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Faculty of Natural Sciences and Mathematics
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BACTERICIDAL, SPOROCIDAL AND FUNGICIDAL ACTIVITY OF A DEVICE USING UVC LIGHT

- report from the evaluation of the device-

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INTRODUCTION

Physical properties of ultraviolet radiation

Ultraviolet radiation (UVR) is electromagnetic radiation that is characterized by its spectrum, which is the distribution of radiation intensity through its wavelength (or frequency). UVR is in the wavelength range of 400 nm to 100nm (respectively with a frequency between 750 THz and 3 PHz).

Table 1. Defining different spectral regions of electromagnetic radiation.

range	wavelength (nm)	frequency (Hz)
radio waves	10^{13}-10^{9}	$3*10^{4}$-$3*10^{11}$
microwaves	10^{9}-10^{6}	3*108-$3*10^{11}$
infrared waves	10^{6}-780	$3*10^{11}$-$3{,}8*10^{14}$
visible light	780-400	$3{,}8*10^{14}$-$7{,}5*10^{14}$
ultraviolet light	400-100	$7{,}5*10^{14}$-$3*10^{15}$
X-rays	< 10	> $3*10^{16}$

The lenghts of UV waves are shorter than those of visible light, but longer than X-rays, i.e. UV wavelengths are in the region 400-100 nm (Table 1). UV is found in sunlight, but can also be produced by some technical devices, such as water and air disinfection devices. To better study the various physical and biological effects, the UV wavelength range is divided into three main ranges A, B and C. According to ISO-21348, the following division is used:

- UV-A (400 nm–315nm)
- UV-B (315nm –280nm)
- UV-C (280nm –100nm)

UV-C is often divided into:

- far UV (FUV) (280 nm –200 nm),
- vacuum UV (VUV) (200 nm –100 nm)

Sunlight is absorbed as it passes through the earth's atmosphere. As a result, radiation at wavelengths below 280 nm (UV-C) is filtered through the stratospheric ozone layer, and UV radiation reaching the earth's surface is mainly composed of UV-A and UV-B. The amount and spectrum of UV radiation reaching the Earth's surface varies around the world and varies depending on altitude, season, time of day, atmospheric ozone and cloudiness.

(http://earthobservatory.nasa.gov/Features/UVB/uvb_radiation3.php; http://www.who.int/uv/uv_and_health/en/).

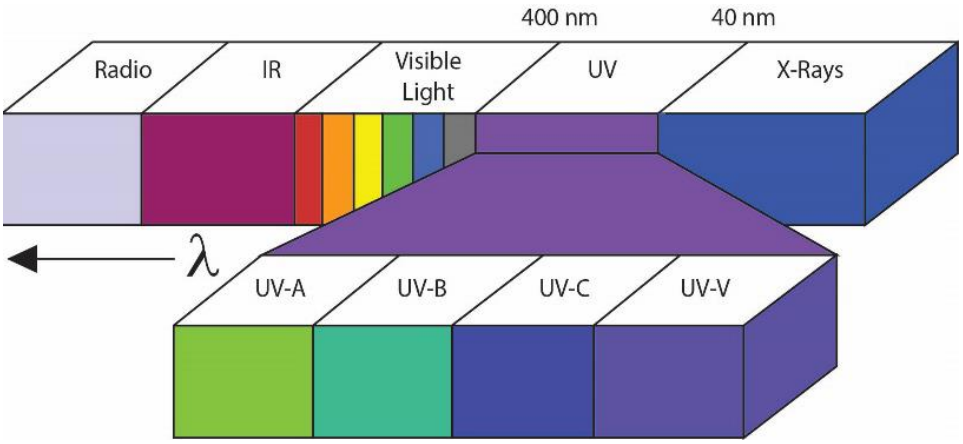

Figure 1. The place of UV light in the electromagnetic spectrum.

Ultraviolet light (UV) has been used as a disinfectant since the mid-20th century, but its microbicide power has been known for a long time, dating back to the mid-19th century. It is used for disinfection of drinking water and wastewater treatment, air disinfection, treatment of fruit and vegetable juices, as well as for a huge number of home disinfection devices, from toothbrushes to tablets and computers. Within the research facilities, UV is used in laboratories and in biological cabinets, in which biological material is manipulated.

Ultraviolet radiation has been known for decades as a terminal disinfection method, widely used in various applications, including health care, agriculture, water and food treatment, in airlines, etc.

Table 2. UV light range and its application.

200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 780 800

UVC | UVB | UVA | VISIBLE LIGHT | INFRARED

UV-C (200–280 nm)	UVB and UVA (280–400 nm)	Visible & Infrared (400–800 nm)	
• Decontamination & disinfection of surfaces	• Bacterial identification	• Chromatography (HPLC)	• Microplate readers
• Water disinfection	• Fluorescence	• Flash chromatography	• Microscopy
• Sterilization	• Medical imaging of cells	• Spectroscopy	• Transilluminator
• DNA analysis	• Medical diagnosis	• Optical detection	• Polarimetry
• Fluorochemistry	• Drug discovery	• UV/Vis	• Ellipsometry
• Mercury detection	• DNA sequencing	• Protein analysis (DNA)	• Reflectometry
• Sulphur detection	• Detection of food contamination	• Nucleic acid analysis	• Atomic absorption
	• Nucleic acid visualization	• Evaporative light scattering detector (ELSD)	
	• UV curing		

UV technology, in order to become safer and more cost-effective to use, has advanced greatly in recent years. Today, appliances that work on this principle are able to maintain the power output of UV lamps much longer than in the past, UV lamps today have a much longer lifespan, and all this allows UV systems to become more cost-effective for widespread use.

