Instituto Valenciano de Microbiología

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ENAC
E N S A Y O S
Nº 648 / LE 1286

Test method for antibacterial activity of semiconducting photocatalytic material with the product PhotoActiva TB.
Film cover method.
(ISO 27447: 2019 Standard)

Report
Registration No.: D/20/604-1

1. **Laboratory identification** ..... Instituto Valenciano de Microbiología

2. **Customer identification** ..... Active Walls S.L.
   Address..... Daoíz I Velarde 22-26 L1. 08980 Sant Feliu de Llobregat (Barcelona).

3. **Sample identification** (information provided by the customer)

   - Product name..... **PhotoActiva TB**.
   - Batch number..... Not indicated.
   - Expiration date..... Does not expire.
   - Manufacturer /Supplier ..... Active Walls S.L.
   - Storing conditions ..... Room temperature.
   - Date of application of the product..... 2020/04/25.
   - Active(s) substance(s) and its concentration(s)..... Applied material. Inorganic

   IVAMI is not responsible for customer-supplied information.

4. **Information about sample reception.**

   - Date of reception of the product..... 2020/05/26.
   - Date of reception of test conditions..... 2020/04/29.
   - Aspect of the received product..... Square ceramic pieces with a glazed face and a porous face.

5. **Method of assay and its validation**

   ISO 27447: 2019 Standard.

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6. Experimental conditions

- Assay period..... 2020/06/22 –2020/06/27.
- Contact time..... 8 hours.
- Intensity of the UV radiation..... 0.1 mW/cm^2.
- Assay temperature..... +18ºC to +25ºC
- Temperature of incubation..... +37ºC ±1ºC.
- Identification of the strains used:
  - *Staphylococcus aureus* CECT-240 (ATCC-6538-P).
  - *Escherichia coli* CECT-516 (ATCC-8739).
- Identification of the test surface area..... Square pieces of 50 ±2 mm. The test is performed on the porous unglazed face.
- Identification of the cover film ..... Square inert pieces of 40 ±2 mm, non-water absorbent, with good-sealing properties, with a transparency rate >85%, with no retention of ultraviolet radiation.
- Identification of the moisture preservation glass..... Rectangular glass panes of 25 by 35±1 cm, with a thickness ≤ 1.1 mm and with a transparency rate >85%, with no retention of ultraviolet radiation.
- Characteristics of the UV lamp..... Fluorescent BLB lamp (Black Light Blue).
- Characteristics of the UV radiometer..... Range: 0-20 mW/cm^2.

7. Results of the assay

- Validation and controls .....See attached tables 1 and 3.
- Evaluation of the bactericidal activity.....See attached tables 2 and 4.
- Discussion of results.....Not needed.
- Interpretation of results.....See attached tables 2 and 4, and conclusion.

8. Special remarks

The photocatalytic antibacterial activity value after UV irradiation (R_L) represents the difference between the number of bacteria of each reference strain on the surface of specimens with the photocatalytic material and the surface of specimens without photocatalytic material after exposure to UV irradiation which activates the photocatalytic material.

The photocatalytic antibacterial value with UV irradiation (ΔR) compares the viable bacteria recovered from the photocatalytic treated-specimens after UV irradiation with those obtained from inoculated specimens of non-photocatalytic treated material exposed to UV irradiation under identical conditions to the treated material, and those obtained from inoculated specimens of both photocatalytic treated and non-treated material kept in the dark for the same period of time.

The ISO 27447: 2019 standard does not indicate ranges of values to consider a product little or very effective according to the values obtained. The higher the values, the greater the photocatalytic activity.

9. Conclusion

The product **PhotoActiva TB,** batch not indicated, when inoculated with a suspension of the test strains, shows R_L values after UV irradiation of 3.0 and 2.5 for the reference strains *Staphylococcus aureus* CECT-240 (ATCC 6538-P) *Escherichia coli* CECT-516 (ATCC 8739), respectively, when assayed according to the ISO 27447: 2019 Standard.

The product **PhotoActiva TB,** batch not indicated, when inoculated with a suspension of the test strains, shows ΔR values with UV irradiation of 2.1 and 1.5 for the reference strains *Staphylococcus aureus* CECT-240 (ATCC 6538-P) *Escherichia coli* CECT-516 (ATCC 8739), respectively, when assayed according to the ISO 27447: 2019 Standard.

Note: The results obtained correspond to the product received in the laboratory.

