Fraunhofer
IVV

Test Report

Microbiological examination and scientific assessment of the disinfection performance of a case with an integrated UV-C lamp

Client:	Lacon Electronic GmbH Mr. Jürgen Sander Hertzstraße 2 85757 Karlsfeld
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The results only relate to the investigated samples and parameters

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1 Background

The company Lacon Electronic GmbH has developed a special case with an integrated UV-C lamp for surface disinfection of objects. The Fraunhofer IVV was commissioned to determine the disinfection efficiency of the UV-C lamp inside the case in dependency of the object position and the duration of the irradiation.

2 Principle of the method

The investigation was performed according to the guidelines of the "Mechanical Engineering Industry Association" (VDMA):

a) *„Code of Practice - Filling Machines of VDMA Hygiene Class V: Testing the Effectiveness of Packaging Sterilization Devices" No. 6/2002; revised 2008, VDMA, Frankfurt am Main, Germany*

b) *„Hygienic Filling Machines of VDMA Class IV for Liquid and Viscous Foods. Minimum requirements and basic conditions for intended operation " No. 10/2005; 2nd Edition, 2016, VDMA, Frankfurt am Main, Germany*

Microbiological tests were performed as a challenge test, more precisely as a count reduction test.

For this purpose, the targeted material is artificially inoculated with a high amount of selected microorganisms. The microorganism of choice needs to possess a defined resistance against the sterilizing agent of the investigated machine or device.

The inoculated material is then exposed to the sterilization process and afterwards microbiologically investigated for germinable spores or viable cells.

From the number of applied spores/cells (initial count), determined by the investigation of untreated control samples, minus the number of surviving spores/cells (survivor count), the mean logarithmic count reduction (MLR) is calculated by equation 1:

Equation 1: Mean logarithmic reduction (log_{red})

$$log_{red} = log\ (mean\ inital\ count) - log\ (mean\ survivor\ count)$$

3 Test microorganisms, sample material and disinfection device

Black-pigmented spores of *Aspergillus niger* DSM 1957 were used as test organism, because of their high resistance against UV radiation. The spores of this mold are generally recommended as test organisms for validation of UV-based disinfection processes. These were applied to sterile stainless steel plates (2 x 4 cm) in a defined concentration using an artificial contamination process.

The experiments were carried out with the test device "UV-C Module KS101; portable" developed by Lacon Electronic GmbH, which is a case with an integrated UV-C lamp (see Figure 1). The inside of the case has a specific forge scale structure.

Figure 1 UV-C Module KS101 (portable) with integrated UV-C emitters for surface disinfection of objects

4 Test procedure

4.1 Artificial contamination of the test specimens

A concentrated spore suspension of *Aspergillus niger* DSM 1957 was used for the artificial contamination. Since this was prepared with physiological saline solution (Ringer's solution), it was washed three times by centrifugation and subsequent resuspension with sterile deionized water. The resulting pellet was vortexed for 60 s and the suspension was then treated in an ultrasonic bath for two minutes in order to dissolve any spore agglomerates.

The pre-sterilized stainless steel plates were placed on a metal plate (carrier) under aseptic conditions and all edges were fixed with adhesive tape. As a result, an area of 1.6 x 3.5 cm was amenable for artificial contamination while cross-contaminations on the edges and the rear can be avoided.

Each sample was homogeneously contaminated with a defined amount of the prepared spore suspension by using a two-substance nozzle. The spray contamination enables a homogeneous distribution of the spores on the surface of the test object. The theoretically applied spore concentration was about $1*10^5$ CFU/object. Following the artificial contamination, the samples were dried under

aseptic conditions for one hour. The dried test specimens were covered with a sterile petri dish until disinfection treatment.

4.2 Irradiation of samples

Five positions were defined on the grid placed in the case for the irradiation treatment. The metal plates were set up with the non-contaminated edge by using a specific holder or directly placed on the grid respectively. In a first test, irradiation times of 200 s, 400 s and 600 s were tested for the selected positions 1 and 2 (see Figures 2 and 3).

