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Dr. Dr/bie-schu

EXPERT'S OPINION

2420 GD 14

for Messrs: Deutsche Steinzeug Cremer & Breuer AG  
Servaisstrasse  
53347 Alfter-Witterschlick / Germany

Product: HT processed file DSCB

The product distributed by the company mentioned above was examined according to ISO 22196:2011 for its antibacterial efficacy. As test germs *Staphylococcus aureus* and *Escherichia coli* were chosen. These organisms can cause infections in humans and animals. Especially in the clinical sector they are responsible for a variety of secondary infectious diseases.

The results are summarized in the test report 4480/8 dated 24 July 2013 of the company ISEGA Forschungs- und Untersuchungsgesellschaft Aschaffenburg, Germany.

After contact of the test germs for 24 h at 36 °C with the products mentioned above, a reduction of the germs of 99.99 % compared to the initial number of germs at the beginning of the test was detectable in case of all listed microorganisms.

The result verifies a very high antibacterial activity of the product against the tested germs.

This expert's opinion consists of 1 page.



  
(Dr. Biester)  
Dipl.-Biologist  
Microbiology



## Test report

### Photocatalytic degradation of bacterial on tiles with HT coating

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21st October 2013



### Summary

The photocatalytic activity regarding the degradation of bacteria was examined for tiles with HT coating compared to tiles without coating, in accordance with ISO standard 27447. The two bacterial strains were used: *staphylococcus aureus* (strain DSM 346) and *escherichia coli* (strain DSM 1576). They were irradiated for 4 hours.

The following performance characteristics have been calculated for the photocatalytic activity of coated tiles (table 1):

Table 1: Results of the photocatalytic activity

Sample	<i>Staphylococcus aureus</i> (strain DSM 346)		<i>Escherichia coli</i> (strain DSM 1576)	
	R <sub>L</sub>	ΔR	R <sub>L</sub>	ΔR
Coated tiles	3.2	0.1	4.8	1.1
	3.2 – 4.2 <sup>*)</sup>	0.1	3.8 – 4.8 <sup>*)</sup>	1.1

<sup>\*)</sup> Calculated taking into account that fact that the undiluted samples of the irradiated, uncoated tiles had not been examined.

R<sub>L</sub> = photocatalytic antibacterial activity after UV irradiation with light strength L

ΔR = photocatalytic antibacterial activity after UV irradiation taking into consideration the bacterial count of the plates incubated in the dark

The determination of the bacterial counts of the irradiated plates with coating was done in the first dilution stage of the extracted bacterial suspension. The bacterial counts in the non-diluted sample were not recorded. This resulted in a R<sub>L</sub> value for both types of bacteria in the region of 3. No bacteria could be detected on the plates in the first dilution stage. It cannot be ruled out that bacteria colonies would also have been detected if the undiluted suspensions had been plated, due to their strong antimicrobial activity. In this case this would result in a R<sub>L</sub> value of 4.2 or 4.8. The photocatalytic activity can therefore be characterised across the range stated in the table.

It has been determined that the coated test specimens showed a reduction in the bacterial count even without UV irradiation. Consequently the coating itself has an antibacterial effect. Differences between the bacterial strains have also been determined. The effect on *S. aureus* was stronger than on *E. coli*. *S. aureus* had already been completely inhibited due to the surface coating of the tiles, while on the other hand *E. coli* bacteria were still evident. The growth of *E. coli* was completely inhibited by the UV irradiation. The tiles thereby exhibited antibacterial photocatalytic activity in addition to their general antibacterial activity.

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## Literature

- 1) ISO 27447: Fine ceramics (advanced ceramics, advanced technical ceramics) – test method for antibacterial activity of semiconducting photocatalytic materials (2009)

The reported tests were performed in accordance with the accepted offer dated 07/08/2013. The evaluated data shown was recorded at the Fraunhofer Institute for Molecular Biology and Applied Ecology. The tests were performed in accordance with the methods described in this report.