10. Comments

The U.V. radiation photocatalytic activity index calculated according to ISO 27447: 2019 can be difficult to understand, then, this data is shown as the percentage (%) of bacterial lethality, based on ASTM standards.

Considering the number of microorganisms recovered from each species, on the surface with photocatalytic product and exposed to U.V. radiation, with respect to those not treated with the product and exposed to U.V. radiation, it is considered that the percentage of bacterial lethality obtained for each microorganism in the photocatalytic treated surfaces would correspond to:

- *Staphylococcus aureus* .... 99.9 %.
- *Escherichia coli* .....99.6 %.

Bétera (Valencia), July 3, 2020.

Signed. Sonia Monteagudo
Responsible Technician

Signed. Encarnación Esteban
Technical Director

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Reference:

- ISO 27447: 2019 – Fine ceramics (advanced ceramics, advanced technical ceramics) - Test methods for antibacterial activity of semiconducting photocatalytic materials].

Results of assay with *Staphylococcus aureus* CECT-240 (ATCC-6538P).

Table 1.- Validation and controls.

Non-treated specimens after inoculation	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	>300	>300	>300	>300	>300	>300
10^{-3}	31	35	39	41	38	36
Value *P*	3.3×10^4		4.0×10^4		3.70×10^4	
Value *A*	3.3×10^5		4.0×10^5		3.70×10^5	
Average *A* (*A*)	3.6×10^5					

Non-treated specimens after incubation in darkness	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-1}	>300	>300	>300	>300	>300	>300
10^{-2}	238	210	162	175	180	169
10^{-3}	<30	<30	<30	<30	<30	<30
Value *P*	2.24×10^4		1.69×10^3		1.75×10^4	
Value *B*	2.24×10^5		1.69×10^4		1.75×10^5	
Average *B* (B_D)	1.89×10^5					

Non-treated specimens after UV irradiation	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-0}	>300	>300	>300	>300	>300	>300
10^{-1}	110	118	131	127	98	83
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	1.14×10^3		1.29×10^2		9.1×10^2	
Value *B*	1.14×10^4		1.29×10^3		9.1×10^3	
Average *B* (B_L)	1.11×10^4					

Test validation:

- **(Lmax - Lmin) / Lmean** ≤ 0.2; Where:
  Lmax is the maximum log value of viable bacteria for non-treated specimens after inoculation.
  Lmin is the minimum log value of viable bacteria for non-treated specimens after inoculation.
  Lmean is the average log value of viable bacteria for non-treated specimens after inoculation.
- **A**. the average number of viable bacteria on non-treated specimens just after inoculation. shall be within 1.0×10^5 to 4.0×10^5 cells range.
- B_D. the viable bacteria of non-treated specimens after light exposure shall be > 1.0×10^3 cells for all 3 specimens.
- "B_L. the viable bacteria of non-treated specimens after being kept in a dark place shall be more than> 1.0×10^3 cells for the 3 samples.

Table 2.-Results of antibacterial activity assay with the test product.

Photocatalytic treated specimens after incubation in darkness	**Piece no. 1**		**Piece no. 2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	>300	>300	>300	>300	>300	>300
10^{-1}	204	212	236	242	239	243
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	2.08×10^3		2.39×10^3		2.41×10^3	
Value *C*	2.08×10^4		2.39×10^4		2.41×10^4	
Average *C* (C_D)	2.29×10^4					
Photocatalytic treated specimens after UV irradiation	**Piece no. 1**		**Piece no. 2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^{-0}	0	0	0	0	0	0
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
Value *P*	1		1		1	
Value *C*	1×10^1		1×10^1		1×10^1	
Average *C* (C_L)	1×10^1					
R_L	3.0					
ΔR	2.10					

Explanations

Vc = counts per mL (one or more plates).
P = is the bacteria concentration (cells/mL).
N = P x 10 (is the number of viable bacteria in 10 mL of medium for washout). This parameter is used to calculate A. B and C values.
A = is the average number of viable bacteria of 3 non-treated specimens. just after inoculation.
B_D = is the average number of viable bacteria of 3 non-treated specimens. after being kept in a dark place.
B_L = is the average number of viable bacteria of 3 non-treated specimens. after UV irradiation.
C_D = is the average number of viable bacteria of photocatalytic treated specimens. after being kept in a dark place.
C_L = is the average number of viable bacteria of photocatalytic treated specimens. after UR irradiation.
R_L = is the photocatalyst bacteria activity value. after UV irradiation.

$$R_L = \log (B_L / C_L)$$

ΔR = is the photocatalyst antibacterial activity value with UV irradiation.

$$\Delta R = \log (B_L / C_L) - \log (B_D / C_D)$$

* **Note**: to avoid non-computable values. the viable counts of 0 ($\log 0 = -\infty$) must be set to 1 ($\log 1 = 0$).

