No-touch automated disinfection: test results using *254nm* UV-C light

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Summary

COVID 19 pandemic has led the world authorities, in particular health authorities, to totally reconsider the infectious problems in all public places and in particular in hospitals, that are also burdened by specific risks, due to the concentration of subjects at risk and the consequences that necessary treatments, such as resistance of infectious agents to the drugs used, may involve. Therefore, traditional and innovative methods were compared to obtain adequate protection from contaminants, paying particular attention to the disinfection method by means of ultraviolet rays emitted by a special robotic instrument named *R2S Robot*. The studies carried out on the subject show that UV-C method represents a rational, effective and economically sustainable choice to ensure adequate disinfection, not only of hospital environments in general but also of frequently visited environments such as public areas and institutions, schools of all types and levels , public and private offices, especially in the light of the new regulations that have totally changed the general approach and the degree of responsibility towards not only the regular visitors of the nosocomial environments, but also the dedicated operators.

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Introduction

The history of man and the biological world are intimately connected with the ancestral presence of an invisible but pervasive entity, sometimes useful but often a deadly enemy, made up of microbial agents.

This evidence was and still is felt and perceived as conditioning and terrible: just think of the notes of Thucydides' description of the plague occurred during the Peloponnesian war or the well-known contemporary pandemics starting from the so-called "*Spanish Flu*" up to SARS, MERS, and, alas, more recently COVID 19.

The urgency to deal effectively with the risk of being infected has pushed all researchers, but especially those who are on the front line against the pandemic to totally reconsider the methods used up to now to reduce the agents responsible for the contamination of the environments, structures, instruments and the same operators whose hands, although salvific, can be carriers of contagion. A particular care should be paid by healthcare professionals who, in addition to treating, have already paid a very high price in terms of human lives.

The pervasiveness of the specific infectious agent COVID 19 has put a strain on health facilities not only for its intrinsic danger but, above all, for the enormous workload required for individual, collective and environmental protection procedures and the consequent upheaval of the usual standard operating times.

Just think of the time needed to sanitize an X-ray room using traditional methods, after having performed an examination on a patient potentially or actually carrying the pathogen; currently it is possible to quantify the time required in about 60 minutes for each deep sanitation.

Equally long times are required for environments such as operating rooms, rooms dedicated to endoscopic procedures of the respiratory and gastrointestinal tract, respiratory physiopathology rooms, clinics for clinical examinations, etc.

International guidelines have identified specific risk conditions in these environments due to the high diffusion potential of aerosols carrying viral agents, capable of spreading at considerable distances from the origin:

> "*Recommendation from ERS Group 9.1 (Respiratory function Technologists/Scientists) Lung function testing during COVID-19 pandemic and beyond. The following is a recommendation for healthcare professionals performing lung function testing during the COVID-19 pandemic. Since transmission of Corona virus is mostly by contact, during lung function procedures transmission can occur also via aerosolized respiratory secretions (during cough and sneezing). Since the safety of our patients and staff is of paramount importance, we recommend additional safety precautions during testing. We are aware that these will lead to longer testing times, need for more consumables, result in*

reorganization of our daily practice and slow down the flow of the patient during his diagnostic workup".

Even the National Institute of Heath (ISS) did not fail to express itself on the matter with its specific document:

"*Ad interim recommendations on the sanitation of non-health facilities in the current COVID-19 emergency: surfaces, interiors and clothing".* May 15, 2020 Version.

National Health Institute Working Group on COVID-19 Biocides 2020, 28 p. ISS COVID-19 Report No. 25/2020

"The report presents an overview on sanitizing non-sanitary surfaces and interiors for the prevention of the spread of COVID-19 infection. The indications are based on the evidence available to date regarding the transmission of SARS-CoV-2 infection, the survival of the virus on different surfaces and the effectiveness of the products used for cleaning and disinfecting/sanitizing the premises. The indications also consider the environmental impact and the risks to human health associated with their use. The document also includes reccomendations on the treatment of textile fibers to be carried out on site (both clothing being tried on and non-hard surfaces such as upholstered furniture, curtains, etc.). The report specifies the terms used in the context of disinfection, clarifying the difference between disinfectant, sanitizer, sanitizer for environments and detergent".

