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The study report was written under a Service Agreement between
University of Siena and Light Progress S.r.l.
Report (version 1.01)

Siena, 22nd July 2016

Microbiological testing of an hygienization device (Stet Clean) for stethoscope/phonendoscope

INDEX

The study report was written under to the Service Agreement (Rep. 1771/2014 Prot. 38520 e Rep. 490/2016; Prot 8995/2016) between the University of Siena and Light Progress. Report (version 1.01)

TARGET

To measure bacterial contamination before and after use of the "Stet Clean" (version 1.0), an hygienization device for stethoscope membranes, *in vitro* and during use.

OPERATIVE SPHERE

The tests were requested by Light Progress S.r.l. and conducted by Dr Comasia RICCI, affiliated with Department of Life Science, University of Siena. Laboratory work was conducted in the laboratories of the Department of Molecular and Developmental Medicine, which are certified for quality control and meet ISO 9001:2008 standards for: titration of antibodies for serological analysis, microbiological diagnosis, epidemiological analysis and chemical/biological/environmental determinations (Annex 1).

STAFF

Dr Comasia RICCI

EQUIPMENT

- Bio Class thermostat bath
- Velp vortex mixer
- Kartell hot plate
- Laminar flow hood with HEPA BIO /4 filter
- KW refrigerator +2 to +8°C
- Sartorius precision balance Gibertini Europe C
- Nichipet EX micropipette
- KW and Isco temperature chambers
- Fedegari sterilising autoclave
- 3M Littmann Classic II SE stethoscope
- Avantes AvaSpec-ULS2048L-USB2-UA spectrophotometer
- Optical fiber, 600 µm UV/VIS/IR Broad band

CULTURE MEDIA, REAGENTS AND LAB WARE

- *Plate Count Agar* culture medium Oxoid
- *Phosphate Buffered Saline* Sigma
- Sterile *90 mm diameter disposable Petri plates*
- Sterile *60 mm diameter, Rodac disposable Petri plates*
- Sterile polypropylene tubes
- Sterile spatula
- Various glassware
- Bacterial strain "*Staphylococcus aureus*" ATCC 13150
- Bacterial strain "*Pseudomonas aeruginosa*" ATCC 27853
- Bacterial strain "*Escherichia coli*" ATCC 25922

OPERATIVE TECHNIQUE

Study design

Cross-sectional study with comparison before and after treatment

Study population

Four hygienization devices for stethoscope/phonendoscope and 32 tests on membranes

Timeline

The experiments were conducted between 28 August 2015 and 21 July 2016.

Setting and presentation of study

Microbes on stethoscopes/phonendoscopes can lead to cross contamination of patients and infection. Correct hygienization of the stethoscope membrane reduces the possibility of microbial transmission.

For the present study, the client, Light Progress S.r.l., provided four devices designed to hold the stethoscope head (see Figure 1) and to sanitize it with ultraviolet C radiation (UV-C) emitted by a LED. The client requested:

i) testing of residual microbial contamination on stethoscope membranes used on several subjects, after the membranes had been exposed to UVC;

ii) simulation of use of the device in time (about 3000 cycles) and subsequent testing the hygienization efficacy for known microbial species;

iii) verification that no UV-C radiation escapes from the device during hygienization.

Data collection and use of the product

The experimental protocol consisted of two steps:

- step A involving four subjects who consented to two auscultations: one, used as control, H(0), was followed by placing the stethoscope membrane, without the use of the device, in direct contact with the culture medium; the second, H(1), had the hygienization treatment of the stethoscope membrane with the device before contact with the culture medium.

  In order to avoid bias and the possibility that the volunteers might modify their personal hygiene habits, the subjects were invited to submit to auscultation on the day of the experiment. All four subjects agreed to take part in the test;

- step B was conducted using an electrical circuit designed ad hoc, that automatically simulated use of the four devices for about 3000 cycles. Each cycle consisted in switching on the LED, supplying it with current for 5 minutes (*on* phase), switching it off, followed by an *off* period of 1 minute. This procedure made it possible to determine:

  i) electrical stresses (switching on, *on* phase, switching off) to assess the electronic circuits;

  ii) hygienization capacity after about 3000 uses lasting 5 minutes.

