Instituto Valenciano de Microbiología

Masía El Romeral
Ctra. Bétera – San Antonio de Benagéber, Km 0,3
46117 Bétera (Valencia)
Tel. 96 169 17 02
Fax 96 169 16 37
e-mail: ivami@ivami.com
www.ivami.com
CIF B-96337217

Test method for antiviral activity of semiconducting photocatalytic material with the product "PhotoActiva S" against Adenovirus type 5 (Based on ISO 27447: 2019 Standard)

Report

Registration No.: D/20/544-1

1. **Laboratory of assay**..... Instituto Valenciano de Microbiología

2. **Customer identification**..... Active Walls, S.L.
   Customer address..... Daoiz i Velarde 22
   08980 Sant Feliu de Llobregat
   Barcelona, Catalunya.

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

   - Product name..... PhotoActiva S.
   - Batch number..... Not indicated.
   - Product application date ..... 2020/04/25.
   - Expiration date..... Not indicated.
   - Manufacturer..... Active Walls S.L..
   - Store conditions..... No specials conditions needed.
   - Diluent recommended by the manufacturer... Not applicable.
   - Active(s) substance(s) and its concentration(s)..... Inorganic.

   IVAMI is not responsible for customer-supplied information.

4. **Information about sample reception.**

   - Date of reception of the product..... 2019/05/26.
   - Date of reception of test conditions..... 2020/04/29.
   - Aspect of the received product..... Ceramic pieces.

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5. Method of assay and its validation

Standard Operation Procedure (SOP) based on ISO 27447: 2019 Standard.

6. Experimental conditions

• Assay period.....	June 02 to June 18, 2020
• Assay temperature.....	35ºC ± 1ºC.
• Titration method	$TCID_{50}$ (Tissue Culture Infective Dose 50%).
• Intensity of the UV-A (315-400 nm wavelength) radiation.....	0.1mW/cm^2.
• Contact time.....	8 hours.
• Contact temperature.....	+18ºC to +25ºC
• Procedure to stop product cytotoxicity..	Molecular sieving.
• Procedure to stop product activity.....	Cooling with ice.
• Solvent of the product used in the assay.....	Not applicable.
• Aspect of the dilutions of the product...	Transparent dilutions.
• Stability of the mixture (interfering substance and product diluted in sterile hard water).....	Stable.
• Identification of the test surface area....	Square pieces of 50 ±2 mm.
• Identification of the adhesion 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-A (315-400 nm wavelength) lamp.....	Fluorescent BLB lamp (Black Light Blue).
• Characteristics of the UV-A (315-400 nm wavelength) radiometer.....	Range: 0-5 mW/cm^2.
• Identification of the origin of viral strains and number of passes.....	Adenovirus type 5 (ATCC VR-5) aliquot: 2019/05/23 passage 2.
• Cell lines (name, origin, number of passes and culture medium).....	VERO ref. FTVE, working aliquot 2, passage 18 and working aliquot 3, passages 10, 13 and 15.

7. Validation of assay results

Sensitivity of cells to virus

- Viral quantification of Adenovirus type 5 with cells not treated with "PhotoActiva S" disinfectant ……..…….....$log10^{-6.16}$
- Viral quantification of Adenovirus type 5 with cells treated with the "PhotoActiva S" disinfectant……..…..….$log10^{-5.91}$

Note: only can be used to determine the infectivity of cells, those dilutions which: a) show a low degree of cellular destruction (< 25% of cell monolayer) and b) produce a reduction of the title of the virus <1 log_{10}.

Control of the effectivity of the disinfectant detection activity

- Viral quantification of Adenovirus type 5 after 30 minutes on bath ice without exposing the virus to the "PhotoActiva S" disinfectant ……...……..….$log10^{-6.58}$
- Viral quantification of Adenovirus type 5 exposing the virus to "PhotoActiva S" disinfectant and incubated 30 minutes on ice bath……..$log10^{-6.41}$

Note: The difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension should be ≤0.5.

8. Special remarks

All controls and validation were between the basic limits.

9. Comments

The antiviral activity values in **irradiation condition (R_L)** represents the difference between the number of viable viruses on the surface with the photocatalytic material and the surface without photocatalytic material after exposure to UV-A (315-400 nm wavelength) irradiation which activates the photocatalytic material.

The antiviral activity values with UV-A (315-400 nm wavelength) irradiation by removing the **effect in the dark (ΔR)** compares the viable viruses recovered from the photocatalytic treated-specimens after UV-A (315-400 nm wavelength) irradiation with those obtained from inoculated specimens of non-photocatalytic treated material exposed to UV-A (315-400 nm wavelength) 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 13125: 2013 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.

