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Laboratory identification number	LI-B-023-007
Study Report	**Testing the bacterial-reduction performance of photodynamic technology against *Staphylococcus aureus* und *Acinetobacter baumannii***
Test	The validation of the anti-bacterial activity on a wood panel carrier coated with **eukula Vir-O-Bac für Holzböden + eukula care matt** using the photodynamic technology. Quantitative determination of the recovered bacterial inoculum according to the following measures: 1. U_t: Dark control, treated test specimen (+ dye / - light / + bacteria) 2. At: Test sample, treated test specimen (+ dye / + light / + bacteria) 3. $U_{0:}$ Recovery control
Sponsor	Dr. Schutz GmbH Steinbrinksweg 30 D-31840 Hessisch Oldendorf
Test method	Quantitative test on non-porous surfaces
Active substance	Singlet oxygen generated in situ from ambient air
Interfering substance	Not applicable

Storage conditions	20 °C, dry
Carrier material	wood panel “Buche Masuria”
	
Strain	***Staphylococcus aureus*:** ATCC 6538; DSM 799, ***Acinetobacter baumannii:*** ATCC 19606; DSM 30007.
Contact time	60 min
Project description	• Bacterial contamination of carriers (4 cm^2) coated with **eukula Vir-O-Bac für Holzböden + eukula care matt** coating • Microbial reduction via light emitting LEDs in combination with **wood panel** coated carrier
Reference documents	Modification of the following test methods: • EN 13697:2019-10 • ISO 22196:2011
	• **SOP-ST-MIK.M.0047** • **SOP-ST-MIK.M.0038**
Reference material	Test specimen without light exposure + bacteria - dye

Written	PD Dr. rer. nat. Maren Eggers	
Test facility	Labor Prof. Dr. G. Enders MVZ GbR Abteilung Technische und angewendete Hygiene Rosenbergstraße 85 70193 Stuttgart	
Dates	Begin of testing:	2023-01-11
	End of testing:	2023-01-13
Technical assistance	Lucija Štauduar Ena Obucic Neira Herenda	

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1. Materials, media and reagents

1.1. Abbreviations

ATCC	American Type Culture Collection
TSA	Trypton-Soja-Agar
CFU	Colony forming units
A. dest.	Deionized water (type 2)
RF	Reduction factor
Max	Maximum
Min	Minimum
RT	Room temperature
SOP	Standard Operating Procedure

1.2. Apparatus

- Incubator 36°C ± 2°C
- Fridge 2 - 8 °C
- Laminar Air Flow
- McFarland measuring device
- Vortexer
- Thermometer
- Pipetting aid (Pipet-Boy)
- 5 ml and 10 ml pipettes
- Eppendorf pipette variable 10 µl - 100 µl
- Eppendorf pipette variable 100 µl - 1000 µl
- sterile pipette tips (white)
- sterile disposable pipettes (1 ml, 5 ml, 10 ml)
  Falcon Test Tubes (50 ml)
- Tweezers
- Petri dishes in sizes from 90 mm to 100 mm
- Inoculation loop
- Glass pearls
- Stopwatch

1.3. Materials

- A. dest.	Deionized water (type 2)
- Tween 20	Polysorbate 20
- 70% EtOH	70% ethanol
- Müller-Hinton-Agar	Mueller Hinton Agar
- Verdünnungsmittel: 0.9% NaCl	diluent: 0.9% NaCl

2. Test methods

The tests were performed according to the modification of the EN 13697:2019-10 and ISO 22196:2019 test methods.

2.1. Test strain

To prepare a culture of the test bacteria, bacteria were transfered with a sterile inoculating loop onto TSA plates from the stock culture (incubation at 36 ± 2 °C for 18 to 24 h). From this culture, a sterile inoculating loop was used to transfer bacteria onto fresh Mueller Hinton plates (incubation at 36 ± 2 °C for 18 to 24 h).

2.2. Preparation of a standardized test suspension

The colony of the test bacteria were removed from the Mueller Hinton plates using a sterile inoculation loop and transfered to a tube with glass pearls and A.dest with 0.1% Tween20 and then vortex it. Set the required microbial density using the Mc-Farland measurement (CFU/ml ca. 10^8). The test suspension shall be CFU/ml ca. 10^8.

