




INTERNATIONAL TESTING & CERTIFICATION LLC

TEST REPORT ref. No. ITC/202110515

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 Melt Blown Graphene
Test Standard: ISO 20743:2021
Name of tests: Evaluation of antibacterial activity of graphene
Place and date of issue: Sheridan, WY Jul 15, 2021




Head of Testing Laboratory

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 STE 1200 – Sheridan, WY 82081 USA - Email: testing@itc-usa.net
Certification Office: Via Alberto Tallone, 84, 00123 Roma Italy - Email: certification@itc-usa.net
www.itc-usa.net

Description and identification of samples:

Table I - Description and identification of samples

| Sample | Description |
|------------------------|---|
| 1. Melt Blown | Melt Blown white 25 g/m ² |
| 2. Melt Blown Graphene | Melt Blown graphene 25 g/m ² |



Figure 1 – Melt Blown



Figure 2 – Melt Blown Graphene

Antibacterial activity effectiveness Testing:

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Test Purpose:

The purpose of this test is to assess whether the samples treated 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 three graphene-treated tissue specimens and three 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 $0.40\text{gr} \pm 0.05\text{gr}$. 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:

| 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 $37^\circ \pm 2^\circ\text{C}$ 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:

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

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.

Test results:

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

Table I – Staphilocco Aureus, registration No. ITC/202110515

| KLEBSIELLA | | | | |
|---|---------------------|--|--------------------------|---------------------------|
| | Average Blank T0 | Average Blank 30 minutes | Average Blank 2 hours | Average Blank 24 hours |
| Log | 569333,3333 | 900000 | 1280000 | 1526666,667 |
| Log | 5,755366612 | 5,954242509 | 6,10720997 | 6,183744223 |
| 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 | | |
| F = AVERAGE BLANK 30 minutes - T0 | | | 0,198875898 | |
| F = AVERAGE BLANK 2 hours T0 | | | 0,351843358 | |
| F = AVERAGE BLANK 24 hours - T0 | | | 0,428377612 | |

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| KLEBSIELLA | | | |
|------------------------|-------------|--------------|-------------|
| AVERAGE T0 | AVERAGE T2 | AVERAGE T4 | AVERAGE T8 |
| 15333,33333 | 220 | 203,3333333 | 200 |
| 4,185636577 | 2,342422681 | 2,30820858 | 2,301029996 |
| G = MEDIA T2 - TEMPO 0 | | -1,843213896 | |
| G = MEDIA T4 - TEMPO 0 | | -1,877427997 | |
| G = MEDIA T8 - TEMPO 0 | | -1,884606581 | |
| A = F-G | | 2,042089794 | |
| A = F-G | | 2,229271355 | |
| A = F-G | | 2,312984193 | |

| STAFILOCOCCUS AURES | | | | |
|---|------------------|--|-----------------------|------------------------|
| | Average Blank T0 | Average Blank 30 minutes | Average Blank 2 hours | Average Blank 24 hours |
| Log | 526666,6667 | 4300000 | 4033333,333 | 7166666,667 |
| Log | 5,721535832 | 6,633468456 | 6,605664116 | 6,855317205 |
| 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 | |
| 2333,333333 | 172,6666667 | 140 | 200 | |
| 3,367976785 | 2,237208505 | 2,146128036 | 2,301029996 | |
| G = MEDIA T2 - TEMPO 0 | | -1,13076828 | | |
| G = MEDIA T4 - TEMPO 0 | | -1,22184875 | | |
| G = MEDIA T8 - TEMPO 0 | | -1,06694679 | | |
| A = F-G | | 2,042700904 | | |
| A = F-G | | 2,105977033 | | |
| A = F-G | | 2,200728163 | | |

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