Techinical Report n° 2004412-001

Customer: DIDA srl
Via Venezia, 73
35129 Padova

Sample type: ***Material and Product***

Required analysis: ***Verification of bactericidal efficacy in prototype air and gas sterilizer***

LabAnalysis code: ***2004412-001***

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1. INTRODUCTION

On 28-10-2020, the company DIDA srl site in Padova provided us with a prototype of LED light sterilizer for air and gas in order to verify the bactericidal efficacy.

2. SAMPLE IDENTIFICATION

Samples tested:

- 2004412-001 sterilizer with LED light source

3. REFERENCES

- UNI EN 13697:2015

4. EXPERIMANTAL PROCEDURE

4.1 Assay of test

The study, performed at the LabAnalysis company, began on 02-11-2020 and ended on 05-11-2020.

4.1.1 identification of microorganisms

In tha Table 1 have been reported the list of microorganisms considered:

Microrganisms	ATCC	Batch
Staphylococcus aureus	6538	04600504
Pseudomonas aeruginosa	9027	484-1128-4
Bacillus subtilis	6633	02900702
Escherichia coli	8739	03800906

Table 1

Pseudomonas aeruginosa: group 2 biological agent; opportunistic microorganism widespread in the environment which occasionally causes disease in plants, animals and humans;

Staphylococcus aureus: biological agent of group 2; ubiquitous microorganism widely spread in nature and easily detectable in the air, considered as an index of anthropogenic contamination, that is, related to the presence of man;

Escherichia coli: group 2 biological agent, pathogenic microorganism indicating organic and / or faecal contamination;

Bacillus subtilis: group 2 biological agent, pathogenic microorganism commonly present in the soil

4.1.2 Maintenance

Standard cultures of microorganisms supplied in lyophilized form have been used. The bacterial strains (Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli) nave been isolated in Casein soya bean digest agar (CASO agar) plates, after incubation, under the optimal growth conditions for the strain in question, they have been stored. at 4 ° C ± 2 ° C and have undergone a maximum of four passages in their respective culture media.

4.1.3 Culture media and reagents

- Casein soya bean digest agar (CASO agar) batch 2972157
- Casein soya bean digest broth (CASO broth) batch 0046335
- Sterile distilled water

  - Diluent:

- KH_2PO_4 3.6 g
- K_2HPO_4 7.2 g
- Meat peptone 1.0 g
- NaCl 4.3 g
- Distilled water 1000 ml
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4.1.4 Main equipment

Steam autoclave Sterilsteam 2	code 1800
Thermostat Memmert: 36°C±1°C	code 4902
Analytical funnel GVS	batch 7086007
Filtration ramp	

4.2 Assay of test

So long as it doesn't exist a technical rule to evaluate the treatment efficacy and we have not available rooms at known concentrations of microbes and bacteria (concentrations of microbes and bacteria in environments are variables), inoculated carriers at known concentrations of bacteria have been employed.

Carriers (steel circled supports) of 1 cm's diameter have been inoculated with pathogen microorganisms in concentration of 10^7.

Such carriers have been exposed to sterilization inside of a prototype with LED lights and at the end of sterilization they have been removed and have been brought in laboratory for the analysis.

Duration of treatment made by prototype has been of 4 cycles of 90 seconds with LED lights as indicated by the manufacturer.

To verify carriers' concentration doesn't change, carriers following the same iter of the sample and not exposed to the treatment system have been preparated.

For the elaboration of experimental system indication of norm UNI EN 13697:2015 “Chemicals disinfectants and antiseptics – Quantitative trial for non-porous surfaces for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, household and institutional field. Trial method and requirements without mechanical action (phase 2/stage 2)”.

Culture media and reagents used are reported in the norm UNI EN 13697:2015.
Bacterial strains have been isolated on TSA plates and have been incubated at 36±1°C for 24 hours. Starting to the generated colonies, a microbical suspension in diluent has been set up and it has been used for the validation test (performed within 2 hours to the dilution). It has been measured the absorbance with the spectrofotometer (λ 625nm) to quantify bacteria species; so, subsequent dilutions to obtain a suspension containing 10^7 UFC/ml (UFC: Unit Formants Colony) have been performed.

Used carriers have been sterilizated through immersion in isopropyl alcohol, so they have been dried under laminar flow hood.
For each microbical strain two carriers have been preparated, inoculated with 50 µl of microbical suspension at concentration of 10^7, dried under laminar flow hood at 37÷1°C for the time required to drying; one carrier has been exposed to sterilizing treatment inside of the prototype and the other has been used as a control.

So the machine has been operated for 4 cycles of 90 seconds, after that carriers have been transferred in a test tube containing 10 mL of diluent and 5g of glass microspheres.
Once returned to the laboratory, test tubes have been shaked vigorously to detach microorganisms from the carrier.

To evaluate the efficacy of treatment system, microorganisms log reduction present of the surface of carriers has been determinated making a comparison with non-treated control carriers, that they haven’t been exposed to the action of the disinfectant.

After a neutralization time of 5 minutes ± 10 seconds, UFC/ml number of the blend has been counted through serial decimal dilutions in diluent and seeding performed in double of the dilutions from 10^{-1} to 10^{-2} for the test and the dilutions from 10^{-2} a 10^{-5} for the control.

After incubation under the optimal growth conditions for each microorganism, it has been registered the number of UFC for each dilution that allowed to calculate the number of UFC/ml for each of the two seeded plates (a, a').

Nc e Nd has been calculated using the following equation:

$$Nc(Nd)=\log_{10}\left[\frac{c}{n}\times\frac{10}{d}\right]$$

Nc: logarithm of the number of UFC/carrier recovered in the control with water
Nd: logarithm of the number of UFC/carrier recovered in the test
c = sum of the values of UFC/ml of the microbical suspension in all considered plates
n = number of the plates considered to calculate c
d = dilution factor of the plates considered for colony counting
10 = neutralizing volume

Antimicrobial activity ME (microbicidial efficacy) has been calculated according to the following formula:

$$ME=Nc-Nd$$

4.3 Results

In the below table log reductions and reductions % obtained compared to untreated controls have been reported.

microorganisms						**Log reduction**	**Reduction %**
	inoculation	control (B2)	log (B2)	samples (B1)	log (B1)	log(B2)-log (B1)	
E.coli	2,50E+07	4,50E+05	5,65	14	1,15	4,51	99,99
S.aureus	5,60E+07	4,00E+04	4,60	9	0,95	3,65	99,98
B.subtilis	1,00E+07	2,60E+05	5,41	32	1,51	3,91	99,99
P.aeruginosa	1,60E+07	1,92E+05	5,28	2	0,30	4,98	99,99

5. CONCLUSIONS

On the basis of the results obtained, the treatment system in question demonstrated bactericidal activity against P.aeruginosa ATCC 9027, S. aureus ATCC 6538, E.coli ATCC 8739 and B.subtilis ATCC 6633 bacteria resulting in a percentage reduction> 99.9% by customer specification.

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End Technical Report