2.3. Test procedure

50 µl of test suspension was pipetted directly into 10 ml of diluent in order to calculate how much inoculum is recovered (t_0).

The precoated and control carriers were inoculated with 5 x 10 µl bacteria. The number of cells were set at approx. 10^8 (CFU/ml). They were left to dry in the dark at a temperature of 36 ± 2 °C for approximately 60 minutes until visible dry.

The test was performed in triplicate. Immediately after drying, the carriers for light treatment were placed on the sample tray. Then the irradiation by LED – modules was started according to following settings:

- Intensity: 4 mW / cm^2
- voltage: 25 V
- current intensity: 0.424 A

The carriers for the dark control were placed in dark at RT.

Immediately after the contact time (within 60 minutes), carriers were transferred into a 10 ml diluent. Then the carriers were afterwards thoroughly shaken for 60 s. Each sample with a carrier was diluted to 10^{-5} and spread on Mueller Hinton plates (2 x 0.5 ml). The Mueller Hinton plates were incubated at 36 ± 2 °C for 20 – 48 hours.

2.4. Calculation

All plates are counted and only the plates up to 330 colonies are documented. Plates with more than 330 colonies are to be documented with "∞".For calculation of the average bacterial count per test specimen, the following applies: maximum upper limit = 330 colonies; minimum lower limit = 14 colonies per plate.

If no colonies were recovered in any of the agar plates for a dilution series, then record the number of colonies counted as “< V” (where V is the volume, in ml, in which the carriers are transferred after contact time). For calculating the average when there are no viable bacteria recovered in a dilution series, consider the number of viable bacteria to be “V”.
(Example: In the case of V = 10 ml, the number used for calculating the average will be 10.)

The mean bacterial count of reference / light control / dark control / main sample in 1 ml plated volume was determined.

The antibacterial activity is calculated by the following formula

$$\mathbf{R = (U_t - U_0) - (A_t - U_0) = U_t - A_t}$$

where

U_0 is the average of the common logarithm of the number of viable bacteria, in $\log_{10}$ CFU/ml, 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 $\log_{10}$ CFU/ml, recovered from the untreated test specimens after contact time;

A_t is the average of the common logarithm of the number of viable bacteria, in $\log_{10}$ CFU/ml, recovered from the treated test specimens after contact time.

Pass criteria for the mean antibacterial activity according to Table B.2 ISO 22196:2011 shall be ≥ 1.72 ± 0.42.

3. Results and Evaluation

The bacterial activity of the wood panel surface coated with **eukula Vir-O-Bac für Holzböden + eukula care matt**, was tested by an inoculation of bacteria following an exposure time 60 minutes.

3.1. Validity of the test

The recovery control (recovery rate) showed that the test is valid since there were over 7 $\log_{10}$ bacteria in the inoculum, Table 1.
As shown in Table 2, the logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens meets the following requirement of ≤ 0.2 $\log_{10}$ for *Staphylococcus aureus* and *Acinetobacter baumannii*.

3.2. Test results

The data of the bacterial efficacy of light emitting LEDs in combination with **eukula Vir-O-Bac für Holzböden + eukula care matt** coated **wood panel** carrier is presented in Table 3.

The photodynamic inactivation of *Staphylococcus aureus* on wood panel carriers by the energy-rich singlet oxygen generated in the test procedure showed a reduction of 1.38 $\log_{10}$ (95.83%) within 60 minutes exposure time.

The photodynamic inactivation of *Acinetobacter baumannii* on wood panel carriers by the energy-rich singlet oxygen generated in the test procedure showed a reduction of 1.99 $\log_{10}$ (98.97%) within 60 minutes exposure time.