However the problem is much wider than the current emergency as hospital infections are now a constant threat to public health, due to the constant growth of microbial agents thar are multi-resistant to antibiotics; therefore it appears indispensable and urgent to adapt sanitation methods of hospital environments to prevent a future catastrophe that could be even worse than the current emergency; in fact interiors and surfaces are regularly contaminated in hospital environments by multi-resistant organisms, responsible for a significant part of mortality due to infectious complications.

Many attempts have been made to develop alternative methods capable of ensuring a sufficient level of sanitation of environments, preventing the uncontrolled proliferation of pathogens that often led to the closure of entire hospital departments.

The necessary strategy to achieve a satisfactory level of safety, especially for the most vulnerable patients such as immunosuppressed and elderly ones, must take into account the imperfection of the correctly used procedures, which often leave even 30% of surfaces still contaminated. From this point of view the methods bases on chemical disinfectants are particularly unsuitable, also for the variation of the application of procedures.

The ideal system to address these serious needs could be a physical device that uses a "*no-touch*" technology not based on direct contact with chemical disinfectants, but rather on the use of UV-C radiation, capable of eliminating not only viruses but also more resistant bacteria.

The advantages of this method are clear, also in terms of savings, operation and result.

This method achieves the necessary result at a speed that represents a driving force and a huge multiplier for the overall operation of the hospital, which otherwise appears substantially blocked by the foregoing.

In this regard, the reader can refer to specific literature (1,2,3).

In the light of the foregoing, the Complex Structure of Pulmonology of USL Umbria 2 (Local Health Authority) has created an integrated working group of clinicians, medical laboratory technicians, engineers and technicians from companies specialized in innovative robotics technologies in Umbria (Italy).

With the synergy of mutual knowledge and excellent operational ability, an original project was developed to demonstrate the effectiveness on the field of innovative methods, such as the use of ultraviolet rays for the sanitization of infected environments actually present in our facilities.

The purpose of the document is to show the results of rapid disinfection tests using 254nm UV-C light performed on June 19 and 30, 2020 on multiple microbial agents obtained from laboratory cultures from real cases report of patients admitted to the Hospital.

The UV-C doses (expressed in mJ/cm^2) irradiated to inactivate the microbial agents tested were far higher than the dose required for the inactivation of SARS-CoV-2 virus (COVID 19), which is approximately 5 mJ/cm^2, as reported by recently published studies. Therefore, the UV-C doses irradiated to inactivate bacteria are also largely sufficient to inactivate the SARS-CoV-2 virus, as demonstrated by the tests carried out by various authors (4).

To say that the reached conclusions are excellent is an understatement. The steps that lead to them will be illustrated here below.

If the methods described were to be applied extensively, there would be a substantial change of the scenario, achieving great results in terms of safety, clinical operating result and also enormous savings that would allow to free-up considerable resources to be reused in Public Health.

Methodologies used for the tests

The first test taken on June 19, 2020 (Tables 1-3) and the second one taken on June 30, 2020 (Tables 3-6), were performed with Alert Organisms, microorganisms of epidemiological significance as they are potentially responsible for serious hospital infections

Table 1. Results of the first test (June 19, 2020): glass.

Material	GLASS				
Irradiation Time	05:20	minutes:seconds			
Distance in cm	120 - 145	50 - 60 mJ/cm²		Result	
Id	Strain		Gram +/-	Irradiat. UV-C	Not Irradiated
C1	Staphylococcus aureus MRSA		+	neg	> 1.000
C2	Pseudomonas aeruginosa MDR		-	neg	> 1.000
C3	Klebsiella pneumoniae KPC		-	neg	> 1.000

which could be transmitted through health workers operations and resistant to multiple classes of antibiotics and can be used as an index for environmental contamination.