  Step B consisted in testing the hygienization efficacy of the device with bacterial strains representative of the microbial flora commonly found in healthcare settings: *Staphylococcus aureus ATCC 13150; Pseudomonas aeruginosa ATCC 27853* and *Escherichia coli ATCC 25922.*

  Samples at H(0), controls, were made from stethoscope membranes that had been contaminated with known bacterial suspensions, and were followed by test samples H(1) from contaminated membranes treated with the device.

In step A and B, after each test, the membrane was sanitized with ethyl alcohol and exposed for 15 minutes to a UV-C lamp mounted in a laminar flow hood. Samples were obtained after this treatment in order to confirm the absence of residual contamination before the control H(0) and testing H(1) phases.

Hygienization prior to the testing phase H(1) was always conducted for a complete 5-minute cycle.

All experimental procedures were standardised for times, materials, sowing of cultures and laboratory procedures in order to minimise variations.

Detailed information from each experiment was recorded in a database for subsequent analysis.

Laboratory analysis

In step A, four subjects underwent heart and lung auscultation. The stethoscope was placed on the subjects' skin a total of six times for the control H(0) and testing H(1) phases, simulating a real clinical auscultation. The membrane was then pressed onto culture medium (Plate Count Agar, PCA) in 60 mm Rodac plates, without UV-C irradiation in the case of H(0) and after UV-C irradiation in the case of H(1). In the second case irradiation was performed for 5 minutes with the stethoscope head lodged in the device in order to check the efficacy of the latter.
In step B, 90 mm Petri plates were prepared with PCA on which bacterial strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 13150 and *Pseudomonas aeruginosa* ATCC 27853 were sown. One or two colonies were obtained from these pure cultures to make 0.5 McFarland standard bacterial suspensions in buffered saline, later diluted to 10^{-3} for the tests.
To prepare the control cultures H(0), 50 µl of each diluted bacterial solution was deposited on a stethoscope membrane and distributed over the whole surface with a sterile pad. The membrane was allowed to dry under the laminar flow hood. It was then pressed directly onto the PCA culture medium in 60 mm Rodac plates, ensuring that the whole membrane surface adhered perfectly to the medium surface.
The same procedure was used to determine the efficacy of the stethoscope hygienization treatment: test phase H(1). In this case, before sowing by direct contact on the culture medium, the infected and dried stethoscope membranes were connected to the device and irradiated with UV-C for 5 minutes.
All Rodac control and test plates were incubated at 36°C. After 24 and 48 hours of incubation the number of colony forming units (CFU) that had developed was counted.

Spectrophotometer measurements

During the hygienization phase, a spectrophotometer was used to detect the presence of any UV-C radiation around the corona of each device with the stethoscope head in place.

Statistical analysis

For each experiment, the number of CFUs was counted before H(0) and after treatment H(1) and the reduction was calculated as a percentage and even expressed as a logarithm base 10.

After checking the data for incongruities, the database was analysed as follows:

i) descriptive statistical analysis stratified for steps A and B and for before/after treatment H(0)/H(1), including mean, standard deviation, median, interquartile interval, minimum and maximum.

ii) inferential statistical analysis to identify possible differences in the degree of microbial contamination before H(0) and after H(1) use of the device; a one-side Wilcoxon test was used to check percentage reductions in microbial load. We also evaluated 95% confidence intervals (95% CI) of the percentage reduction in microbial load by the *bootstrap bias corrected and accelerated* resampling method. Finally the reduction in microbial load and corresponding 95% CI were expressed in $\log_{10}$ units.

Stata 12.1 (Stata Corporation, College Station, TX, USA) software was used for all statistical analysis, setting significance at 95% ($p<0.05$).

RESULTS

Table 1 shows the results of control H(0) and treatment H(1) cultures for steps A and B of the experiment with mean, standard deviation, median, interquartile interval, minimum, maximum, percentage and $\log_{10}$ reduction and the corresponding 95% CI for bacterial load measured in CFU.

For step A we found:

- a mean reduction in bacterial load of 97.7% ($\log_{10}$ = 1.64) after 24 h, 95% CI = 90.9-100% ($\log_{10}$ > 1) for the four subjects who underwent auscultation. In three of them, bacterial load was reduced by 100% and in the fourth only one CFU remained. At 48 h, CFUs in control plates were uncountable and a second calculation of the reduction in CFU between H(0) and H(1) was therefore impossible.
- One Rodac plate exposed to treatment went from zero CFU at 24 h to 1 CFU at 48 h; another went from 0 to 5 CFU.