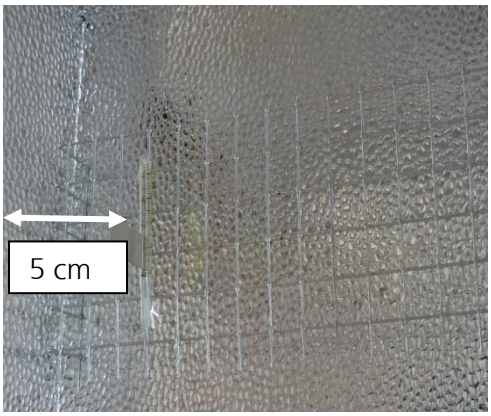

Figure 2 Position 1 in the case; contaminated surface facing the UV lamp

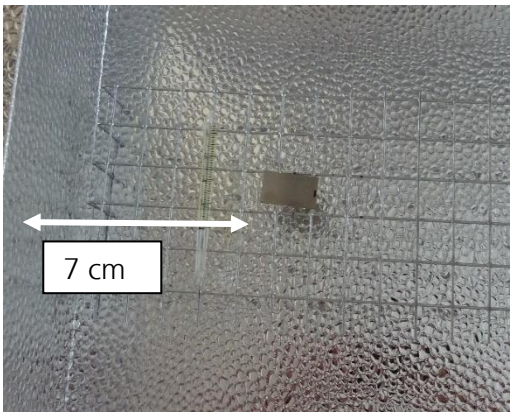

Figure 3 Position 2 in the case; contaminated surface facing upwards

Position 1 was about 5 cm away from the case wall opposite the UV lamp and the contaminated surface of the metal plate was facing the UV lamp. An irradiance of 3 mW/cm^2 was measured for this position. The sample on position 2 was positioned flat on the grid about 7 cm from the case wall opposite the UV tube. The contaminated area was facing up.

Three other positions, which are shown in Figure 4, were examined in a second trial. Based on the results of the first test, 180 s was chosen as treatment time. All contaminated surfaces of the samples in the three selected positions faced the case wall. The distances between the samples and the case wall were adjusted to 4 cm in all three positions.

After the samples had been positioned, the case lid was closed and the UV irradiation started. The treatment was stopped by opening the lid.

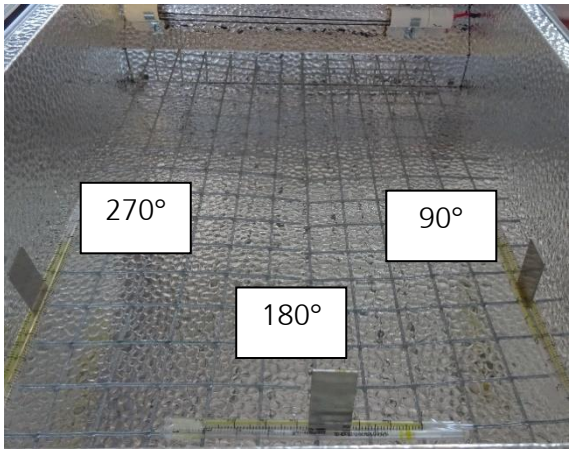

Figure 4 Positions of the contaminated sample in the second irradiation trial. The stainless steel plates were each 4 cm away from the wall of the case and their contaminated surfaces were aligned at 90 °, 180 ° and 270 ° to the UV lamp (i.e. each pointing to the wall of the case).

4.3 Microbiological handling of the treated samples

Immediately after the treatment with UV-C radiation, the stainless steel plates were transferred to sterile bags with 30 ml of sterile Ringer's solution supplemented with 0.1% Tween 80. The samples were rubbed manually for one minute in order to detach the spores from the surface.

The sample suspension was then analyzed for germinable spores. For this purpose, 20 ml were examined by using the membrane filtration method, and further 2 ml using the Koch's pour plate method. The detection limit was 1.4 CFU/object.

The pour plates as well as the membrane filters were incubated at 30 °C on YGC agar (Yeast Glucose Chloramphenicol) and their evaluation was carried out after 54 hours.

In order to be able to determine the exact initial count, reference samples were used as positive controls. These were treated in the same way as the samples, but without UV radiation. Their microbial load was determined using the pour plate method. The references were incubated at 30 °C on YGC agar and evaluated after 35 hours.

