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Investigation of disinfection success of an UV-C source on the moving model of robot „Puductor-2“ in hospital ICU rooms, assessment from a hygienic-microbiological point of view

Dear Dr. Sha He,

On February 2nd 2021 a realistic practical test was carried out in the intensive care unit of Bad Brückenau city hospital. The test was accompanied by bioindicators and contact tests, to be able to examine the effectiveness. The recommendation of the Commission for Hospital Hygiene and Infection Prevention at Robert-Koch-Institute (Federal Institute of Infection prevention) “Requirements of Hygiene at cleaning and disinfection of surfaces“ (2004) is used as legal basis. The on-site tests were carried out by the cooperation institute Hygieneinstitut Mainfranken, Maßbach. The production and evaluation of the bioindicators was in the responsibility of the Laboklin GmbH, an accredited laboratory in Bad Kissingen.
The robot presented for the test is the „Puductor-2“ version of the Pudu disinfection robot. According to the manufacturer’s instructions, the wavelength of the UV-C lamps is 254 nm. The energy (radiation intensity) emitted is 180 µW/cm = 0.18 mJ/cm^2 s = 10.8 mJ/cm^2 min at a distance of 1 meter.

1. Material

1.1 Bioindicators

The usual panel of potential pathogens for disinfection trials was partly not chosen here. Instead, *Enterococcus faecium* as a representative of the gram-positive cocci, *Escherichia coli* as a representative of gram-negative rods and mostly avirulent grampositive cocci *Micrococcus luteus*.

Justification for the selection of microorganisms

Enterococcus faecium is a fairly environmentally stable bacterium, that can survive up to 30 minutes even at 60 °C. In addition, it is comparatively antibiotic-resistant and has spread in southern Germany with the formation of the multi-resistant variant VRE. The bioindicators were deployed with an initial bactarial count of 5,5 x 10^7 Colony-forming units (CFU)/ indicator plate (see 1.2).

Escherichia coli is a comparatively environmentally stable bacterium, which is therefore used as a faecal indicator in drinking water samples. In hospitals, this bacterium usually tops the list of the most common pathogens. Resistant variants are referred to as MRGN. The bioindicators were applied with an initial bacterial count of 2,6 x 10^5 CFU / indicator plate (see 1.2).

Micrococcus luteus is a generally apathogenic inhabitant of mucous membranes of the nasal-throat space and the skin. It is particularly suitable for the study of UV-C radiation as surface sanitizer, as it has a pigmentation that protects it comparatively from the influence of these rays. Therefore, it is quite useful as “worst case” – indicator.

The bioindicators were applied with an initial bacterial count of 1,8 x 10^6 CFU / indicator plate (see 1.2).

1.2 Production of the indicator surfaces (plates)

The bacteria were taken from a bouillon with late-logaritmic inoculum and distributed by the louse on COS agar plates. The transport controls were carried out in exactly the same way (5 in each case for averaging). Subsequently, the respective starting germ count from the bouillon was determined and documented by means of standard dilution methods.

1.3 UV-C exposure

The bioindicators were placed in a set of three (one plate each with a germ) at the measuring points and the robot was started. The control of robots way was done manually.

The rooms were classic intensive care rooms, from which the typical furniture with bed, night box, perfusor tree, etc. had not been removed, but were equipped with the indicators as measuring points (5 each per room).

1.4 Contact tests

In addition, before and after surveys were carried out using the RODAC method (Replicating Organisms Detecting And Counting, contact plate). The natural contamination was used, so no other microorganisms were brought out as additional contamination.

1.5 Air ion measurement

During the disinfection process, air ionization was measured and differences were taken into account.

2. Results

2.1 Bioindicators

ID-Nr.	testgerm	CFU medium Colony-forming units (CBE)	rounded reduction in %
Removal location: room 249			
1	E.coli	0	> 99,999
1	E.faecium	572	99,98
1	M.luteus	12	99,99
2	E.coli	0	> 99,999
2	E.faecium	544	99,99
2	M.luteus	29	99,99
3	E.coli	0	> 99,999
3	E.faecium	11	99,999
3	M.luteus	5	99,99
4	E.coli	2	99,99
4	E.faecium	487	99,99
4	M.luteus	18	99,99
5	E.coli	0	> 99,999
5	E.faecium	1	> 99,999
5	M.luteus	0	> 99,999
Removal location: room 248			
11	E.coli	0	> 99,999
11	E.faecium	89	99,999
11	M.luteus	10	99,99
11 a	E.coli	0	> 99,999
11 a	E.faecium	21	99,999
11 a	M.luteus	4	99,999
12	E.coli	0	> 99,999
12	E.faecium	21	99,999
12	M.luteus	1	99,999
13	E.coli	0	> 99,999
13	E.faecium	284	> 99,999
13	M.luteus	21	99,99
14	E.coli	0	> 99,999
14	E.faecium	106	> 99,999
14	M.luteus	8	99,999