The use of UV has also emerged as an invaluable option for preventing the spread of nosocomial infections, providing disinfection of them in addition to existing cleaning methods. The use of ultraviolet light for surface disinfection is constantly increasing due to its ease of use, short time doses and wide efficiency.

How does UV light work?

Ultraviolet light exists within the light spectrum between 10 and 400 nm. The germicidal range of UV is in the wavelength range 100-280nm, known as UVC; This radiation is used in a growing number of appliances that include water and air disinfection, food processing and air conditioning.

Among these wavelength ranges, UVC has been shown to be most effective against microorganisms. The UVC wavelength, 250-270nm, is strongly and primarily absorbed by the nucleic acids of microbial cells leading to DNA damage and cell death. UVC is known to be a safer choice compared to UVA and UVB light which have been shown to be carcinogenic. This is because UVC radiation is absorbed by the outer dead layer of human skin, while UVB and UVA radiation penetrate deeper.

The maximum germicidal activity was observed at 265 nm. This range of UV light is absorbed by the DNA and RNA of microorganisms, causing changes in the structure of DNA and RNA, making them unable to replicate. A cell that cannot reproduce is considered dead because it is unable to multiply to an infectious number of cells, which could cause a particular infection and disease in the host. This is why UV disinfection is sometimes called ultraviolet germicidal irradiation (UVGI).

The microbicidal mechanism of UV-C occurs by damaging their RNA and DNA, often leading to the formation of dimers between pyrimidine residues in nucleic acid strands. The consequence of this modification is the appearance of dimers of cyclobutane pyrimidine (CPD), which causes deformation of the DNA or RNA molecule, which in turn can cause defects in cell replication, which will lead to cell death.

Damage of DNA by UVC

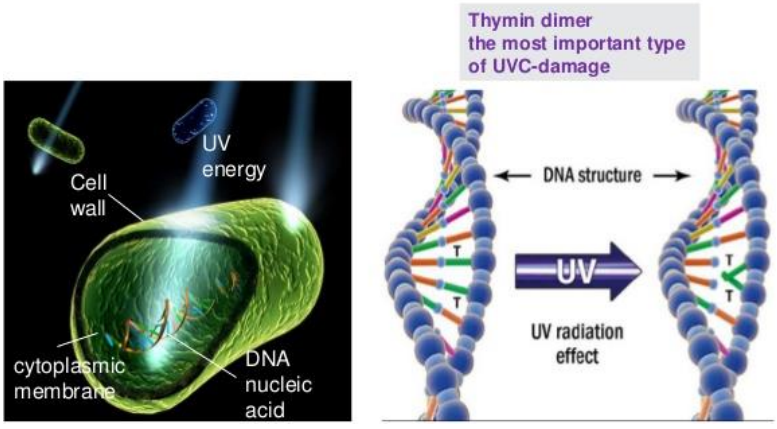

Figure 2. Mode of action of UV light on microbial cells.

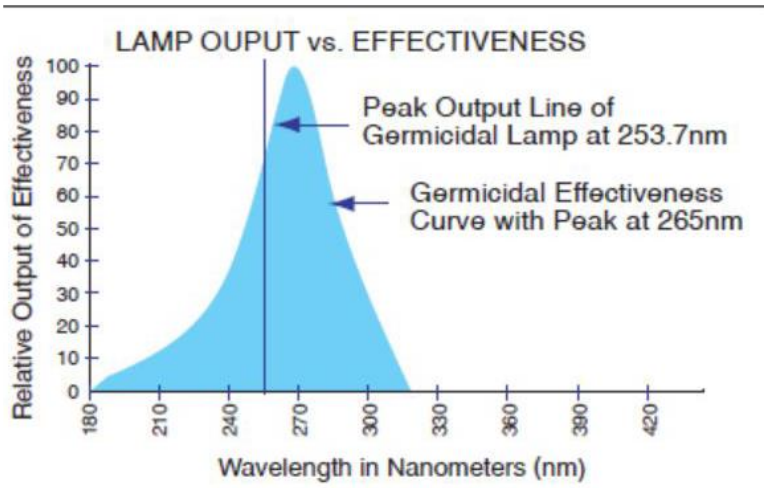

Figure 3. Overview of the relative microbicidal efficiency of the UV spectrum.

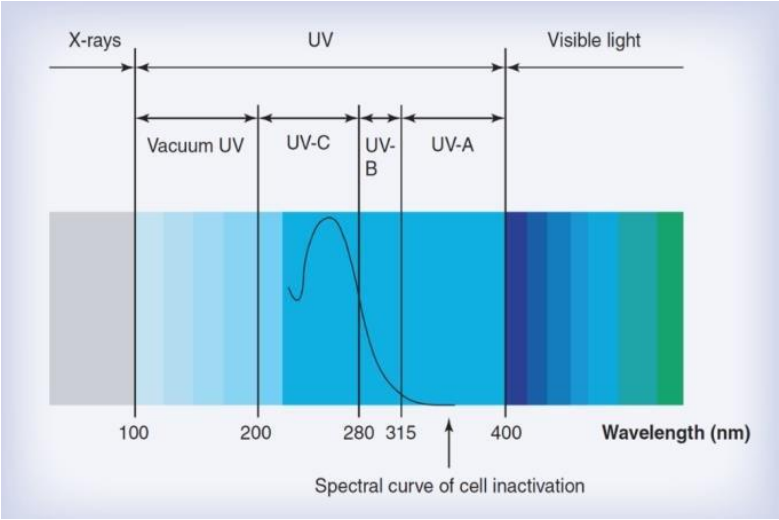

Figure 4. Spectral line of microbial inactivation with peak at 265 nm.