5 Results and Discussion

First trial on 15.07.2020

In a first experiment, the inactivation performance of the UV-C lamp against the spores of *Aspergillus niger* DSM 1957 was determined at two positions in the case under variation of the exposure time.

As shown in Table 1, the mean initial load of *Aspergillus niger* DSM 1957 spores on the stainless steel plates was $4.0*10^5$ CFU/object. This concentration corresponded to the targeted contamination level and was sufficiently high to demonstrate a mean logarithmic reduction of the initial number of spores by 4 log.

Table 1 Initial concentration of *Aspergillus niger* DSM 1957 conidiospores on untreated stainless steel plates (reference samples)

Sample no.	CFU/Object
0.1	1.9E+05
0.2	5.7E+05
0.3	4.4E+05
Mean (N_0)	**4.0E+05**
SD	1.9E+05

The results of the UV-C treatment in the case are shown below, depending on the duration of the treatment (200-600 s), with the samples placed on the one hand with the contaminated surface facing the UV lamp (Table 2) and on the other hand directed upwards (Table 3).

Table 2 Reduction of *Aspergillus niger* DSM 1957 conidiospores on the stainless steel plates after UV treatment in the case under variation of the exposure time. The contaminated surfaces of the samples faced the UV lamp (about 5 cm from the wall of the case opposite the UV lamp).

200 s / faced to UV	CFU/Object	log_{red}
1.1	3.5E+01	4.1
1.2	1.2E+01	4.5
1.3	5.5E+00	4.9
arithm. mean	**1.7E+01**	**4.4**

400 s / faced to UV	CFU/Object	log_{red}
2.1	1.7E+01	4.4
2.2	6.8E+00	4.8
2.3	<1.4E+00	>5.5
arithm. mean	**8.2E+00**	**4.7**

600 s / faced to UV	CFU/Object	log_{red}
3.1	<1.4E+00	>5.5
3.2	2.7E+00	5.2
3.3	<1.4E+00	>5,5
arithm. mean	**1.8E+00**	**5.3**

In both cases, mean logarithmic reductions of the initial spore concentration by an average of 4.4 log (facing the radiator) and 4.2 log (directed upwards) could be achieved after a treatment time of 200 s. An extended UV treatment resulted in a higher inactivation. After 600 s, the mean logarithmic reductions were 5.3 log (facing the radiator) and 5.5 log (directed upwards) respectively.

Table 3 Reduction of *Aspergillus niger* DSM 1957 conidiospores on the stainless steel plates after UV treatment in the case under variation of the exposure time. The contaminated surfaces of the samples were directed upwards (about 7 cm from the wall of the case opposite the UV emitter).

200 s / directed upwards	CFU/Object	log_{red}
1.1	2.3E+01	4.2
1.2	2.0E+01	4.3
1.3	3.9E+01	4.0
arithm. MW	**2.7E+01**	**4.2**

400 s / directed upwards	CFU/Object	log_{red}
2.1	1.5E+01	4.4
2.2	2.7E+00	5.2
2.3	<1.4E+00	>5.5
arithm. MW	**6.4E+00**	**4.8**

600 s / directed upwards	CFU/Object	log_{red}
3.1	<1.4E+00	>5.5
3.2	1.4E+00	5.5
3.3	<1.4E+00	>5.5
arithm. MW	**1.4E+00**	**5.5**

Second trial on 21.07.2020

The inactivation performance against the spores of *Aspergillus niger* DSM 1957 was determined at three additional positions in the case and an irradiation time of 180 s.

The mean initial load of spores on the stainless steel plates was $3.0*10^5$ CFU/object. This concentration corresponded to the targeted contamination level and was sufficiently high to demonstrate a mean logarithmic reduction of 4 log. There were no significant deviations in the initial concentration of the reference samples observed (Table 4).

Table 4 Initial concentration of *Aspergillus niger* DSM 1957 conidiospores on untreated stainless steel plates (reference samples)

Sample no.	CFU/Object
0.1	1.4E+05
0.2	3.9E+05
0.3	3.8E+05
arith. mean	**3.0E+05**
SD	1.4E+05

The results of the UV-C exposure for a treatment time of 180 s are shown below. The samples were placed on the opposite side of the lamp with the contaminated areas facing to the left (90 ° to the lamp), middle (180 ° to the lamp) and right side of the case (270 ° to the lamp) (Table 5).