Laboratory manager:

21/10/2013

Date



(Dr. Kerstin Hund-Rinke)

## **1 Background**

Photocatalysis is becoming increasingly interesting as a procedure for reducing the bacterial count on surfaces. ISO guidelines (ISO 27447) exist regarding the quantitative evidence of the effectiveness of the corresponding surfaces. This guideline covers the effect on pathogenic bacteria.

This procedure determines the antibacterial activity of the active photocatalytic material due to the contact of the test specimens with bacteria during UV irradiation. Depending on the test specimen material, two procedures are available. The film adhesion method is applied to examine plate-shaped materials, while on the other hand the glass adhesion method is used for textiles.

## **2 Materials and methods**

### **2.1 Test specimens**

Test specimens (tiles) both with coating and without coating) were supplied by Deutsche Steinzeug Cremer & Breuer AG. The dimensions were 5 x 5 cm. The test specimens had the following names:

- With HT coating) or
- Without coating respectively.

### **2.2 Test set-up**

Test apparatus was used in accordance with ISO standard 27447 [1]. The apparatus consisted of a light source under which the test specimens were placed in a chamber. The light strength was regulated using two perforated sheets which can be slid towards each other. The height of the perforated sheets compared to the test specimens was sufficient to prevent any shadowing, which would result in an unequal irradiation of the test specimens and therefore in a reduction in the photocatalytic effectiveness.

Light source: Cleo Performance 40 W-R (Philips);

Test device for detecting the intensity of the irradiation: LUTRON UV-340A (290-390 nm)

The test set-up can be seen in the annex in figure 2.

The spectrum of the fluorescent lamps used is depicted in the annex (figure 3).

### 2.3 Bacterial strains

The two test bacterial strains stated in the test guidelines for the film adhesion method were used.

- *Staphylococcus aureus* (strain DSM 346)
- *Escherichia coli* (strain DSM 1576)

The bacteria were obtained in August 2013 from the German Collection of Microorganisms (Braunschweig) as cryogenically preserved samples and were cultivated on agar plates using the nutrient agar specified in the guideline at  $37 \pm 1$  °C. The strains were passaged twice as soon as the test begun.

### 2.4 Test execution

The film adhesion method was used to examine the test specimens of Deutsche Steinzeug Cremer & Breuer AG. Nine uncoated test specimens and six coated test specimens were examined. The examination sketch can be seen below.

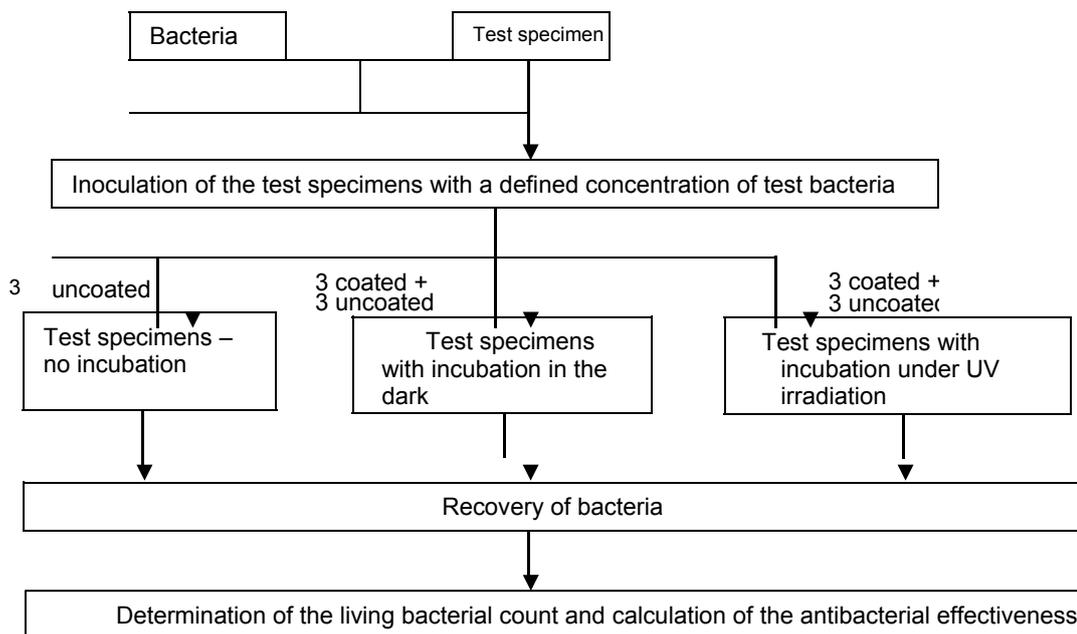


Figure 1: Workflow for the film adhesion method to determine the antibacterial effect of photocatalytic active test specimens

