



**INTERNATIONAL  
TESTING & CERTIFICATION LLC**



**TEST REPORT  
ref. No. 202201/04/1**

**Manufacturer:** C&S Italy Srl  
**Address:** Via Setteponti, 133 52100 Arezzo (AR) ITALY  
**Legal Representative:** C&S Italy Srl  
**Address:** Via Setteponti, 133 52100 Arezzo (AR) ITALY  
**Sample:** Melt Blown (as blank) and AV MASK PRO  
**Test Standard:** ISO 20743:2021  
**Name of tests:** Evaluation of antibacterial activity of graphene  
**Place and date of issue:** Sheridan, WY Jan 26, 2022

  
Certification Manager

**Note: The results given in this Test Report apply only to the sample tested by our laboratory!**  
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**Testing Laboratory:** 1309 Coffeen Avenue Suite – 1512, Sheridan, WY 82081 USA - Email: [testing@itc-usa.net](mailto:testing@itc-usa.net)  
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**Description and identification of samples:**

**Table I - Description and identification of samples**

Sample	Description
1. Melt Blown	Melt Blown white 25 g/m <sup>2</sup>
2. AV MASK PRO	AV MASK PRO



**Figure 1 – Melt Blown**

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**Figure 2 – Melt Blown Graphene**

**Antibacterial activity effectiveness Testing:**

**Test Purpose:**

The purpose of this test is to assess whether the AV MASK PRO with graphene have antibacterial activity against the tested strains.

**Sampling method:**

The samples intended for the tests were selected by the client. The testing laboratory shall not be held liable for any faults caused by an incorrect sampling.

**Testing methods used:**

A test method for determining antibacterial activity according to ISO 20743:2021.

**Test conditions:**

The test samples were conditioned for the time period of minimum 4 hours at the temperature of  $(21 \pm 5) ^\circ\text{C}$  and a relative humidity of air of  $(85 \pm 5) \%$ .

**Test Sample:**

The parent solutions of the strains of interest have been prepared. The tests were carried out on AV MASK PRO with graphene-treated tissue specimens and specimens of un treated tissue, for each bacterial strain, for each different timing (time 0, after 30 minutes from the inoculum, after 2h from the inoculum, after 24 hours from the inoculum). Samples must be weighing  $5.40\text{gr} \pm 0.05\text{gr}$ . The mother solution containing bacterial strains is diluted several times until it reaches a target concentration between  $1 \times 10^5 \text{CFU} / \text{ml}$  and  $3 \times 10^5 \text{CFU} / \text{ml}$ . You use this solution as an inoculum for testing. The first six samples treated and not, for each strain, are used to measure vital cells immediately after inoculation and later expressed as CFU at zero time. The remaining samples, treated and not, are used to evaluate vital cells after incubation for 30 minutes, 2 hours, 24 hours.

**Acceptance Criteria:**

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Effectiveness of antibacterial activity	Value of antibacterial activity (A)
Significant	$2 \leq A < 3$
Strong	$A \geq 3$

### Test Procedure:

The samples were placed in different vials, sterilized in an autoclave at 121° for 15 minutes to eliminate any contaminants and left to dry for at least an hour under biohazard hood before proceeding with the test. The inoculum of 0.2 ml for each strain, is placed in separate places on each tissue sample. Immediately after inoculation some of the tissue samples, treated and not, were washed with 20 ml of neutralizing solution, these will constitute the samples at time 0. The recovered solution undergoes serial dilutions to determine its initial bacterial concentration by the plate counting method. The remaining samples, on the other hand, after inoculum, are placed to incubate for 30 minutes, 2 hours, 24 hours at 37° ± 2°C in incubator.

After incubation, the samples are treated, as indicated above, adding 20 ml of neutralizing solution, diluting the recovered solution, and proceeding to the determination of the bacterial concentration.

The value for antibacterial activity is calculated using the following formula:

$$A = (\lg C_t - \lg C_0) - (\lg T_t - \lg T_0) = F - G$$

Where:

- A is antibacterial activity.
- F is the growth value on the unaddressed control sample.
- G is the growth value on the sample whose antibacterial activity needs to be tested.
- $\lg C_t$  is the common logarithm of the arithmetic mean of the number of bacteria, obtained from untreated control samples after 30 minutes, 2 hours, 24 hours of incubation.
- $\lg C_0$  is the common logarithm of the arithmetic mean of the number of bacteria, obtained from untreated control samples immediately after the transfer of the inoculum to the tissue.
- $\lg T_t$  is the common logarithm of the number of vital bacteria, obtained from the treated samples after 30 minutes, 2 hours, 24 hours of incubation.
- $\lg T_0$  is the common logarithm of the number of vital bacteria, obtained from the samples treated immediately after the transfer of the inoculum to the tissue.

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**Test results:**

The test results obtained are given in the tables as follows:

**Table I – Staphilocco Aureus, registration No. 202201/04/1**

KLEBSIELLA				
	Average Blank T0	Average Blank 30 minutes	Average Blank 2 hours	Average Blank 24 hours
Log	8283333,333	163333333,3	208000000	175533333,3
Log	6,918205138	8,213074825	8,318063335	8,2443596
Conditioning Parameters		85 ± 5% relative humidity (RH) and 21 ± 5°C for a minimum of 4 hours		
Number and Date of this European Standard		Internal Procedure		
<b>F = AVERAGE BLANK 30 minutes - T0</b>			<b>1,294869687</b>	
<b>F = AVERAGE BLANK 2 hours T0</b>			<b>1,399858197</b>	
<b>F = AVERAGE BLANK 24 hours - T0</b>			<b>1,326154462</b>	

KLEBSIELLA			
AVERAGE T0	AVERAGE T2	AVERAGE T4	AVERAGE T8
300	40	46,66666667	8,333333333
2,477121255	1,602059991	1,669006781	0,920818754
<b>G = MEDIA T2 - TEMPO 0</b>		<b>-0,875061263</b>	
<b>G = MEDIA T4 - TEMPO 0</b>		<b>-0,808114474</b>	
<b>G = MEDIA T8 - TEMPO 0</b>		<b>-1,556302501</b>	
<b>A = F-G</b>		<b>2,16993095</b>	
<b>A = F-G</b>		<b>2,20797267</b>	
<b>A = F-G</b>		<b>2,882456962</b>	

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STAFILOCOCCUS AURES				
	Average Blank T0	Average Blank 30 minutes	Average Blank 2 hours	Average Blank 24 hours
Log	693333,3333	5300000	4433333,333	9166666,667
Log	5,84094208	6,72427587	6,646730386	6,962211439
Conditioning Parameters		85 ± 5% relative humidity (RH) and 21 ± 5°C for a minimum of 4 hours		
Number and Date of this European Standard		ISO 20743:2021		
F = AVERAGE BLANK 30 minutes - T0			0,911932623	
F = AVERAGE BLANK 2 hours T0			0,884128283	
F = AVERAGE BLANK 24 hours - T0			1,133781373	
AVERAGE T0	AVERAGE T2	AVERAGE T4	AVERAGE T8	
2000	123,3333333	70	26	
3,301029996	2,091080469	1,84509804	1,414973348	
G = MEDIA T2 - TEMPO 0		-1,209949526		
G = MEDIA T4 - TEMPO 0		-1,455931956		
G = MEDIA T8 - TEMPO 0		-1,886056648		
A = F-G		2,093283316		
A = F-G		2,261720262		
A = F-G		3,007326007		

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