18.01.2023
Date

PD Dr. rer. nat. Maren Eggers
Head of disinfectant testing and applied / technical hygiene

Table 1 Recovery rate of test organism from the test specimen

S. aureus	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**	**R**
recovery control	0	7.13	7.05	7.22	**7.13**	0.09	-
dark control	60	7.09	7.12	7.11	**7.11**	0.02	0.03

A. baumannii	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**	**R**
recovery control	0	7.18	7.23	7.08	**7.16**	0.08	-
dark control	60	5.52	5.29	5.45	**5.42**	0.12	1.74

Table 2 Test validity (dark control) according to ISO 22196:2011

S. aureus	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**
L_{max}	60	7.09	7.12	7.11	**7.11**	0.02
L_{min}	60	7.11	7.23	7.15	**7.16**	0.06
L_{mean}	60	7.10	7.18	7.13	**7.14**	0.04
$(L_{max} - L_{min}) / L_{mean} \leq 0.2$	60	0.00	-0.02	-0.01	**-0.01**	0.01
pass criteria ≤ 0.2	60	valid	valid	valid	-	-

A. baumannii	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**
L_{max}	60	5.52	5.29	5.45	**5.42**	0.12
L_{min}	60	5.70	5.30	5.60	**5.53**	0.21
L_{mean}	60	5.61	5.30	5.53	**5.48**	0.16
$(L_{max} - L_{min}) / L_{mean} \leq 0.2$	60	-0.03	0.00	-0.03	**-0.02**	0.02
pass criteria ≤ 0.2	60	valid	valid	valid	-	-

L_{max} is the common logarithm (i.e. base 10 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 the specimens.

Table 3 **Antibacterial activity against *Staphylococcus aureus* and *Acinetobacter baumannii* on wood panel carrier coated with eukula Vir-O-Bac für Holzböden + eukula care matt, according to ISO 22196:2011**

Test organism: *S. aureus*

S. aureus	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**	R	Kill rate (%)
U_0	**0**	7.13	7.05	7.22	7.13	0.09	1.38	**95.83**
U_t	60	7.09	7.12	7.11	7.11	0.02		
A_t	60	5.73	5.65	5.80	5.73	0.08		

Test product	Calculation	R	Kill rate (%)	Activity
eukula Vir-O-Bac für Holzböden + eukula care matt	R= (Ut – U0) – (At – U0) = Ut–At =	**1.38**	**95.83**	**active**

Test organism: *A. baumannii*

A. baumannii	**Contact time (min)**	**Test 1** log_{10} CFU/ml	**Test 2** log_{10} CFU/ml	**Test 3** log_{10} CFU/ml	**Mean**	**SD**	R	Kill rate (%)
U_0	**0**	7.18	7.23	7.08	7.16	0.08	1.99	**98.97**
U_t	60	5.52	5.29	5.45	5.42	0.12		
A_t	60	3.46	3.52	3.32	3.43	0.10		

Test product	Calculation	R	Kill rate (%)	Activity
eukula Vir-O-Bac für Holzböden + eukula care matt	R= (Ut – U0) – (At – U0) = Ut–At =	**1.99**	**98.97**	**active**

Raw data:
Test 1, *Staphylococcus aureus*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Staphylococcus aureus ATCC 6538***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	161	173	334	6.64	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	25	25	50			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.13	-	-
10^{-4}	72	63	135			
10^{-5}	8	7	15			
dark control (Ut)						
10^{0}	>330	>330	>660	7.09	-	-
10^{-1}	>330	>330	>660			
10^{-2}	>330	>330	>660			
10^{-3}	>330	>330	>660			
10^{-4}	64	58	122			
10^{-5}	3	10	13			
test sample (A_t)						
10^{0}	>330	>330	>660	5.73	1.36	-
10^{-1}	>330	>330	>660			
10^{-2}	246	292	538			
10^{-3}	38	41	79			
10^{-4}	1	2	3			
10^{-5}	0	0	<10			

Test 2, *Staphylococcus aureus*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Staphylococcus aureus ATCC 6538***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	161	173	334	6.64	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	25	25	50			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.05	-	-
10^{-4}	53	60	113			
10^{-5}	7	8	15			
dark control (Ut)						
10^{0}	>330	>330	>660	7.12	-	-
10^{-1}	>330	>330	>660			
10^{-2}	>330	>330	>660			
10^{-3}	>330	>330	>660			
10^{-4}	66	67	133			
10^{-5}	6	11	17			
test sample (A_t)						
10^{0}	>330	>330	>660	5.65	1.47	-
10^{-1}	>330	>330	>660			
10^{-2}	259	277	>660			
10^{-3}	26	19	45			
10^{-4}	4	5	9			
10^{-5}	0	0	<10			

