 200 cm | $3.80 \times 10^5$ |
| Rack in an open cupboard | $1.68 \times 10^6$ |

* cfu: colony forming unit




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**Table 4:** Recovered viable cells from differently positioned tiles after treatment with evaporated Bacoban® (water-based, 3 %) and a 5 days storage at room temperature in relation to the applied microorganisms ($3.8 \times 10^7$) and respectively in relation to the control tiles

| Position of tiles in the room with 5 days storage at room temperature | Cell recovery rate after 30 minutes in relation to the applied microorganisms | Cell recovery rate after 30 minutes in relation to the control tiles |
|---|---|---|
| Control (not stored in the room) | 131 % | 100 % |
| Floor | 0.03 % | 0.02 % |
| Table | 1.68 % | 1.28 % |
| At a height of approximately 200 cm | 1.00 % | 0.76 % |
| Rack in an open cupboard | 4.42 % | 3.35 % |

*cfu: colony forming unit

### *III. Conclusion*

In order to determine the antimicrobial long-term efficiency of evaporated Bacoban® (water-based, 3 %), glazed tiles were positioned at different places in the room (floor, table, at a height of approximately 200 cm, rack in an open cupboard); subsequently, Bacoban® (water-based, 3 %) was evaporated in the room by help of an evaporator, according to the instructions of the manufacturer (control tiles were not treated). Afterwards, the treated tiles were stored at room temperature for 5 days. The antimicrobial efficiency of the applied Bacoban® on *Staphylococcus aureus* was determined according ASTM E 2180 directly after surface-drying of the suspension in the agar film (after about 30 minutes). Independently of the position of the test surfaces in the room, the viable cells were reduced by at least 96.65 % in relation to the recovered viable cells from the non-treated control tiles (table 4).

Kirkel-Limbach, 20th January 2011

(Dipl.-Biol. E. -M. Schmees, Assistant head of laboratory)

Reviewed and approved

(Dr. Heiko Ewen, Head of laboratory / Quality control)





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