Legal background for the use of UV rays

The marketing of UVC lamps is regulated by Directive 2014/35 / EC, the so-called Low Voltage (LDV) Directive, for electrical equipment designed for use in defined voltage ranges. The main product safety directive 2001/95 / EC applies to UV-C lamps whenever the LVD is not applicable. Consumer products are required to provide reasonably expected safety throughout the life of the product. The authorities of the Member States responsible for implementing these Directives are required to carry out controls to ensure compliance by the relevant economic operators.

European harmonized standards related to UV-C lamps are voluntary, but if published in the Official Journal of the European Union (OJEU), they provide a presumption of compliance with the relevant basic safety requirements in the relevant EU legislation. However, applicable product standards still do not address some specific safety risks of UVC lamps. For example, the standard EN 60335-2-109 for UVC water treatment

appliances, which includes pond filters, excludes repair and replacement kits from its range.

To whom is UV light effective?

Within the distribution of ultraviolet light (10-400 nm), UVC (100-280nm) has the largest disinfection capacity (with the greatest effect at 265nm). UVC light is absorbed by RNA and DNA in cells, causing a change (apoptosis) in the structure of DNA / RNA as a result of their inability to replicate. Many microorganisms have been shown to be susceptible to inactivation using UVC light, including (in order of ease of inactivation) bacteria, viruses, molds, and spores. The amount of inactivation is directly proportional to the UVC dose received, which in turn is a result of its intensity and duration of exposure. The further away the light source is, the less UVC will reach the target. UVC radiation has a short wavelength and high energy, compared to other UV radiation, which allows it to work best in a direct line and over a short distance. Due to the high energy of UVC radiation, it is bound by the law of inverse squares, where the propagation of light intensity decreases exponentially with increasing distance from the light source. This means that objects near the light source will have greater exposure, hence shorter disinfection cycles compared to more distant objects. The reflection rate of UV radiation is also low, so shaded areas are likely to require longer periods of exposure to reach the same level of disinfection as in areas in a straight line and at the same distance from the light source. Any object between the light source and the target will block UVC. Conversely, to some extent, UV light can be reflected from surfaces to reach even the back of objects. This reflection capacity is highly dependent on the surface material. For example, organic material will absorb penetration and block UVC reflection, so surfaces need to be cleaned by hand to remove organic matter before decontamination.

UV light has been proven to be effective against a wide range of microorganisms. Viruses contain RNA or DNA and are therefore susceptible to radiation. Bacteria and molds contain DNA and are also vulnerable to UV light. Spores are also susceptible to UV. Bacteria are generally easier to inactivate than viruses, while molds and spores are a little harder to inactivate with UV.

Protection

Because UVC provides radiation, it is not safe to be in the room while disinfection is taking place. UVC is classified as "reasonably predicted to be human carcinogen" by the National Toxicology Program. It poses a danger to the skin and eyes, so direct exposure to UVC should always be avoided. UVC is blocked by a number of materials, including glass (but not quartz glass) and the clearest plastic, so it is possible to safely observe UVC if goggles are provided as protection.

The disinfection process itself is environmentally friendly because there are no hazardous or toxic chemicals that require special storage or handling. As no chemicals are added to the air / water / surface, there are no by-products that would have a detrimental effect. UV lamps do not require special handling or removal either, which makes the system a green alternative to chemical disinfectants. UVC provides residue-

free disinfection, so there is no concern about hazardous residues that should be removed or neutralized after disinfection.

There is concern about the odor generated after the premises are disinfected with ultraviolet light. This odor is associated with the presence of ozone. In reality, this odor is due to a UVC reaction with human dead skin cells and hairs found in room dust.

Up to 80% of airborne dust in homes, offices and other indoor environments consists of dead human skin and hair. Skin and hair cells consist of keratin, a protein, while hair also contains cysteine, an amino acid. When high-energy UVC light hits keratin / cysteine molecules, it has enough power to break their internal chemical bonds, creating smaller sulfur-containing compounds that fall into the thiol category. The human nose is extremely sensitive to thiols and can detect them in concentrations up to 1 billion. Concentrations of thiol molecules after UVC disinfection are negligible when compared to the acceptable exposure limit. This means that any odor present after UVC disinfection is not dangerous, so the room can be entered immediately after UVC disinfection..

Benefit from using UV rays

Although there are definite limitations to UVC disinfection technologies, there are many benefits. Disinfection time is fast, with a typical disinfection cycle lasting up to 15 minutes. This allows extremely fast turnaround times for rooms that are disinfected. Due to its simplicity, UVC disinfection is extremely easy to perform. All surfaces at a certain distance for a certain time will be successfully disinfected until the light is blocked to radiate on that surface.

UV systems require little maintenance due to their simplified nature. UV bulbs have a long service life, reducing the need for routine replacement and maintenance of consumables.

Disadvantages

While UV is effective in inactivating a wide range of microorganisms, there are limitations to its use. Because it involves light waves, UV works in a "visible" way, irradiating only surfaces that are in the field of view of the rays. The rays could be blocked if various objects were found in the path of the UV rays, much like a beach umbrella blocks the sun and offers protection from it. These areas in which UV light is blocked are called "shadow areas". Surfaces in these "shadow areas" are not properly disinfected because UV light does not have the ability to act at full effect.