The test specimens were conditioned as soon as the test began. For this purpose they were washed with pure (reagent-grade) ethanol without any denaturing and stabilisation agents, irradiated with UVA ( $10 \text{ W/m}^2$  or  $1 \text{ mW/cm}^2$ , 20 h), rinsed with TOC-free ultrapure water and dried under the laminar airflow cabinet.

The test bacteria were mixed into the nutrient solution, the cell count was determined in a hemocytometer and the bacterial suspension was set to a value of approx.  $1.0 \times 10^6$ . 0.15 mL of the bacterial suspension was discarded per test specimen and was covered with an adhesive film (from Office Point Germany, no. 3400010-50, OPTEX GmbH, Berlin). The test specimens not used immediately to determine the bacterial count, were put into petri trays which were lined with moist filter paper and incubated as per figure 1. To determine the living bacterial count in the form of colony-forming units, the bacteria were extracted using a freshly-created sterile SCDLP medium in accordance with the standard, adding polysorbate 80 (2 g/L), and different dilution phases were created in the sterile physiological NaCl<sub>2</sub> solution. The bacterial count was examined at suitable dilution stages during the pour plate method.

## **2.5 Calculation of the photocatalytic antibacterial activity**

The photocatalytic activity of the test specimens was calculated as follows:

$$R_L = \log[B_L/C_L]$$

$R_L$  = Photocatalytic antibacterial activity after UV irradiation with light strength L

$B_L$  = Average number of living bacteria in the uncoated test specimens after UV irradiation with light strength L

$C_L$  = Average number of living bacteria in the coated test specimens after UV irradiation with light strength L

$$\Delta R = \log[B_L/C_L] - \log[B_D/C_D]$$

$\Delta R$  = Photocatalytic antibacterial activity after UV irradiation taking into consideration the bacterial count of the plates incubated in the dark

$B_D$  = Average number of living bacteria in the uncoated test specimens after incubation in the dark

$C_D$  = Average number of living bacteria in the coated test specimens after incubation in the dark

### 3 Results

In the examinations the conditions listed in table 2 were prevalent.

Table 2: Conditions during the examinations

Parameter	Actual condition in the test	Conditions according to the guideline
Light strength	0.25 mW/cm <sup>2</sup>	0.25 mW/cm <sup>2</sup>
Duration of light	4 h	4 – 8 h
Temperature during the test	22 °C	No information

On the basis of the cell counts determined during the dilution series, the following photocatalytic activity of the test specimens can be calculated:

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
R <sub>L</sub>	3.2	3.8
ΔR	0.1	1.1

The individual bacterial counts per plate can be seen in table 3 (*S. aureus*) and table 4 (*E. coli*).

The determination of the bacterial counts of the irradiated plates with coating was done in the first dilution stage of the extracted bacterial suspension. The bacterial counts in the non-diluted sample were not recorded. This resulted in a R<sub>L</sub> value in the region of 3. No bacteria could be detected on the plates in the first dilution stage. It cannot be ruled out that if the undiluted suspensions had been plated bacteria colonies would also have not been detected due to the strong antimicrobial activity. In this case this would result in a R<sub>L</sub> value of 4.2 or 4.8. The photocatalytic activity can therefore be characterised across the following range.

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
R <sub>L</sub>	3.2 – 4.2	3.8 – 4.8
ΔR	0.1	1.1

It can be determined that the test specimens with coating showed a reduction in the bacterial count even without UV irradiation. Consequently the coating itself has an antibacterial effect. The effect on *S. aureus* was stronger than on *E. coli*.