Results of assay with *Escherichia coli* CECT-516 (ATCC-8739).

Table 3.- Validation and controls.

Non-treated specimens after inoculation	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	>300	>300	>300	>300	>300	>300
10^{-1}	190	198	185	171	167	174
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	1.94×10^3		1.78×10^3		1.71×10^3	
Value *A*	1.94×10^4		1.78×10^4		1.71×10^4	
Average *A* (*A*)	1.81×10^4					

Non-treated specimens after incubation in darkness	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	>300	>300	>300	>300	>300	>300
10^{-1}	122	1119	136	138	153	149
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	1.21×10^3		1.37×10^3		1.51×10^3	
Value *B*	1.21×10^4		1.37×10^4		1.51×10^4	
Average *B* (B_D)	1.36×10^4					

Non-treated specimens after UV irradiation	Piece no. 1		Piece no. 2		Piece no. 3	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	>300	>300	>300	>300	>300	>300
10^{-1}	114	110	91	82	96	97
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	1.12×10^3		8.7×10^2		9.7×10^2	
Value *B*	1.12×10^4		8.7×10^3		9.7×10^3	
Average *B* (B_L)	9.87×10^3					

Test validation:

- **(Lmax - Lmin) / Lmean** ≤ 0.2; Where:
  Lmax is the maximum log value of viable bacteria for non-treated specimens after inoculation.
  Lmin is the minimum log value of viable bacteria for non-treated specimens after inoculation.
  Lmean is the average log value of viable bacteria for non-treated specimens after inoculation.
- **A.** the average number of viable bacteria on non-treated specimens just after inoculation. shall be within 1.0×10^5 to 4.0×10^5 cells range.
- B_D. the viable bacteria of non-treated specimens after light exposure shall be > 1.0×10^3 cells for all 3 specimens.
- "B_L. the viable bacteria of non-treated specimens after being kept in a dark place shall be more than> 1.0×10^3 cells for the 3 samples.

Table 4.-Results of antibacterial activity assay with the test product.

Photocatalytic treated specimens after incubation in darkness	**Piece no. 1**		**Piece no. 2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	142	138	138	129	137	141
10^{-1}	<30	<30	<30	<30	<30	<30
10^{-2}	<30	<30	<30	<30	<30	<30
Value *P*	1.40×10^2		1.34×10^2		1.39×10^2	
Value *C*	1.40×10^3		1.34×10^3		1.39×10^3	
Average *C* (C_D)	1.38×10^3					
Photocatalytic treated specimens after UV irradiation	**Piece no. 1**		**Piece no. 2**		**Piece no. 3**	
	Vc_1	Vc_2	Vc_1	Vc_2	Vc_1	Vc_2
10^0	6	3	4	2	3	3
10^{-1}	0	0	0	0	0	0
10^{-2}	0	0	0	0	0	0
Value *P*	4.5×10^0		3.0×10^0		3.0×10^0	
Value *C*	4.5×10^1		3.0×10^1		3.0×10^1	
Average *C* (C_L)	3.5×10^1					
R_L	2.5					
ΔR	1.5					

Explanations

Vc = counts per mL (one or more plates).
P = is the bacteria concentration (cells/mL).
N = P x 10 (is the number of viable bacteria in 10 mL of medium for washout). This parameter is used to calculate *A*. *B* and *C* values.
A = is the average number of viable bacteria of 3 non-treated specimens. just after inoculation.
B_D = is the average number of viable bacteria of 3 non-treated specimens. after being kept in a dark place.
B_L = is the average number of viable bacteria of 3 non-treated specimens. after UV irradiation.
C_D = is the average number of viable bacteria of photocatalytic treated specimens. after being kept in a dark place.
C_L = is the average number of viable bacteria of photocatalytic treated specimens. after UR irradiation.
R_L = is the photocatalyst bacteria activity value. after UV irradiation.

$$R_L = \log (B_L / C_L)$$

ΔR = is the photocatalyst antibacterial activity value with UV irradiation.

$$\Delta R = \log (B_L / C_L) - \log (B_D / C_D)$$

* **Note**: to avoid non-computable values. the viable counts of 0 ($\log 0 = -\infty$) must be set to 1 ($\log 1 = 0$).