Shaded areas can be resolved by moving the UV light source to another position to accommodate disinfection of surfaces blocked by the first disinfection cycle. UV light also does not penetrate well into organic materials, so for best results, UVC should be used after standard room cleaning to remove organic materials from surfaces.

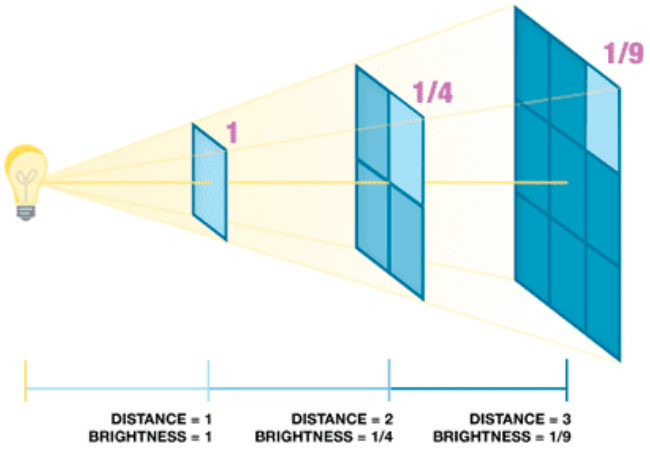

Figure 5. Law of inverse squares.

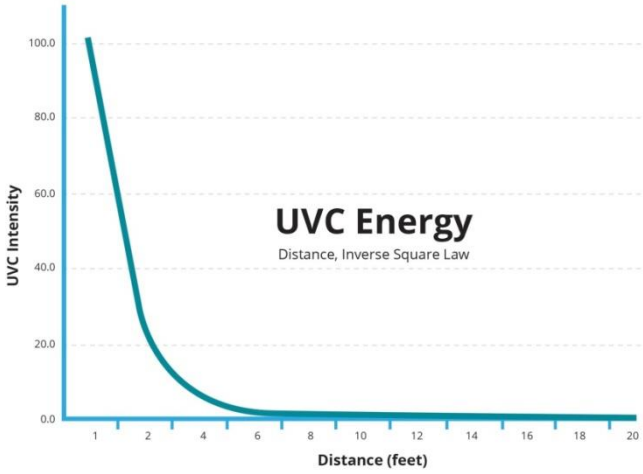

Figure 6. Action of UV light depending on the distance from the source.

EVALUATION DEVICE

A device from the company CLEAN MAX is used to evaluate the germicidal ability of certain microorganisms, in conditions when they are in the form of pure suspension, or applied to a certain type of material. The mobile automated UV-C device we use in this study was developed for decontamination and disinfection of mattresses, and is expected to enable rapid, automatic disinfection of mattresses.

It has the following performance:

Germinator 1 - Device for disinfection of surfaces and objects with the help of Ultraviolet radiation in C electromagnetic spectrum (UVC).

- 8 UV lamps with power of 40W per lamp (total 320W)- UV-C- wavelength is 254 nm

- disinfection area is 0.172 square meters

- distance of lamps from the surface: 2,5 cm

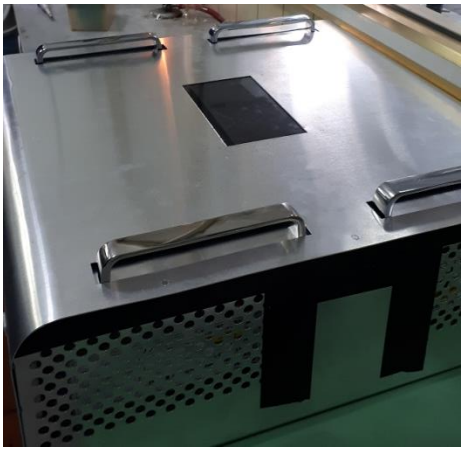

Figure 7. Evaluation device.

METHODS FOR EVALUATING THE EFFICIENCY OF THE DEVICE

Test microorganisms

The microorganisms used for these studies are *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, spores of *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404.

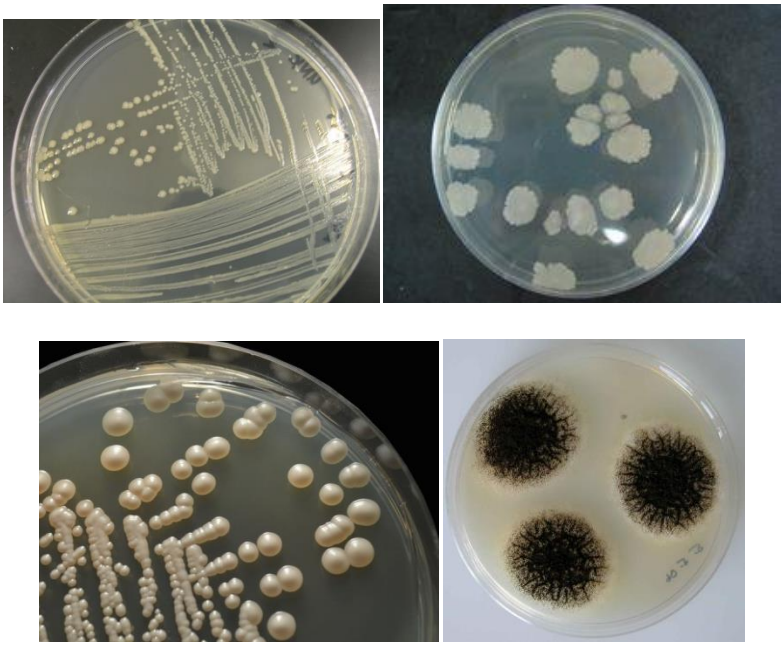

Figure 8. Test microorganisms used in this study: Escherichia coli ATCC 8739, Bacillus subtilis ATCC 6633 (vegetative cells and spores), Candida albicans ATCC 10231, Aspergillus niger ATCC 16404.