The B_L is the average viral suspension titre of the three non-treated pieces after the UV-A (315-400 nm wavelength) irradiation for several hours. Then, if the values (**R_L**) and (**ΔR**) are next to the **B_L** value, means that the lethality percentage due to photocatalytic activity is high, and with a 100% of virus lethality **R_L** and **ΔR** are $\approx$ **B_L**. With a 100% of viral lethality, the **C_L** is zero and then **R_L** = **B_L**.

10. Assay results

The disinfectant product, **"PhotoActiva S"**, batch not indicated, when inoculated with Adenovirus type 5 (ATCC VR-5) and during 8 hours of exposure of UV-A (315-400 nm wavelength) irradiation shows antiviral activity values in UV-A (315-400 nm wavelength) irradiation condition (R_L) of 5.22 log, when the activity is assayed according with the internal procedure DESIN-9407 based on the ISO 27447: 2019 Standard.

The product "**PhotoActiva S"**, batch not indicated, when inoculated with Adenovirus type 5 (ATCC VR-5), shows antiviral activity values in the dark (ΔR) of 5.06 log with UV-A (315-400 nm wavelength) irradiation, after subtracting the effect in the dark, when assayed with the internal procedure DESIN-9407 based on the ISO 27447: 2019 Standard.

11. Conclusion

The disinfectant product, **"PhotoActiva S"**, batch **not indicated,** when inoculated with Adenovirus type 5 (ATCC VR-5) and during 8 hours of exposure shows antiviral activity values in UV-A (315-400 nm wavelength) irradiation condition (R_L) of 5.22 log and antiviral activity values (**ΔR**) of 5.06 log with UV-A (315-400 nm wavelength) irradiation, after subtracting the effect in the dark, when assayed with the internal procedure DESIN-9407 based on the ISO 27447: 2019 Standard.

Considering the viable viruses recovered on the surface with a photocatalytic product exposed to UV-A (315-400 nm wavelength) radiation, compared to those untreated and not exposed to UV-A (315-400 nm wavelength) radiation and considering the difference between the titre of the viral suspension and the cytotoxicity level of the product in the assay, it is considered that the percentage of virucidal lethality, due to the photocatalytic activity, obtained for Adenovirus type 5 (ATCC VR-740) is **79.52%**.

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

Bétera (Valencia), July 03, 2020.

Signed. Ruth Novella
Responsible Technician

Signed. Encarnación Esteban
Technical Director

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Reference:

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

Results of activity of the product "PhotoActiva S", batch not indicated with Adenovirus type 5 (ATCC VR-740)

Table 1.- Results of assay with the non-treated test pieces following inoculation.

Non-treated test pieces after inoculation	Piece no. 1	Piece no. 2	Piece no. 3
Log $TCID_{50}$	6.41	6.32	6.16
Maximum level of virus inactivation detectable	5.91	5.82	5.66
Value S	2.5×10^6	2.09×10^6	1.4×10^6
Value F_A	5.14×10^6	4.17×10^6	2.9×10^6
Value A_X	4.07×10^6		

Table 2.- Results of assay with the non-treated test pieces after incubation in the dark.

Non-treated test pieces after incubation in the dark	Piece no. 1	Piece no. 2	Piece no. 3
Log $TCID_{50}$	6.25	6.07	6.24
Maximum level of virus inactivation detectable	5.75	5.57	5.74
Value S	1.78×10^6	1.17×10^6	1.74×10^6
Value F_B	3.56×10^6	2.35×10^6	3.48×10^5
Value B_D	3.12×10^6		

Table 3.- Results of assay with the non-treated test pieces after UV-A (315-400 nm wavelength) irradiation.

Non-treated test pieces after UV-A (315-400 nm wavelength) irradiation	Piece no. 1	Piece no. 2	Piece no. 3
Log $TCID_{50}$	6.16	5.91	6.08
Maximum level of virus inactivation detectable	5.66	5.41	5.58
Value S	1.44×10^6	8.12×10^5	1.20×10^6
Value F_B	2.89×10^6	1.63×10^6	2.40×10^6
Value B_L	2.31×10^6		
log B_L	6.36		

Verification of the methodology:

- **A**, Average of viral suspension titre on non-treated test pieces following inoculation shall be over 1.0×10^4 and under 1.0×10^5.
- Number of surviving viruses on all-treated test pieces after UV-A (315-400 nm wavelength) irradiation (**F_B**) is over 5.0×10^3.
- $(L_{max} - L_{min}) / L$ medio ≤ 0.2
  L_{max} is the value of the maximum logarithm of vial viruses of the untreated pieces after inoculation
  L_{min} is the value of the minimum logarithm of vial viruses of the untreated pieces after inoculation
  L_{medium} is the value of the medium logarithm of vial viruses of the untreated pieces after inoculation.

Table 4.- Results of assay with the photocatalytic treated test pieces after incubation in the dark.