The strains were taken from biological materials of hospitalized patients to simulate the potential of treatment in a realistic epidemiological situation in our hospital facility.

The materials that were chosen to evaluate the bactericidal power of this treatment are common in the healthcare environment, and usually have to undergo disinfection.

Such materials (glass, plastic and metal) were contaminated by bacterial suspensions.

In the days, preceding the simulation, various strains with genetic characteristics of resistance to multiple classes of antibiotics or all of them, a control strain and a bacillus, were selected from a variety of biological materials and cultivated on different mediums:

- *E. coli ATCC8739* (control strain used to standardize laboratory equipment)
- *Klebsiella pneumoniae* with genetic mechanism of resistance to KPC carbapenems
- *Pseudomonas aeruginosa* with genetic mechanism of resistance to MDR carbapenems
- *Acinetobacter baumanii MDR*
- *Staphylococcus aureus MRSA*
- *Bacillus cereus*
- *Enterococcus faecium VRE*

Table 2. Results of the first test (June 19, 2020): plastic.

Material	PLASTIC				
Irradiation Time	05:20	minutes:seconds			
Distance in cm	120 - 145	50 - 60 mJ/cm²		Result	
Id	Strain		Gram +/-	Irradiat. UV-C	Not Irradiated
C1	Staphylococcus aureus MRSA		+	neg	> 100
C2	Pseudomonas aeruginosa MDR		-	neg	2
C3	Klebsiella pneumoniae KPC		-	1	26

Table 3. Results of the first test (June 19, 2020): metal.

Material	METAL				
Irradiation Time	05:20	minutes:seconds			
Distance in cm	120 - 145	50 - 60 mJ/cm²		Result	
Id	Strain		Gram +/-	Irradiat. UV-C	Not Irradiated
C1	Staphylococcus aureus MRSA		+	neg	neg *
C2	Pseudomonas aeruginosa MDR		-	neg	neg *
C3	Klebsiella pneumoniae KPC		-	neg	neg *

*The protection on the strains that were not meant to be irradiated was insufficient: a single sheet of aluminum foil, not perfectly joined on its four sides. It is possible that the cultures meant to be kept protected for comparison purposes have been partially irradiated with UV-C light which caused their inactivation.

Table 4. Results of the second test (June 30, 2020): glass.

Material	GLASS				
Irradiation Time	06:20	minutes:seconds			
Distance in cm	125 - 150	60 - 70 mJ/cm²		Result	
Id	Strain		Gram +/-	Irradiaz. UV-C	Not Irradiated
C1	Escherichia coli ATCC		-	neg	neg*
C2	Klebsiella pneumoniae KPC		-	neg	85
C3	Acinetobacter baumannii MDR		-	3	> 100
C4	Staphylococcus aureus MRSA		+	65	>1.000.000
C5	Bacillus cereus		+	neg	20
C6	Enterococcus faecium VRE		+	39	> 100.000

*The protection on the strains that were not meant to be irradiated was insufficient: a single sheet of aluminum foil, not perfectly joined on its four sides. It is possible that the cultures meant to be kept protected for comparison purposes have been partially irradiated with UV-C light which caused their inactivation.

The day before the test the colonies were again plated to prepare fresh microbial cultures, and on the test day, about 2 hours before starting, the materials in use were contaminated with a standardized 0.5 Mac Farland solution (using a nephelometer made by Thermo scientific).

Small circles were drawn on the materials in order to circumscribe the contaminated points and recover with certainty the germs where they had been placed.

The various contaminated materials were then divided into two groups: the first without shielding to make them perfectly reachable by the treatment, and the second wrapped three times in foil to shield it from the irradiation.

During the first test with only three strains, the material was shielded with a single sheet of aluminum foil and not perfectly joined at its four sides; therefore, the bacteria were partially irradiated.