2.2 RODAC-contact plates

There were different measuring points (n=20, 10 per room) here. In order to obtain a statistically reasonably relevant statement in such investigations, the results of all measuring points are drawn together in one room. As a result, the following results of reduction were achieved in relation to the individual bacterial groups:

room	**before (CFU)**	**after (CFU)**	**difference %**
249	51	27	47 %
248	127	35	73 %
total	**178**	**62**	**65 %**

As expected, the smallest reduction was found in *Micrococcus luteus* and in the very robust spore-forming Bacillus group. *Staphylococcus aureus,* only very few isolates, also equipped with a UV-C-reducing pigment, however, was reduced quite well. For the individual bacteria, a summary was made by species or group (Bacillus, skin staphylococci = Coagulase Negative Staphylococci, CoNS) in order to be able to present possible differences in percentage.

The following picture emerged for the groups shown:

	before CBE	**after CBE**	**reduction %**
M. luteus	95	34	64
CoNS*	53	1	98
S.aureus	2	1	50
Bacillus group	4	4	0
Corynebacteria	7	7	0
fungi	0	0	-
Mix	**161**	**47**	**71**
Mix without M. luteus	*66*	*13*	*80 %*

*) group of skin staphylococci

2.3 Measurement of air ions

A shift to negative air ions is observed. Negative air ions are perceived as pleasant, as also produced by (camping) fires and represented in a high proportion in forest air.

3. Discussion and conclusion

UV irradiation was previously used in surgical areas to clean up areas and only left when there were new, potent, low-toxic liquid surface sanitizers with remanence. In principle, the surface disinfection method by UV-C is suitable if there are no shadows. Therefore, the approach of mounting the radiation source on a moving robot is rational and comprehensible. An official norm is not available.

Therefore, a test setup based on literature source 1 was chosen.

The bioindicators show the good effectiveness of the method in principle, with the values from source 1 still exceeding. The values of RODAC plates, which are less favorable to the bioindicators, result from the attachment of the pathogens to particles such as clothing fibers, skin scales and additionally protective droplet residues, skin fat, etc.

Compared to *Staphylococcus* and fungi, a disinfection effect according to literature source 2, compared to the spore-forming Bacillus group does not arise, nor against Micrococcus luteus, which was the literature based expected value.

The effectiveness of a UV-C-based method depends on the radiation intensity and the exposure time. During the first wave of the pandemic, UV-C was used to disinfect FFP2 and KN 95 masks, with both bacteria and viruses being targets (3). Subsequently, the reaction of a typical pathogen of pandemics and epidemics, namely the influenza virus, was tested for UV light (3). The reduction of viruses was also achieved with contamination with usually log > 3 (99.9%) the type of contamination was less relevant. The authors stress that prior to the establishment of such a procedure, careful evaluation is required.

In the meantime, there are increasing numbers of studies that directly address the coronavirus mentioned. Every time, a high sensitivity of the SARS-CoV-2 virus was detected (4, 5, 6, 7). The UV-C dose of 1,048 mJs/cm2 used in the study (4) was able to inactivate a number of 5×10^6 /ml in a applied suspension within 9 min.

An in vitro study determined the lethal (inactivating) doses of UV-C (254 nm) on SARS-CoV-2 viruses, but no test contamination was applied that would simulate saliva components in droplets, for example. The values collected were:

- LD_{90}: 0.016 mJ/cm^2.
- $LD_{99.999}$: 108.714 mJ/cm^2,

In both cases, the time of entry was less than 50 seconds (6).

In a meta-analysis, a large part of the available studies was evaluated. The aim was, among other things, to determine the limits of the required radiation. The authors concluded that the different results of the studies studied were due less to the UV sensitivity of the coronavirus, but to the different experimental setups. Ultimately, a 90% reduction is assumed to be achieved with a radiation of about 10.6 mJ/cm^2 (median), according to the study results, while the authors suspect that the required dose is actually 3.7 mJs/cm^2 (7).

The results of the bioindicators and bacterial killing in conjunction with the literature data suggest that enveloped viruses (influenza, coronavirus) are also inactivated. Statistically, a reduction of active viruses of at least 99 %, but depending on factors like exposition time, less dirty conditions an inactivation of > 99.9% is to be assumed (based on requirement of 2, data of study (6) and results of *Micrococcus luteus* indicators).

This is possible because the "Puductor 2" can reach the target areas closely for a chosen time, instead of just beaming stationary into the environment.

4. Literature

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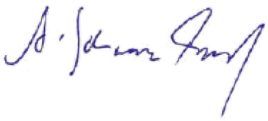
Kind regards



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