*S. aureus* had already been completely inhibited due to the surface coating of the tiles, while on the other hand *E. coli* bacteria were still evident. Due to the UV irradiation the growth of *E. coli* was also completely inhibited. The tiles thereby exhibited

antibacterial photocatalytic activity in addition to their general antibacterial activity. The control plates with coating showed comparable bacterial counts on the UV-irradiated as well as the non-irradiated samples and those stored in the dark, meaning that there was no incorrect measuring of the effect observed on the coated tiles.

#### 4 Validity

Apart from the bacterial count on the samples without coating before and after incubation, all the validity criteria were met. In the two tests the upper threshold of the bacterial count was  $4.0 \times 10^5$  (*S. aureus*:  $5.1 \times 10^5$ ; *E. coli*:  $1.1 \times 10^6$ ). As there was still a very high (*E. coli*) or complete elimination (*S. aureus*) of the inoculation suspension, despite the increased bacterial count, a repeat of the examination is not deemed necessary. The other validation criteria which allow direct conclusions to be drawn about the quality of the examination (the spread of the bacterial counts; the bacterial counts of the uncoated samples after exposure in light and darkness), have all been met properly.

- The spread of the bacterial counts of the samples without coating after inoculation:  $(L_{\max} - L_{\min}) / (L_{\text{mean}}) \leq 0.2$  (actual count: *S. aureus*: 0.176; *E. coli*: 0.169)  
 $L_{\max}$ : Logarithm for the maximum living bacterial count  
 $L_{\min}$ : Logarithm for the minimum living bacterial count  
 $L_{\text{mean}}$ : Logarithm for average of the living bacterial count
- Bacterial count of the samples without coating after inoculation:  $1.0 \times 10^5 - 4 \times 10^5$  (actual count: *S. aureus*:  $5.1 \times 10^5$ ; *E. coli*:  $1.1 \times 10^6$ )
- Bacterial count of the samples without coating after exposure to light:  $> 1.0 \times 10^3$  (actual count: *S. aureus*:  $1.8 \times 10^5$ ; *E. coli*:  $6.9 \times 10^5$ )
- Bacterial count of the samples without coating after exposure to darkness:  $> 1.0 \times 10^3$  (actual count: *S. aureus*:  $1.5 \times 10^5$ ; *E. coli*:  $8.4 \times 10^5$ )