Table 5 Reduction of *Aspergillus niger* DSM 1957 conidiospores on the stainless steel plate after UV treatment in the case for 180 s

180 s / opposite the lamp	CFU/Object	$\log_{red}$
1.1	1.4E+02	3.3
1.2	1.4E+02	3.3
1.3	5.0E+02	2.8
arith. mean	**2.6E+02**	**3.1**

180 s / left of the lamp	CFU/Object	log_{red}
2.1	1.6E+02	3.3
2.2	1.5E+02	3.3
2.3	2.0E+02	3.2
arith. mean	**1.7E+02**	**3.3**

180 s / right to the lamp	CFU/Object	log_{red}
3.1	6.6E+01	3.7
3.2	1.7E+02	3.3
3.3	3.2E+02	3.0
arith. mean	**1.8E+02**	**3.2**

The UV-C exposure for 180 s on the opposite side of the lamp resulted in a mean logarithmic reduction of approx. 3.2 log at all three positions.

6 Conclusion

The performed test trials enabled to quantify the disinfection performance of a case (hygiene module KS101) with integrated UV-C lamp from Lacon Electronic GmbH. Black-pigmented spores of the mold *Aspergillus niger* DSM 1957 were used as test microorganism since they are known to exhibit a high resistance against UV radiation. Furthermore, they are recommended by the Association of German Machinery and Plant Engineering (VDMA) as test organisms for UV based sterilization processes (VDMA guidelines No. 11 / 2006). Sterile stainless steel plates were used as test specimens.

In the middle of the case, a mean logarithmic reduction of 4.4 log (99.9958%; contaminated area were faced to the UV lamp) or 4.2 log (99.9933%; contaminated area were upwards directed) could be demonstrated after 200 s. An extension of the irradiation time resulted in a higher inactivation. After 600 s, the proven mean reduction was 5.3 log (99.9996%; contaminated area were faced to the UV lamp) and 5.5 log (99.9997%; contaminated area were upwards directed).

On the opposite side of the UV-C lamp (furthest position), the mean logarithmic reduction after 180 s averaged 3.2 log, with the contaminated areas on the left (90 ° to the emitter), middle (180 ° to the emitter; facing away) and right wall (270 ° to the radiator) of the case.

Own studies with the spores of *Aspergillus niger* DSM 1957 and a UV-C low-pressure lamp (70 mW/cm² at 2 cm) have indicated a decimal reduction dose (D

value, reduction by 90%) of 134.4 mJ/cm². Cortesão et al. (2020) determined a D value of 103.8 mJ/cm² for spores of *Aspergillus niger* ATCC 64974.

According to Hessling et al. (2020), the dose for a reduction of coronaviruses by 90% (D value; UV radiation at 254 nm) is between 3.7-10.6 mJ/cm², depending on the virus strain used and the test conditions examined (e.g. various surfaces, aerosol). These data indicate that the black pigmented spores of *Aspergillus niger* are about 10 times more resistant to UV-C radiation (based on the higher D value).

Objects must be placed in the hygiene module in such a way that the radiation is not shielded, as shadow effects can reduce the disinfection efficiency.

Literature:

Heßling M, Hönes K, Vatter P, Lingenfelder C. Ultraviolet irradiation doses for coronavirus inactivation - review and analysis of coronavirus photoinactivation studies. GMS Hyg Infect Control. 2020;15

Cortesão M, de Haas A, Unterbusch R, Fujimori A, Schütze T, Meyer V and Moeller R (2020) Aspergillus niger Spores Are Highly Resistant to Space Radiation. Front. Microbiol. 11:560.

7 Signatures

Fraunhofer Institute for Process engineering and Packaging

Freising, 01.10.2020

Peter Muranyi
Digital unterschrieben von Peter Muranyi
Datum: 2020.10.02 11:32:09 +02'00'

Dr. Peter Muranyi
(Deputy Head Dept. Retention of Food Quality)

Johanna Weidlich
Digital unterschrieben von Johanna Weidlich
Datum: 2020.10.02 11:53:29 +02'00'

B. Sc. Johanna Weidlich
(Dept. Retention of Food Quality)