Test 3, *Staphylococcus aureus*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Staphylococcus aureus ATCC 6538***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	161	173	334	6.64	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	25	25	50			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.22	-	-
10^{-4}	91	74	165			
10^{-5}	10	10	20			
dark control (Ut)						
10^{0}	>330	>330	>660	7.11	-	-
10^{-1}	>330	>330	>660			
10^{-2}	>330	>330	>660			
10^{-3}	>330	>330	>660			
10^{-4}	74	56	130			
10^{-5}	10	4	14			
test sample (A_t)						
10^{0}	>330	>330	>660	5.80	1.31	-
10^{-1}	>330	>330	>660			
10^{-2}	307	325	632			
10^{-3}	43	69	112			
10^{-4}	4	11	15			
10^{-5}	0	0	<10			

Test 1, *Acinetobacter baumannii*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Acinetobacter baumannii ATCC 19606***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	187	174	361	6.66	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	16	23	39			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.18	-	-
10^{-4}	66	84	150			
10^{-5}	8	10	18			
dark control (Ut)						
10^{0}	>330	>330	>660	5.52	-	-
10^{-1}	>330	>330	>660			
10^{-2}	134	194	328			
10^{-3}	24	11	35			
10^{-4}	2	3	5			
10^{-5}	0	0	<10			
test sample (A_t)						
10^{0}	157	132	289	3.46	2.05	-
10^{-1}	21	19	40			
10^{-2}	4	2	6			
10^{-3}	0	0	<10			
10^{-4}	0	0	<10			
10^{-5}	0	0	<10			

Test 2, *Acinetobacter baumannii*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Acinetobacter baumannii ATCC 19606***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	187	174	361	6.66	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	16	23	39			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.23	-	-
10^{-4}	73	96	169			
10^{-5}	9	6	15			
dark control (Ut)						
10^{0}	>330	>330	>660	5.29	-	-
10^{-1}	>330	>330	>660			
10^{-2}	101	96	197			
10^{-3}	12	20	32			
10^{-4}	2	0	2			
10^{-5}	0	0	<10			
test sample (A_t)						
10^{0}	179	151	330	3.52	1.78	-
10^{-1}	38	38	76			
10^{-2}	5	7	12			
10^{-3}	0	0	<10			
10^{-4}	0	0	<10			
10^{-5}	0	0	<10			

Test 3, *Acinetobacter baumannii*

Testmethode / *test method:*	SOP-ST-MIK.M.0038 / SOP-ST-MIK.M.0047
Laboridentifikationsnummer / *identification number:*	LI-B-023-007
Zeitraum der Prüfung / *test period:*	2023-01-11 - 2023-01-13
Prüfobjekt / *test object:*	eukula Vir-O-Bac für Holzböden + eukula care matt
Trägermaterial / *carrier material:*	wood panel
Lichtquellenintensität / *intensity of light source:*	4 mW/cm^2
Einwikzeit / *contact time:*	60 min
Prüftemperatur / *test temperature:*	20.0 °C ± 0.5 °C
rel. Luftfeuchte / *humidity:*	50%
Verdünnungsmittel / *diluent:*	0.9% NaCl
Prüfkeim / *test strain:*	***Acinetobacter baumannii ATCC 19606***
Inkubationstemperatur / *temperature of incubation:*	36.0 °C ± 2.0 °C
Inkubationszeit / *time of incubation:*	2 days

dilution	CFU 0.5 ml	CFU 0.5 ml	CFU 1 ml	log10	log10 R	Validation
Verification of the test						
Test suspension						
10^{-6}	187	174	361	6.66	-	6.57 ≤ lg N ≤ 7.10
10^{-7}	16	23	39			yes
recovery control (U_0)						
10^{-3}	>330	>330	>660	7.08	-	-
10^{-4}	61	58	119			
10^{-5}	3	9	12			
dark control (Ut)						
10^{0}	>330	>330	>660	5.45	-	-
10^{-1}	>330	>330	>660			
10^{-2}	149	133	282			
10^{-3}	13	9	22			
10^{-4}	1	3	4			
10^{-5}	0	0	<10			
test sample (A_t)						
10^{0}	118	89	207	3.32	2.13	-
10^{-1}	38	27	65			
10^{-2}	6	4	10			
10^{-3}	0	0	<10			
10^{-4}	0	0	<10			
10^{-5}	0	0	<10			