The first test was performed with 3 materials (glass, plastic, metal) and 3 bacterial strains (*P. aeruginosa MDR, K.pneumoniae KPC, Staphylococcus aureus MRSA*).

The second test was performed with 3 materials (glass, plastic, metal) and 6 bacterial strains (*E.coli ATCC87,39, K. pneumoniae KPC, A. baumanii complex, S. aureus MRSA, B.cereus, E. faecium VRE*).

At the end of the test, the treated materials were recovered by means of a swab and spread on plates with COS blood medium from the company Biomerieux and incubated in aerobiosis for 18-24h (Figure 1).

Figure 1. The areas within the circumferences were contaminated with the strains used during the test. The surface of the material not irradiated was wrapped 3 times in aluminum foil for protection. The surface of the material irradiated was exposed to UV-C light.

Table 5. Results of the second test (June 30, 2020): plastic.

Material	**PLASTIC**				
Irradiation Time	**06:20**	minutes:seconds			
Distance in cm	**125 - 150**	60 - 70 mJ/cm²		Result	
Id	**Strain**		**Gram +/-**	**Irradiaz. UV-C**	**Not Irradiated**
C1	Escherichia coli ATCC		-	neg	6
C2	Klebsiella pneumoniae KPC		-	neg	> 100
C3	Acinetobacter baumannii MDR		-	neg	> 100
C4	Staphylococcus aureus MRSA		+	neg	>1.000
C5	Bacillus cereus		+	neg	15
C6	Enterococcus faecium VRE		+	25	> 100

Table 6. Results of the second test (June 30, 2020): metal.

Material	**METAL**				
Irradiation Time	**06:20**	minutes:seconds			
Distance in cm	**125 - 150**	60 - 70 mJ/cm²		Result	
Id	**Strain**		**Gram +/-**	**Irradiaz. UV-C**	**Not Irradiated**
C1	Escherichia coli ATCC		-	neg	> 1.000
C2	Klebsiella pneumoniae KPC		-	neg	14
C3	Acinetobacter baumannii MDR		-	neg	102
C4	Staphylococcus aureus MRSA		+	2	63
C5	Bacillus cereus		+	neg	36
C6	Enterococcus faecium VRE		+	neg	92

On July 31, 2020, a third test was carried out, performing environmental tests in the room used for spirometry (Table 7, Figure 2): here a morning cleaning had been carried out and large flow of patients had passed through for several hours, therefore a first sampling was carried out, then a disinfection treatment with irradiation and we performed a second sampling.

The samples were taken with count agar plates with neutralizer; this non-selective medium allows the growth of all aerobic bacteria and is useful for evaluating the effectiveness of cleaning and sanitizing programs.

Several points in the room were swabbed, which were numbered and identified in order to repeat a second sampling in the same places after irradiation.

A first reading was carried out 24 hours later for a first view of the results and a second one 72 hours later as required by the environmental control procedure. The plates were collected and incubated in a thermostat at 30° in aerobiosis.

Note: Sample 12 (metal parts of the bed) was removed from the study due to two errors that occurred in the pre-analytical phase: the pre-irradiation plate remained inside the room, then it received a dose of radiation; also in the incubation phase the second plate fell and was subject to inevitable contamination.

Table 7. July 31, 2020: the third test was carried out.