For step B overall we found that:

- after 2918 simulated cycles the four devices did not show any alterations in switching on, *on* phase of UV-C and automatic switching off after 5 minutes.
- the experiments with bacteria showed an overall statistically significant (Wilcoxon test $p<0.001$) decrease in CFU of 97.1% ($\log_{10}$ = 1.54), 95% CI = 94-99% (95% CI, $\log_{10}$ = 1.22-2.00). This was based on a hypothetical 200 CFU in the case of *Pseudomonas a.*, where the number of colonies was too great to count. Excluding the *Pseudomonas a.* count, the percentage reduction was 98.2% ($\log_{10}$ = 1.74), 95% CI = 91.1-100% (95% CI, $\log_{10}$ = 1.05-∞).

In detail, the experiments conducted with the different strains showed the following results:

Escherichia coli

- H(1): all four devices recorded zero CFU
- The percentage reduction in CFU between H(0) and H(1) was 100%.

Staphylococcus aureus

- H(1): three devices recorded zero CFU; the other recorded 1 CFU.
- The percentage reduction in CFU between H(0) and H(1) was 96.9% (log_{10} = 1.51), 95% CI = 87.5-100% (95% CI, log_{10} = 0.90-∞).

Pseudomonas aeruginosa

- All four control plates showed too many CFU to count.
- H(1): the maximum number of CFU was 15, the minimum 3.
- The general percentage reduction in CFU between H(0) and H(1) was estimated at more than 95% (log_{10} > 1.33).

Figures 2 and 3 show culture plates illustrating the reduction in CFU between H(0) and H(1) for the experiment on volunteers and those with selected bacteria, respectively.

Check for escaping UV-C radiation

When the detector was passed around the entire perimeter of the lodging of the device during irradiation of stethoscope heads, no escaping UV-C was detected.

CONCLUSIONS AND CONSIDERATIONS

Many scientific studies confirm the role of stethoscope membranes as vectors of microbial contamination. The controls in step A of the present experiment confirm the transfer of microbes between surfaces.

The devices tested were demonstrated to significantly sanitize the stethoscope membrane after the tests performed with volunteers, in which the stethoscope was used for auscultation, as well as after purposeful contamination of the membrane with bacteria.

Several considerations regarding the device can be summarised as follows:

- The device is automatic and is activated when the stethoscope head is placed in the appropriate lodging. This operation proved to be simple and immediate.
- The colour and structure of the membrane were observed to be not altered during or at the end of the experiment.
- When the stethoscope was properly lodged in the device, a blue light on the device began to flash, indicating that the UV-C LED was operating.
- During irradiation, many spectrophotometer readings taken around the heads of stethoscopes coupled to the device indicated an absence of escaping UV-C radiation.
- No sanitization (cleaning and disinfecting) steps were taken before exposure to UV-C in H(1) phase, both in step A, with volunteers, or, in step B, when bacterial strains were used. These measures could increase the hygienizing efficacy of the device, especially in step A, where for example the presence of perspiration on the stethoscope membrane may reduce the biocidal effect of UV-C, so that the simple expedient of wiping the membrane with a cloth or paper napkin could enhance hygienization.
- Lower exposure times in H(1) phase could reduce the biocidal effect of the device. This aspect was not investigated because the device was designed to irradiate for 5 minutes.
- Biocidal activity could presumably be increased by: i) increasing exposure time in H(1) phase; ii) using more than one UV-C source in the device; iii) increasing the power of the UV-C source.
- UV-C radiation has a biocidal effect on objects irradiated but it does not remove dirt or biological material that may accumulate if normal cleaning is neglected. The device is not designed to replace the whole sanitization procedure.
- Certain aspects of the study could be further investigated: i) increasing the number of samples tested; ii) checking the effect of the device on other species of microbe; iii) testing the efficacy of the device for a greater number of cycles (so far about 15,000 minutes); iv) seeking correlations between the power of UV-C emitted by the device and microbiological efficacy; v) conducting a longer experiment in a healthcare setting.

