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Department of Molecular and Developmental Medicine

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between the University of Siena and ISO ITALIA GROUP s. r. l.

Report (version 1.0)

Siena, 22th March 2021

TEST ON REAL SETTING WITH CLEANING AIR T12 BY ISO GROUP ITALIA s.r.l.

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TARGET

The Cleaning air T12 (Figure 1 and 2), produced by ISO ITALIA GROUP s. r. l., is composed of 3 parts: i) the lower base, that allows air to enter; ii) a central germicidal chamber, with 12 UV-C lamps and iii) two grids, in the upper part, which allow the air, treated by UV radiation to exit. The central chamber is divided into 2 independent canals (with 6 lamps each) that allow the air to flow from the lower inlets to the upper outlet grids after UV-C irradiation.
This study aimed to assess the effectiveness of Cleaning air T12 in disinfecting air in a real office setting, with presence of subjects, located in the Department of Molecular and Developmental Medicine at the University of Siena.

Figure 1. Cleaning air T12 - front view

Figure 2. Cleaning air T12 - lateral view

OPERATIVE SPHERE

The verifications were requested by ISO ITALIA GROUP s. r. l. and produced under the supervision of Prof. Gabriele Messina, Department of Molecular and Developmental Medicine, University of Siena, on the basis of internal tests and all technical documentation provided by ISO ITALIA GROUP s.r.l.

EQUIPMENT

- **Cleaning air T12** (lamp type: OSRAM PURITEC HNS UV-C, 254 nm)
- **Plate Count Agar (PCA) medium;**
- **Sas Microflow ALPHA Aquaria;**
- **Climet Ci-550 particle counter**
- **Spectrophotometer Avantes, model AvaSpec-ULS2048CL-EVO-USB3 UV/VIS/NIR irradiance measurement bundle** (200-1100 nm)
- **Sterile 60 mm Ø disposable Petri plates**;
- **Laminar flow hood with HEPA BIO/4 filter, KW Refrigerator +2 to +8°C, Sartorius precision balance, Nichipet EX micropipette, KW and Isco temperature chambers, Fedegari sterilizing autoclave, sterile glass bottles, various glassware;**
- **Microsoft Excel 2016** for data collection
- **Stata SE/16.0** for statistical analysis

OPERATIVE TECHNIQUE

Timeline

The study was conducted between December 2020 and February 2021 and was organized in stages:

- Preliminary stage conducted to evaluate the percentage of direct microbiological abatement in air contamination;
- Experimental stage conducted to evaluate the performance of the device in real environments with the presence of people.

Study design and Experimental Set up

Preliminary stage:
The Cleaning Air T12 was positioned in the Department microbiological laboratory and turned ON during working hours for two days. The incoming and outgoing air was sampled simultaneously with two microbiological air samplers (Sas Microflow α Aquaria). It was built a setting so as we collected the untreated air close to the inlet grid, and flowing from the outlet grids, after UV-C treatment (Figure 3).
For each sample, 2 m^3 of air were aspirated with Sas Microflow α, at speed of 120 L/min, and collected in 60 mm Ø Petri plates, with PCA medium. After sampling, the plates were then removed and incubated for 48hs at 22 and 36°C.

In addition, the particulate matter (>0,3, >0,5, >1,0, >3,0 >5,0 and >10 µm), was measured with a Climet CI-550 by placing the detection probes close to both the inlet grill and the outlet area of the device.

Finally, photometric measurements, radiant flux (µW) and Irradiance (µW/cm^2), of the internal sections of the device were taken at 6 different positions of the device, 3 on each side, at a distance of 20 cm from the UV-C lamps and approximately halfway along their longitudinal axes.

Figure 3. Setting around the device: the microbiological air samplers were placed next the inlet grid and on the outgoing air stream.

Experimental stage:

The Cleaning Air T12 was placed in an office setting, located in the Department of Molecular and Developmental Medicine at the University of Siena. Air samples were collected during working hours while people were present in the room. Initially, to obtain a reliable baseline; the samples were taken for three days inside the office with the device turned OFF, and with a different number of people each day. This was useful to assess the level of microbial contamination in the real environment, alternating changes in the main variables taken into account for the study: i) the number of people present inside the office, ii) the state of isolation of the environment and iii) the actions carried out by the staff inside the office. Subsequently, the samples were collected having the device turned on and with a fixed number of people inside the office. In every experiment, the first samplings correspond to T(0)

(time zero, or starting time), in which the device was OFF. In this way, once the device was switched ON, it was possible to verify whether the device was able to determinates changes of microbial air contamination. Finally, in the final experiment, the level of microbial contamination when the device was switched OFF again was also studied.
The office volume, where this part of the investigation took place, was 65 m^3. There were two desks and 5 seats, two doors and one window. For each Petri dish, samples of 0.5 m^3 of air were aspirated with two Sas Microflow α, at a rate of 120 L/min, and collected in 60 mm Ø Petri dishes, with PCA medium. After sampling, the dishes were removed and incubated for 48hs at 22 and 36°C.