Preparation of test microorganisms

E. coli ATCC 8739 and B. subtilis ATCC 6633 were cultured on Nutrient Agar Medium (NA) at 24 h / 37 ° C.
C. albicans ATCC 10231 was cultured on Sabouraud Dextrose Agar (SDA), 3 days / room temperature, while Aspergillus niger ATCC 16404 was cultured on Sabouraud Dextrose Agar (SDA), 5-7 days / room temperature. At the end of the incubation period, the bacterial and yeast cultures were collected in sterile saline (0.85% NaCl), while Aspergillus niger ATCC 16404 was collected in sterile saline with 0.05% Tween 80. The density of the suspensions was adjusted to 0 , 5 McFarland standard (~1.5 × 108 CFU / ml).

Preparation of bacterial spores

The suspension of Bacillus subtilis ATCC 6633 was heated to 70 ° C for 30 min. to inactivate vegetative forms. Spore suspension density was adjusted to 0.5 McFarland standard (~1.5 × 108 CFU / ml).

CONDUCTED TESTING

1. DETERMINING APPROPRIATE CONTACT DEVICE OPERATION TIME

Use a 10 ml suspension of the appropriate microorganism test which is placed in a sterile Petri dish. The suspension thus prepared is exposed directly to the UVC effect of the test device. The exposure time being tested is 5 sec, 10 sec, 30 sec, 60 sec, 90 sec.

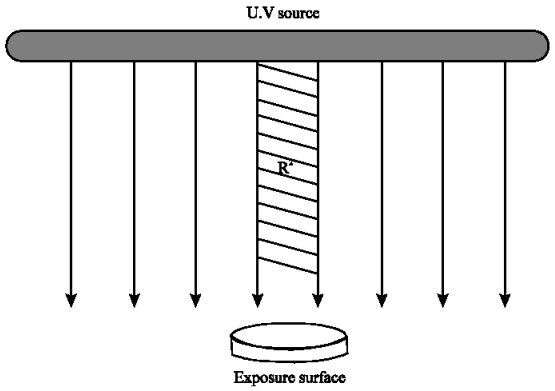

Figure 9. Setting up the experiment to determine the appropriate contact time.

To determine the exact number of surviving cells after UV-C treatment, a dilution series of the treated suspension is prepared, and seed is diluted from each dilution.

For each microorganism, 10x serial dilutions were made (10−1 to 10−8 for bacteria and 10−1 to 10−6 for eukaryotes) in sterile 0.85% saline. For all microorganisms, 1 ml of the appropriate suspension dilution was used as an inoculum for further sowing of microorganisms.

The pour plate technique was used to count the colony-forming units (CFUs) of the tested microorganisms. After homogenization and solidification, the Petri dishes are

incubated at an appropriate temperature. Vessels containing between 10 and 300 colonies are counted. The number of microorganisms / spores in mL is calculated by multiplying the amount of inoculum (1 mL) by the number of m.o counted. (CFU) and divided by dilution. The test is repeated three times.

Figure 10. Pour plate technique for counting colony-forming units (CFU) of tested microorganisms.

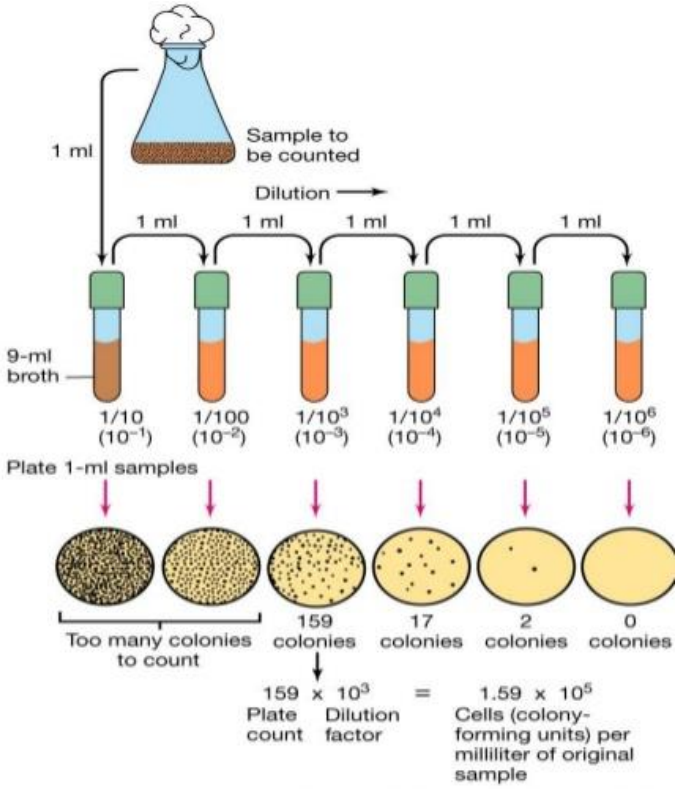

Figure 11. Pour plate method protocol for determining the number of microorganisms in a given sample.