**Annex**

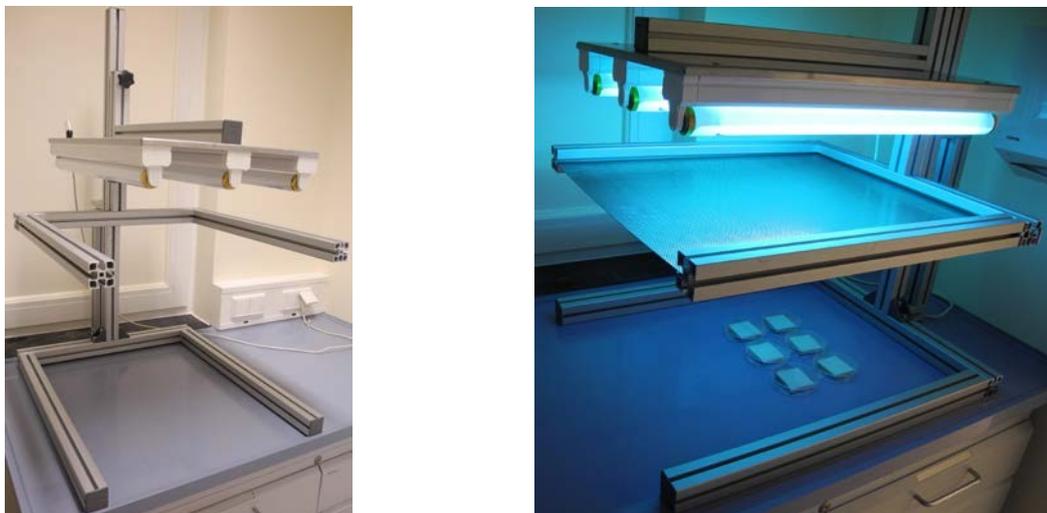


Figure 2: Pictures of the irradiation apparatus

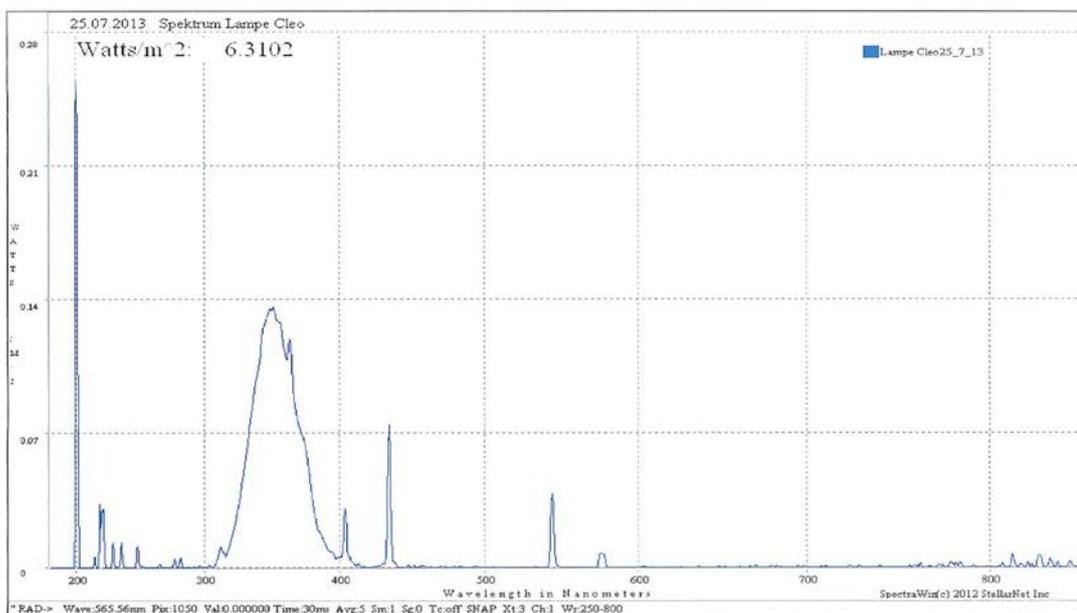


Figure 3: Spectral energy distribution of the light source used

Table 3: *Staphylococcus aureus* – Bacterial counts of the individual plates in the test

Incubation conditions	Sample	CfU/sample <sup>1</sup> sample 1	CfU/sample <sup>1</sup> sample 2	CfU/sample <sup>1</sup> sample 3	Average (CfU/sample) <sup>1</sup>	Standard deviation (CfU/sample) <sup>1</sup>	RL	ΔR
Control	Uncoated	500,000	525,000	490,000	505,000	18,027.8	3.2	0.1
UV [0.25 mW/cm <sup>2</sup> ]	Uncoated	173,000	176,000	182,500	177,167	4,856.3		
	Coated	100	100	100	100	0.0		
Dark	Uncoated	133,000	67,000	235,500	145,167	84,906.3		
	Coated	100	100	100	100	0.0		

<sup>1</sup> CfU = colony-forming units

Table 4: *Escherichia coli* – Bacterial counts of the individual plates in the test

Incubation conditions	Sample	CfU/sample <sup>1</sup> sample 1	CfU/sample <sup>1</sup> sample 2	CfU/sample <sup>1</sup> sample 3	Average (CfU/sample) <sup>1</sup>	Standard deviation (CfU/sample) <sup>1</sup>	RL	ΔR
Control	Uncoated	1,120,000	1,150,000	905,000	1,058,333	133,635.1	3.8	1.1
UV [0.25 mW/cm <sup>2</sup> ]	Uncoated	735,000	675,000	650,000	686,667	43,684.5		
	Coated	100	100	100	100	0.0		
Dark	Uncoated	975,000	870,000	675,000	840,000	152,233.4		
	Coated	2,600	700	950	1,417	1,032.4		

<sup>1</sup> CfU = colony-forming units