		Growth after 24h				Growth after 72h			
Sample	Surfaces	Before UV-C bacteria	Before UV-C fungi	After UV-C bacteria	After UV-C fungi	Before UV-C bacteria	Before UV-C fungi	After UV-C bacteria	After UV-C fungi
1	Door	4	0	0	0	4	1	0	1
2	Door handle	20	0	0	0	37	0	0	0
3	Switch near the door	7	0	0	0	>1000	0	0	0
4	Washbasin mirror	0	0	0	0	6	0	1	0
5	Washbasin	44	0	1	0	104	0	21	1
6	Metallic trolley	38	0	3	0	57	0	10	1
7	Blue wardrobe	0	0	0	0	2	0	0	0
8	Green wardrobe	2	0	0	0	5	0	0	1
9	Fridge	0	0	0	0	0	0	0	0
10	Floor	53	0	6	0	>1000	0	17	0
11	Bed	41	0	3	0	102	0	16	2
~~12~~	~~Metal Parts of bed~~	~~15~~	~~0~~	~~4~~	~~0~~	~~nval~~	~~nval~~	~~nval~~	~~nval~~
13	SmartPhone	98	0	0	0	107	0	0	0
14	Spirometer bench	2	0	0	0	2	0	1	0
15	Spirometer keyboards	84	0	0	0	106	0	1	6
16	Desk (Below area)	9	0	2	0	25	0	2	0
17	Computer keyboard	52	0	17	0	108	0	24	0
18	Paper	1	0	0	0	4	0	0	0
19	Plastic Chair	24	0	1	0	35	0	1	0
20	Armchair fabric	13	0	0	0	23	0	1	0

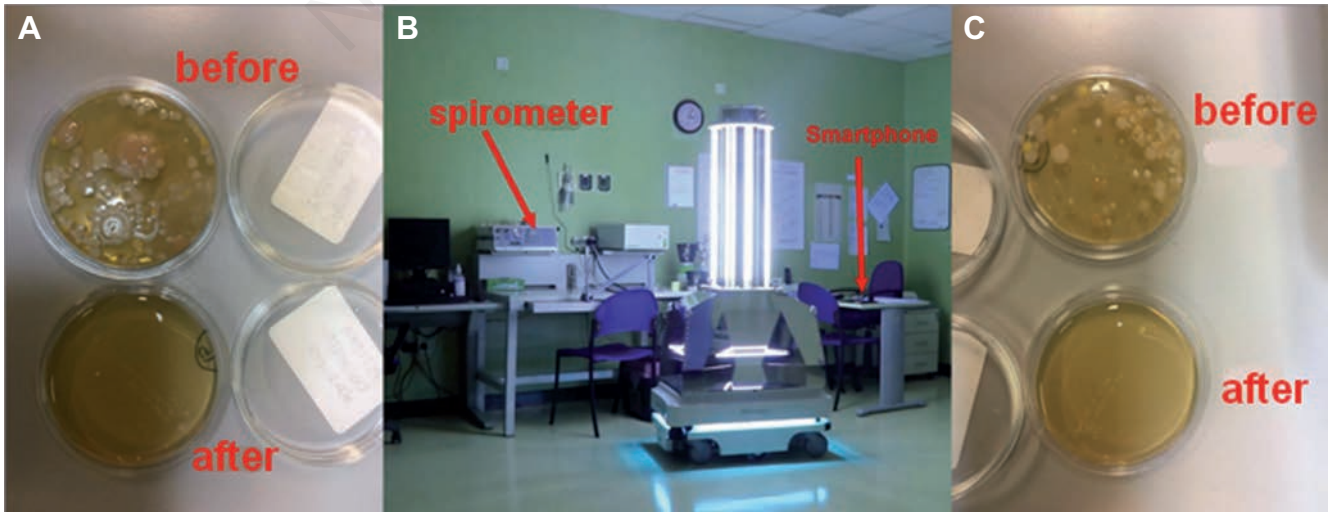

Figure 2. A) Spirometer; B) spirometry clinic; C) smartphone.

Reason for reflection and revision: In the reading made after 72 hours it was noted that, among the colonies that survived irradiation, some belonged to the category of fungi (for example, on the keyboard of the spirometry tool) out of 7 colonies 6 were fungi. Fungi are more complex structures at evolutionary level and with different growth characteristics compared to bacteria) which require a higher radiation dose.

UV-C device used for the test: *R2S Robot*

UV-C radiation with a wavelength of 253.7*nm* has the power to alter the DNA/RNA of microorganisms, given that it reaches the UV-C dose sufficient to prevent the restoration of molecular bonds caused by the phenomenon of photoreactivation.