DETERMINATION OF BACTERICIDAL, SPOROCIDAL AND FUNGICIDAL EFFECT OF THE DEVICE ON TEST MICROORGANISMS APPLIED ON DIFFERENT MATERIALS

The test is performed in two different experimental environments, in the presence and absence of organic matter, according to the standards EN14561 and EN14562. In the absence of organic matter, the test is carried out by adding 0.3 g / L bovine serum

albumin as an interfering substance. To test the effect in the presence of organic matter, 3 g / L bovine serum albumin and 3 mL / L erythrocytes are used.

For this purpose, 0.9 mL of inoculum (~1.5 × 108 CFU / ml) is mixed with 0.1 mL of the interfering substance for each treatment individually (with and without organic matter).
50 ?L of the mixture is applied on an area of 1 cm2 of the tested material (canvas, wood and stainless steel) and left to dry at room temperature (15-45 min).

The test material is exposed to UVC (contact time depends on the test organism - predetermined). Then, the pieces of material are transferred to sterile Petri dishes and washed with 10 mL of phosphate buffer / 5 min. Then, a dilution series is prepared and sown. CFUs are determined after appropriate incubation.

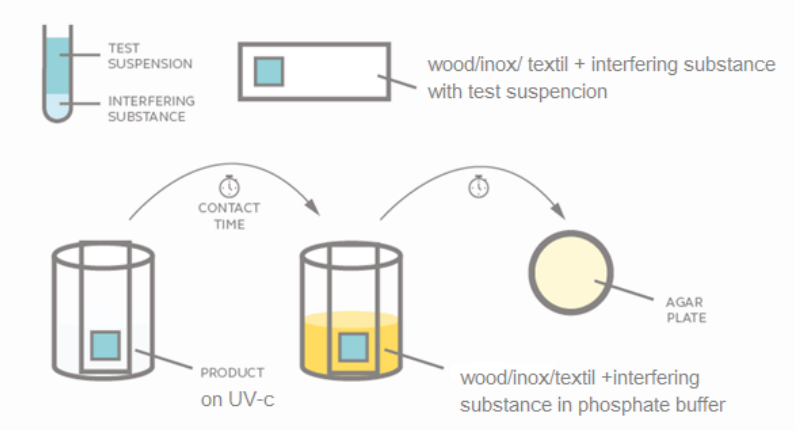

Figure 12. Schematic representation of test EN14561 and EN14562.

RESULTS FROM DEVICE EVALUATION

The mobile automated UVC device we use in this study was developed for decontamination and disinfection of mattresses, and is expected to enable rapid, automatic disinfection of mattresses. For that purpose, the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Sciences and Mathematics, UKIM, Skopje, conducted a series of tests in order to perform a complete evaluation of the submitted test device, an innovation of the company CLEAN MAX.

In order to determine and evaluate the activity of the delivered device on certain microorganisms, two sets of tests were conducted;

1. test in conditions where the test microorganisms are in the form of pure suspension, in order to determine the appropriate contact time required for UV-C treatment to carry out disinfection of the same

2. test in conditions when the test microorganisms are applied to a certain type of material (textile, stainless steel, wood) as carriers of the same, in order to determine the efficiency of the device for bactericidal, fungicidal and sporocidal activity.

1. DETERMINING APPROPRIATE CONTACT DEVICE OPERATION TIME

In order to determine the contact time required for exposure of the test micro-organisms when they are in the form of pure culture, each examined micro-organism, in suspension at a precisely determined concentration (1.5 * 108 CFUs / ml), was individually exposed to the action of UVC. Five different contact times (5, 10, 30, 60, and 90 seconds) were tested, followed by a dilution series, and three replicates were sown from each dilution to determine the number of surviving vegetative cells/ spores, after each time of exposure, i.e. to calculate the % of reduction in the number of vegetative cells / spores.

Table 3. Results of the conducted test to determine the appropriate contact time.

tested microorganisms	untreated sample (CFUs/ml)	treated sample (CFUs/ml)					% of reduction
		Contact time (sec)					
		5	10	30	60	90	
vegetative cells from bacteria							
E. coli ATCC 8739	$1,5*10^8$	2960	/	/	/	/	99.998027%
B subtilis ATCC 6633	$1,5*10^8$	3860	/	/	/	/	99.997427%
bacterial endospores							
B subtilis ATCC 6633	$1,5*10^8$	/	3820	/	/	/	99.997453%
eukaryotic microorganisms							
C. albicans ATCC 10231	$1,5*10^8$	/	5280	/	/	/	99.99648%
A. niger ATCC 16404	$1,5*10^8$	/	/	720	/	/	99.99952%

According to the results shown in Table 3, it can be clearly seen that the contact time in vegetative cells of bacteria is 5 seconds with a reduction percentage of 99.998027% for E. coli ATCC 8739, i.e. 99.997427% for B. subtilis ATCC 6633, while for reduction of spores of B. subtilis ATCC 6633 take 10 seconds, as these are resistant forms that allow the bacterium to survive in adverse conditions. Regarding the exposure time of the tested eukaryotic microorganisms to UVC, it is 10 seconds for C. albicans ATCC 10231, and slightly higher, 30 seconds for A. niger ATCC 16404, with a reduction rate of 99.99648% or 99.99952 %, respectively. These slightly increased exposure times of microorganisms to UVC are to be expected, as they are eukaryotic microorganisms that require slightly longer exposure times.