Treated test pieces after incubation in the dark	Piece no. 1	Piece no. 2	Piece no. 3
Log $TCID_{50}$	5.91	6.07	6.08
Maximum level of virus inactivation detectable	5.41	5.57	5.58
Value S	8.12×10^5	11.75×10^5	12.02×10^5
Value F_C	16.26×10^5	23.49×10^5	24.04×10^5
Value C_D	21.27×10^5		

Table 5.- Results of assay with the photocatalytic treated test pieces after UV-A (315-400 nm wavelength) irradiation.

Treated test pieces after UV-A (315-400 nm wavelength) irradiation	Piece no. 1	Piece no. 2	Piece no. 3
Log $TCID_{50}$	0.83	0.91	0.75
Maximum level of virus inactivation detectable	0.33	0.41	0.25
Value S	6.76	8.13	5.62
Value F_C	13.52	16.25	11.25
Value C_L	13.67		
R_L	5.22		
ΔR	5.06		

Note: Maximum level of virus inactivation detectable is the difference between the titre of the viral suspension and the cytotoxicity level.

Explanations

$TCID_{50}$: Tissue Culture Infective Dose 50%.
S = Viral suspension titre.
F = S x 2 (number of surviving viruses in 2 mL of medium for washout). This parameter is used to calculate A, B and C values.
A = Average of viral suspension titre for non-treated test piece following inoculation.
B_D = Average of viral suspension titre of non-treated test pieces incubated in the dark several hours.
B_L = Average of viral suspension titre of non-treated test pieces after UV-A (315-400 nm wavelength) irradiation several hours.
C_D = Average of viral suspension titre of photocatalytic treated test pieces incubated in the dark several hours.
C_L = Average of viral suspension titre of photocatalytic treated test pieces after UV-A (315-400 nm wavelength) irradiation several hours.
R_L = Antiviral activity value in irradiation condition L.

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

ΔR = Antiviral activity value with UV-A (315-400 nm wavelength) irradiation by removing the effect in the dark.

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

Porcentaje de letalidad = (ΔR x 100) / B_L

Table 6. Results of the activity of the product "**PhotoActiva S**", batch not indicated, with Adenovirus type 5 (ATCC VR-740) (Assay of titration with 12 wells).

Product	Sample*	Time of contact (min/hour)	Dilutions (log10)[a,b]							
			1	2	3	4	5	6	7	8
Photocatalytic treated specimens after UV-A (315-400 nm wavelength) irradiation	Piece no. 1	8 h	0002 2200 2000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000
	Piece no. 2	8 h	0032 2002 2000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000
	Piece no. 3	8 h	0000 2202 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000
Photocatalytic treated specimens after incubation in darkness	Piece no. 1	8 h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2200 2000	0000 0000 0100	0000 0000 0000	0000 0000 0000
	Piece no. 2	8 h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2202 2000	0000 0000 0110	0000 0000 0000
	Piece no. 3	8 h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0032 2002 2200	0000 0000 0100	0000 0000 0000
Cytotoxicity	Piece	NA	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	0000 0000 0000	NR
Non-treated specimens after UV-A (315-400 nm wavelength) irradiation (Virus control)	NA	8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2022 2000	0000 2100 1000	0000 0000 0000
		8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2002 2000	0000 0000 1000	0000 0000 0000
		8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2202 2200	0000 0000 0100	0000 0000 0000
Non-treated specimens after incubation in darkness (Virus control)	NA	8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2202 2200	0002 1000 1000	0000 0000 0000
		8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0003 2202 2000	0000 0000 2100	0000 0000 0000
		8h	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0032 2222 0200	0000 0010 1000	0000 0000 0000
Non-treated specimens after inoculation in darkness (Virus control)	NA	0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0322 2222 3200	0000 0010 2000	0000 0000 0000
		0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0032 2222 2020	0000 0101 0000	0000 0000 0000
		0	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	4444 4444 4444	0032 2022 0200	0000 0100 0100	0000 0000 0000

Sensitivity control of cells to virus	NA	Cells not treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	000C CC0C CC00	0000 000C C000	0000 0000 0000
		Cells treated	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	000C CC0C 0000	0000 000C 0000	0000 0000 0000
Effectiveness control of the disinfectant detection activity	NA	Without product	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0000 0000 2000	0000 0000 0000
		With product	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	CCCC CCCC CCCC	0CCC 0CCC CCCC	0000 0000 C000	0000 0000 0000

a): 1 to 4, virus present and grade of the cytophatic effect in 12 units of cellular culture, or grade of cellular lesions in the cytotoxicity assay.
C = cytopathic effect with presence of virus (in this case and according to guideline does not take into account the degree of cytopathic effect only, the presence or absence of the same).
0 = no virus present or absence of cellular lesions in the cytotoxicity assay.
NA: not applicable; NR: not realized.
sec: minutes; min: minutes; h: hours