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CONTACTS

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Dr. Comasia Ricci

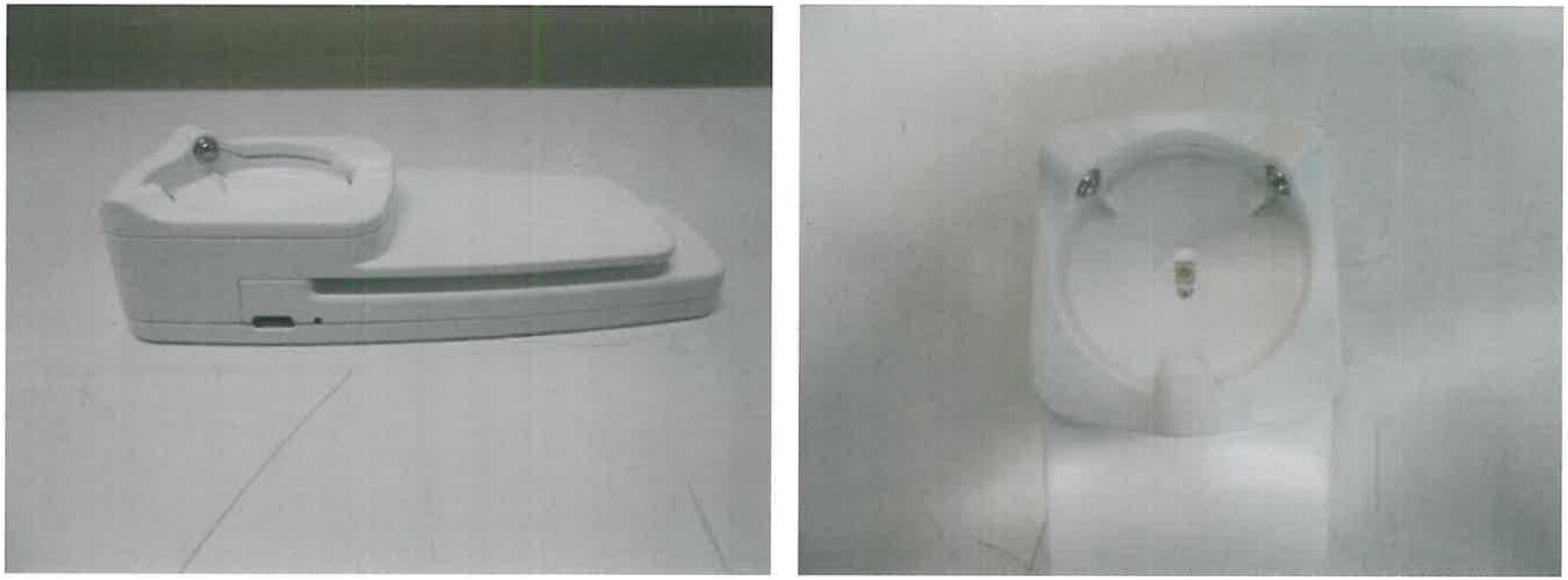
Table 1 Mean, standard deviation , median, interquartile interval , minimum and maximum , mean reduction (percentage and log10 with their confidence intervals at 95%) of the bacteria/subjects in control H(0) and after treatment H(1)

	Mean (DS)		25°,50°,75°		Min-Max		Mean percentage reduction [95% CI]	Mean log10 reduction [95% CI]
Test on	***Control H(0)***	***Treatment H(1)***	***Control H(0)***	***Treatment H(1)***	***Control H(0)***	***Treatment H(1)***		
Escherichia coli	19(16.6)	0	7-12-38	0-0-0	7-38	0	100 [-]	- [-]
Staphylococcus aureus	25.8(26.4)	0.3(0.5)	9.5-15-52.8	0-0-0.8	8-65	0-1	96.9 [87.5-100]	1.51 [0.90 -]
Pseudomonas aeruginosa	uncountable	9.5(52)	uncountable	4.3-10-14.3	uncountable	3-15	95.3 [93.3-97.4]	1.33 [1.17 - 1.59]
E.Coli+Staf.+Pseud.*	87.3(90.1)	3.5(5.5)	12-38-200	0-0-8	7-uncountable	0-15	97.1 [94.0-99.0]	1.54 [1.22-2.00]
Subjects**	20.5(9.14)	0.3(0.5)	13-19-29.5	0-0-0.8	11-33	0-1	97.7 [90.9-100]	1.64 [1.04 -]

* overall calculation using the tested bacteria and based on the hypothetical quantity of 200 CFU in the Controls of the Pseudomonas aeruginosa.