However, during this research we showed that the CLEAN MAX device successfully inactivates the tested microorganisms and reduces the number of living vegetative cells, as well as spores up to 99.99%, with a contact time of only 30 seconds, which is quite short exposure time, taking into account the nature of the microorganisms tested. Namely, the bacterium E. coli belongs to the group of Gram-negative bacteria, which are generally known as more resistant bacteria, since they have, in addition to the peptidoglycan layer in the cell wall, an additional outer membrane. On the other hand, the bacterium B. subtilis ATCC 6633, although it belongs to the group of Gram-positive bacteria, which are generally considered to be more sensitive bacteria, is still a genus that has the ability to create spores when the bacterium itself is in unfavorable conditions, which among other things is this UVC radiation.

The ability of partial resistance of bacterial endospores is reflected in the time it takes for them to be exposed to this radiation, which differs from the time of exposure of vegetative cells - five versus ten seconds - for bacterial endospores.

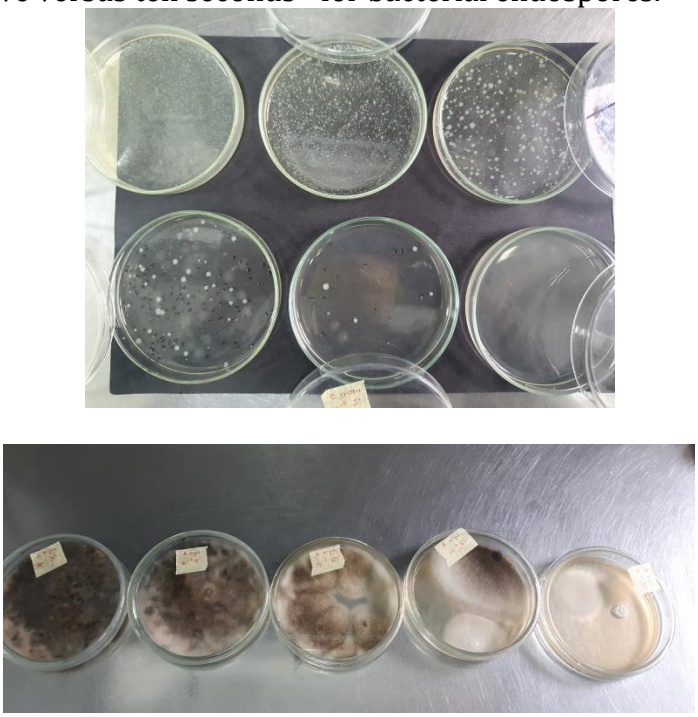

Figure 13. Reduction of the number of tested microorganisms after UVC treatment.

As for the eukaryotic microorganisms, given the complexity of the structure of their cells, a slightly longer contact time was to be expected, which was proven during this experiment. To achieve a 99.99% reduction for C. albicans ATCC 10231 took 10 seconds of exposure, while for A. niger ATCC 16404 it took 30 seconds, which however, given the nature of the microorganisms (A. niger ATCC 16404 is considered for a fairly resistant mold that is difficult to eradicate) is a very short exposure time.

1. DETERMINATION OF BACTERICIDAL, SPOROCIDAL AND FUNGICIDAL EFFECT OF THE DEVICE ON TEST MICROORGANISMS APPLIED ON DIFFERENT MATERIALS

This test is performed in accordance with the standards EN14561 and EN14562, according to which the test is performed in two different experimental environments, i.e. in the presence and absence of organic matter. In the absence of organic matter, the test is carried out by adding 0.3 g / L bovine serum albumin as an interfering substance. To test the effect in the presence of organic matter, 3 g / L bovine serum albumin and 3 mL / L erythrocytes are used. After the appropriate exposure to UVC (for each microorganism according to the results obtained from the first test) a dilution series is made, and from each dilution three replicates are sown, in order to determine the number of remaining living vegetative cells / spores, which were previously applied on different materials, i.e.% of reduction of the number of vegetative cells / spores is calculated.

Table 4. Results of the conducted test to determine the bactericidal, sporocidal and fungicidal effect of the device on test microorganisms applied to different materials

tested microorganisms	untreated sample	treated sample (CFUs/ml)					
		material with BSA			material with BSA + blood		
	(CFUs/ml)	canvas	wood	stainless steel	canvas	wood	stainless steel
vegetative cells from bacteria							
E. coli ATCC 8739	$1,5*10^8$	$469*10^3$	$188*10^3$	$23*10^1$	$256*10^4$	$132*10^4$	$58*10^3$
% of reduction		99.6874	99.8747	99.9999	98.2934	99.12	99.9614
B subtilis ATCC 6633	$1,5*10^8$	$695*10^3$	$422*10^3$	$59*10^1$	$490*10^4$	$330*10^4$	$69*10^3$
% of reduction		99.5367	99.7187	99.9996	96.7334	97.8	99.954
bacterial endospores							
B subtilis ATCC 6633	$1,5*10^8$	$779*10^3$	$503*10^3$	$86*10^1$	$661*10^4$	$449*10^4$	$87*10^3$
% of reduction		99.4807	99.6647	99.9994	95.5934	97.0067	99.942
eukaryotic microorganisms							
C. albicans ATCC 10231	$1,5*10^8$	$271*10^3$	$313*10^3$	$74*10^3$	$612*10^4$	$415*10^4$	$121*10^3$
% of reduction		99.8194	99.7914	99.9507	95.92	97.24	99.9194
A. niger ATCC 16404	$1,5*10^8$	$391*10^3$	$212*10^3$	$99*10^3$	$660*10^4$	$522*10^4$	$231*10^3$
% of reduction		99.7394	99.8587	99.934	95.6	96.52	99.846

From the results that were implemented according to EN14561 and EN14562 method, which includes examination of the bactericidal, sporocidal and fungicidal effect of the device on test microorganisms applied to different materials, in the absence and presence of interfering organic matter we can conclude the following:

Of the three tested materials (canvas, wood and stainless steel) on which the correct concentration of test organism was applied (1.5 * 108 CFUs / ml of Escherichia coli ATCC 8739, Bacillus subtilis ATCC 6633, spores of Bacillus subtilis ATCC 6633, Candida albic 10231, Aspergillus niger ATCC 16404) the canvas proved to be the most difficult material to remove the tested microorganisms, while stainless steel proved to be the material from which all the tested microorganisms were most easily removed, whether the material contained organic matter as an interfering material or not.