The UV-C dose is obtained by multiplying the intensity of UV-C radiation by the time expressed in seconds. The unit of measurement of the UV-C dose is expressed in mJ/cm^2

For a UV-C disinfection system to be effective, it is very important that the dose necessary to inactivate the pathogenic microorganisms is irradiated in such a way as to reach all surfaces within a working range normally between 100 and 250 cm.

R2S Robot (Figure 3) is equipped with a powerful group of UV-C lamps, with an overall length of the lamp arcs of approximately 758 cm. The lamps and the surfaces of the body, which has a high capacity of reflecting UV-C light, are positioned so as to radiate the light from top to bottom and from bottom to top, thanks to a system of deflectors (patent pending) in order to reach even those surfaces that would otherwise be in the shadow.

Each room subject to *R2S Robot* disinfection process is identified with a special QR code by which it is possible to mark each room distinctively, even if this is part of complex structures located on large geographical areas consisting of several groups of buildings organized by blocks, floors, departments, areas.

Each mission consists of a variable number of stops (steps) of the *R2S Robot*. The duration of each stop depends on the UV-C dose to be irradiated in order to inactivate the most resistant pathogen potentially existing in the environment to be disinfected.

Once the disinfection process is finished, *R2S Robot* communicates the data of the work performed to the *SafetyMe* back-end (Figure 4). The report with the work done is then stored in an unchangeable way in a blockchain system.

Figure 3. R2S Robot.

Conclusions

COVID 19 pandemic has led the world authorities, in particular health authorities, to totally reconsider the infectious problems in all public places and in particular in hospitals, the latter also burdened by specific risks, consisting of their main function that induces concentration of sick subjects, but also mainly for the consequences of the necessary treatments, *i.e.* the resistance of infectious agents to the drugs used.

General methods and observations are the subject of a much more extensive discussion, which is part of the general protocol and the overall study.

From these results it clearly appears that the effectiveness of UV-C sterilization method is absolutely the best choice for the sanitation of environments that are highly contaminated and continuously visited by patients and operators.

The strong points of this method are summarized as follows:

– very high sterilization capability and suppression of bacterial growth after irradiation
– very limited time required for procedures, about 6-10 minutes,

Figure 4. QR code and blockchain system.

compared to much longer times needed by other methods, quantifiable in a range of many tens of minutes, without taking into consideration the ventilation time required afterwards
- safety in use without fear of generating resistance to infectious agents, which could potentially occur with chemical suppression methods
- capability to sterilize every single surface (including the parts under the tables, chairs, various supports) in the areas reached by the rays, made even larger by the mobility programmed in the robot carrying UV-C) lamps
- sterilization capability that extends to suspended gaseous particles and droplets generated by aerosols of various origins, especially in critical environments such as operating rooms, endoscopic procedure rooms, respiratory physiopathology rooms, clinics etc.
- reduction to a minimum of operators compared to traditional chemical and mechanical methods
- minimization of downtime between one procedure at risk and the next (operating theaters, radiology rooms, endoscopy rooms, medical clinics, especially for those with poor ventilation)
- increase of productivity, which at the moment seems to be seriously reduced due to the need to maintain safety procedures that take at least 30 minutes for clinics and well beyond for the other environments listed above
- significant reduction of staff assigned to sanitation procedures
- considerable overall savings
- significantly higher overall safety for users and operators
- perceived quality absolutely not comparable with other procedures
- elimination of possible disputes that could arise due to inadequate application of sterilization procedures
- versatility of the method, which can be extended to any other environment visited by people, not only health related, such as schools, kindergartens, supermarkets, public environments of all kinds.

The above illustrated method is continually subject to further checks, which will certainly establish its greater ability to perform in all conditions, also in the light of the new rules that will regulate access to any environment, both public and private, from now on.

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