** counts of the CFU, on the 4 subjects, at 24h. Counts at 48h were not possible because controls were uncountable.

Figure 1



The device tested to hygienize the stethoscope membrane. On the left, a side view; on the right, a frontal view showing the UV-C source, in the centre.

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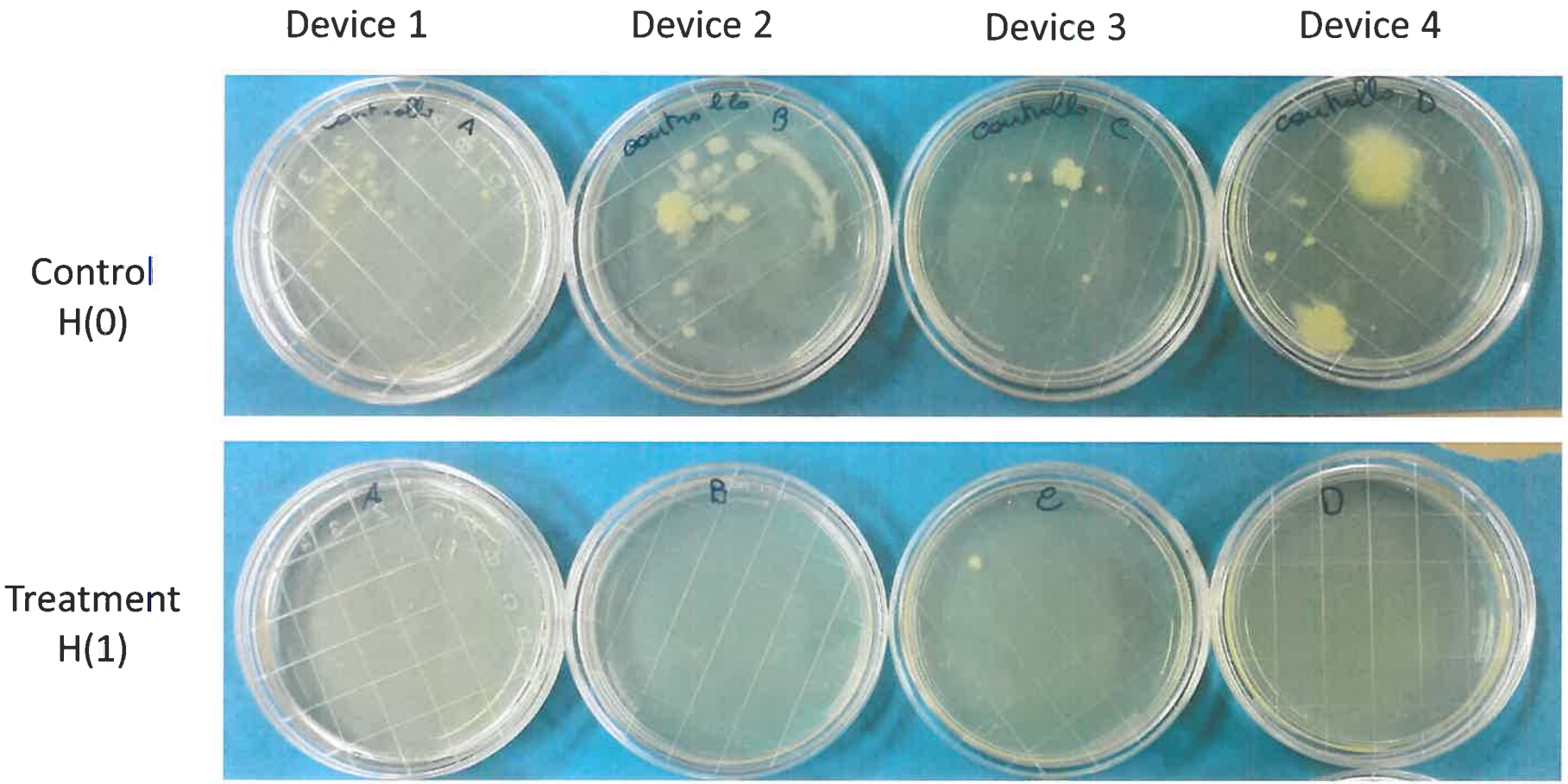
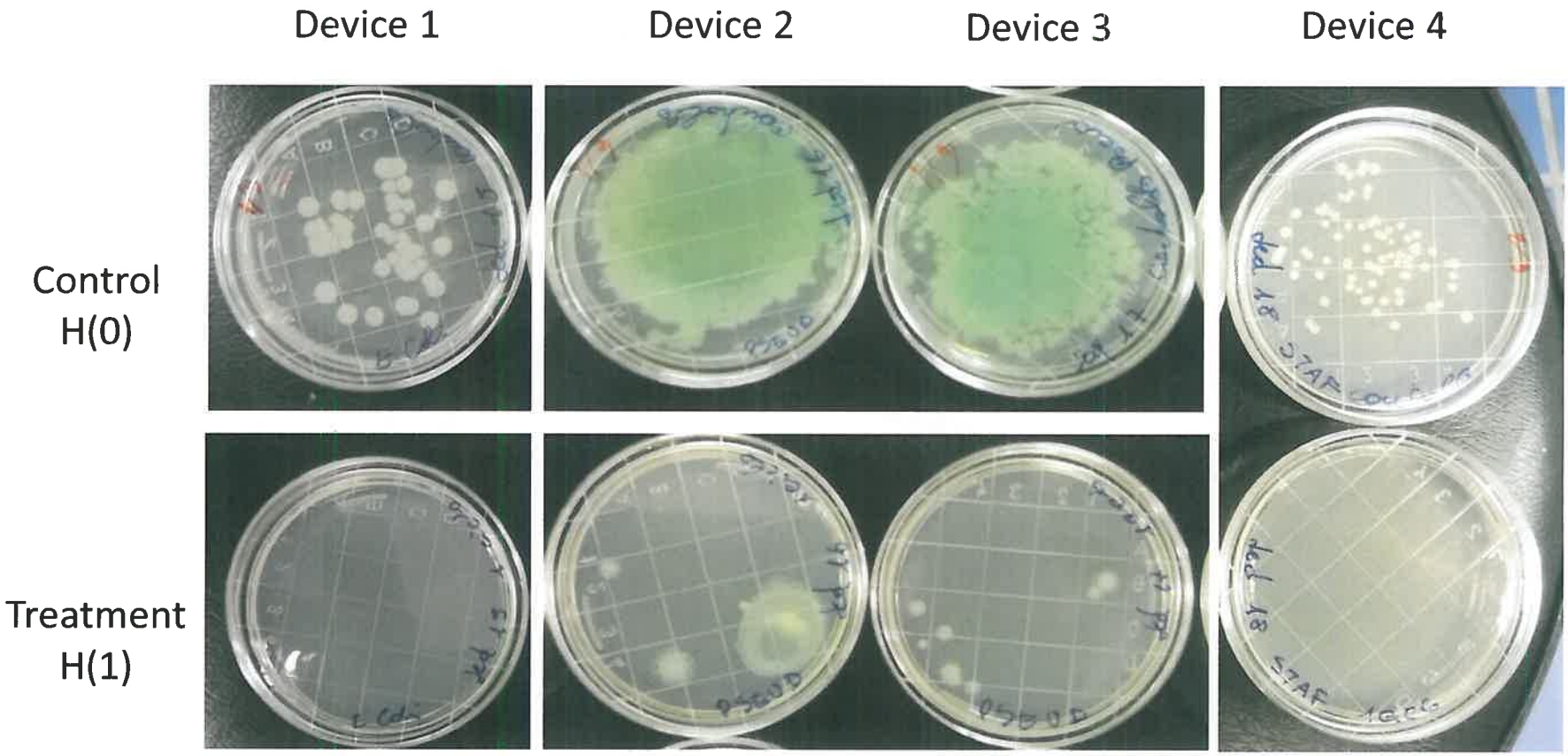
Figure 2



Petri plates with CFU at 24 h. Tests made on 4 subjects (A,B,C,D) using 4 different devices. In the superior part, controls H(0); in the inferior part, treatments H(1).

Figure 3



Petri plates, with CFU at 48h, after 2.918 cycles (14.558 minutes), in order to simulate the LED consumption. Examples of tests made on Escherichia Coli (left), Pseudomonas aeruginosa (centre), Staphylococcus aureus (right). In the superior part, controls H(0); in the inferior part, treatments, H(1).

ANNEX 1

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Prima emissione *First Issue*	21.01.2010
Emissione corrente *Current Issue*	08.01.2016
Data scadenza *Expiry Date*	15.09.2018

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