As for the tested microorganisms, Escherichia coli ATCC 8739 proved to be the most easily removed microorganism from any material.

As for the interfering material, as expected, the organic matter to some extent has a protective effect on the survival of all tested microorganisms, and therefore for all

tested materials, in the presence of interfering organic matter the reduction was slightly reduced. , and ranged from 95.5934% in Bacillus subtilis ATCC 6633 spores to 99.9614% in Escherichia coli ATCC 8739 applied on stainless steel.

CONCLUSION

From the evaluation of the germicidal ability on certain microorganisms, in conditions when they are in the form of pure suspension, or applied to a certain type of material, with the help of a UVC device, CLEAN MAX innovation can be determined that the mobile automated UVC device is compatible with the function for which it was developed, ie it shows a high percentage of efficiency in terms of decontamination and disinfection of mattresses, which would achieve fast, automatic disinfection of the same.

The tested device requires an efficiency in the range of 99.4807- 99.9999% of reduction of microorganisms when they are in conditions without the presence of interfering organic matter incorporated in the material in which the tested microorganisms could be found, and from 95.5934-99.9614 microorganism reduction when they are in the presence of interfering organic matter incorporated into the material in which the tested microorganisms are located.

According to the results obtained from this evaluation, the bactericidal, sporocidal and fungicidal activity of the device that uses UVC light in order to obtain completely decontaminated and disinfected mattresses can be confirmed, which would achieve fast, automatic disinfection of the same.

REFERENCES

Bolton J.R. and Linden, K.G. 2003. Standardization of methods for fluence (UV Dose) determination in benchscale UV experiments. J. Environ. Eng. 129(3): 209-216.

Chang, J.C.H., Osoff, S.F., Lobe, D.C., Dorfman, M.H., Dumais, C.M., Qualls, R.G. and Johnson, J.D. 1985. UV inactivation of pathogenic and indicator microorganisms, Appl. Environ. Microbiol., 49(6): 1361-1365.

Giese, N. and Darby, J. 2000. Sensitivity of microorganisms to different wavelengths of UV light: implications on modeling of medium pressure UV systems, Wat. Res., 34(16): 4007-4013.

Harris, G.D., Adams, V.D., Sorensen, D.L. and Curtis, M.S. 1987. Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria, Wat. Res.,21(6): 687-692.

Light Sources Inc and American Ultraviolet Company. UV Irradiation Dosage Table. Accessed from http://www.americanairandwater.com/uv-facts/uv-dosage.htm.

Mamane-Gravetz, H. and Linden, K.G. 2004. UV disinfection of indigenous aerobic spores: Implications for UV reactor validation in unfiltered waters, Wat. Res.,38(12): 2898-2906.

Maya, C., Beltran, N., Jimenez, B. and Bonilla, P. 2003. Evaluation of the UV disinfection process in bacteria and amphizoic amoebae inactivation, Wat. Sci. Technol.: Wat. Supply, 3(4): 285-291.

Sommer, R., Weber, G., Cabaj, A., Wekerle, J., Keck, G., and Schauberger, G. 1989. UV inactivation of micro- organisms in water. Zbl. Hyg. 189: 214-224.

Sommer, R., Haider, T., Cabaj, A., Pribil, W. and Lhotsky, M. 1998. Time dose reciprocity in UV disinfection of water, Water Sci. Technol., 38(12): 145-150.

Sommer, R., Cabaj, A., Sandu, T. and Lhotsky, M. 1999. Measurement of UV radiation using suspensions of microorganisms, J. Photochem. Photobiol., 53(1-3): 1-5.

Sommer, R., Lhotsky, M., Haider, T. and Cabaj, A. 2000. UV inactivation, liquid-holding recovery, and photoreactivation of *E. coli* O157 and other pathogenic *E. coli* strains in water, J. Food Protection, 63(8): 1015-1020.

UV-Light.co.UK, UV Light Technology Limited, https://www.uv-light.co.uk/uv-dose-required-for-inactivation-of-viruses-bacteria-moulds-etc/ accessed on 2-20-2018.

https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014L0035&from=EN

https://www.coi.cz/userdata/files/dokumenty-ke-stazeni/ec-decision-dangerous-products.pdf

https://www.etics.org/doc/doc_12607_main1_LVD%20Guidelines.pdf

https://www.ojeu.eu/

Со својот печат и своерачен потпис потврдувам дека овој превод правилно и верно го извршив од македонски на англиски јазик. Постојан судски преведувач Анета Јованоска Прилеп 07-ми јуни 2021	I, The undersigned, hereby confirm that the foregoing is a true and correct translation from Macedonian to English Language. Sworn Court Translator Aneta Jovanoska Prilep,07th of June 2021