DATABASE ORGANIZATION

All the data collected during the study were entered into a database and included the following variables:

- Date of test
- Petri dish ID
- CFUs
- Particulate counts at >0,3, >0,5, >1,0, >3,0 >5,0 and >10 µm
- Photometric measurements
- Notes of relevant variables for the test

DATA ANALYSIS AND STATISTICS

Data analysis and statistical computations were supervised by Prof. Gabriele Cevenini, Department of Medical Biotechnologies, Bioengineering Lab, University of Siena. The Microsoft Excel software (ver. 16) has been used for preliminary statistical evaluations from empirical data and to organize a database. Parametric (t-test for paired and unpaired data) and not parametric (Wilcoxon and Mann Whitney) tests were used to assess differences between samples as needed. Inferential statistical aanalysis was carried out using Stata software Ver 16.

RESULTS

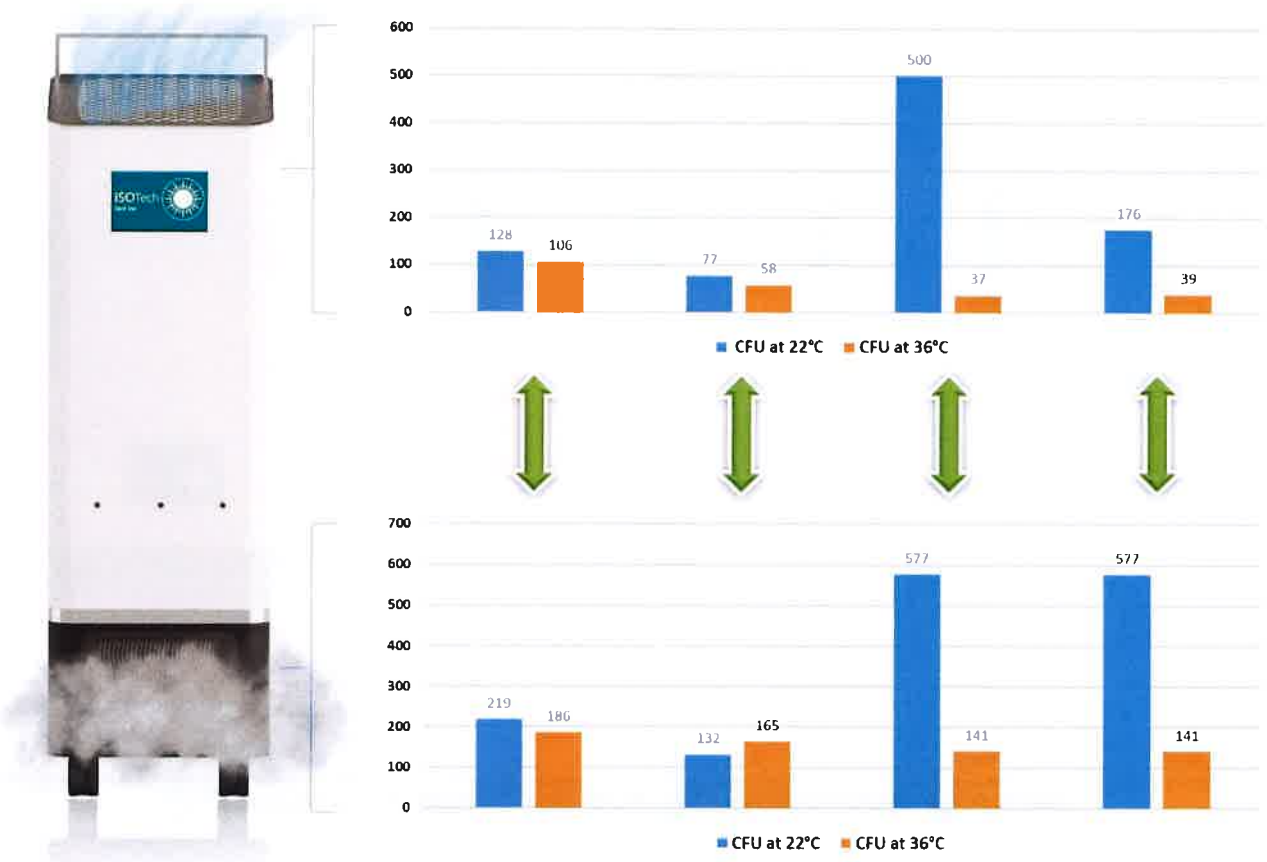
Preliminary stage:
36 Petri dishes were used in this stage. The laboratory tests have shown a significant mean CFU reduction (p=0.0023) of 51 CFU (SD+-34,9) (95% CI 24.3 – 78.0) corresponding to a 33%, for samples incubated at 22° C, and a significant mean CFU reduction (p=0.001) of 77,9 (+- 46.5 SD) (95% CI 42.2 – 73.3) corresponding to a 62.5% for samples incubated at 36° C after treatment with UV-C radiation. The results obtained from the experiments are described in Tables 1A and 1B and in Figures 4 and 5 (see ANNEX for details, separate document).

Table 1 A. Preliminary test: Day 1 results

Number air sampling		CFU 22°C	CFU 36°C
1	Incoming air	219	186
	outcoming air	128	106
2	Incoming air	132	165
	outcoming air	77	58
3	Incoming air	577	141
	outcoming air	500	37
4	Incoming air	265	70
	outcoming air	176	39

Table 1 B. Preliminary test: Day 2 results

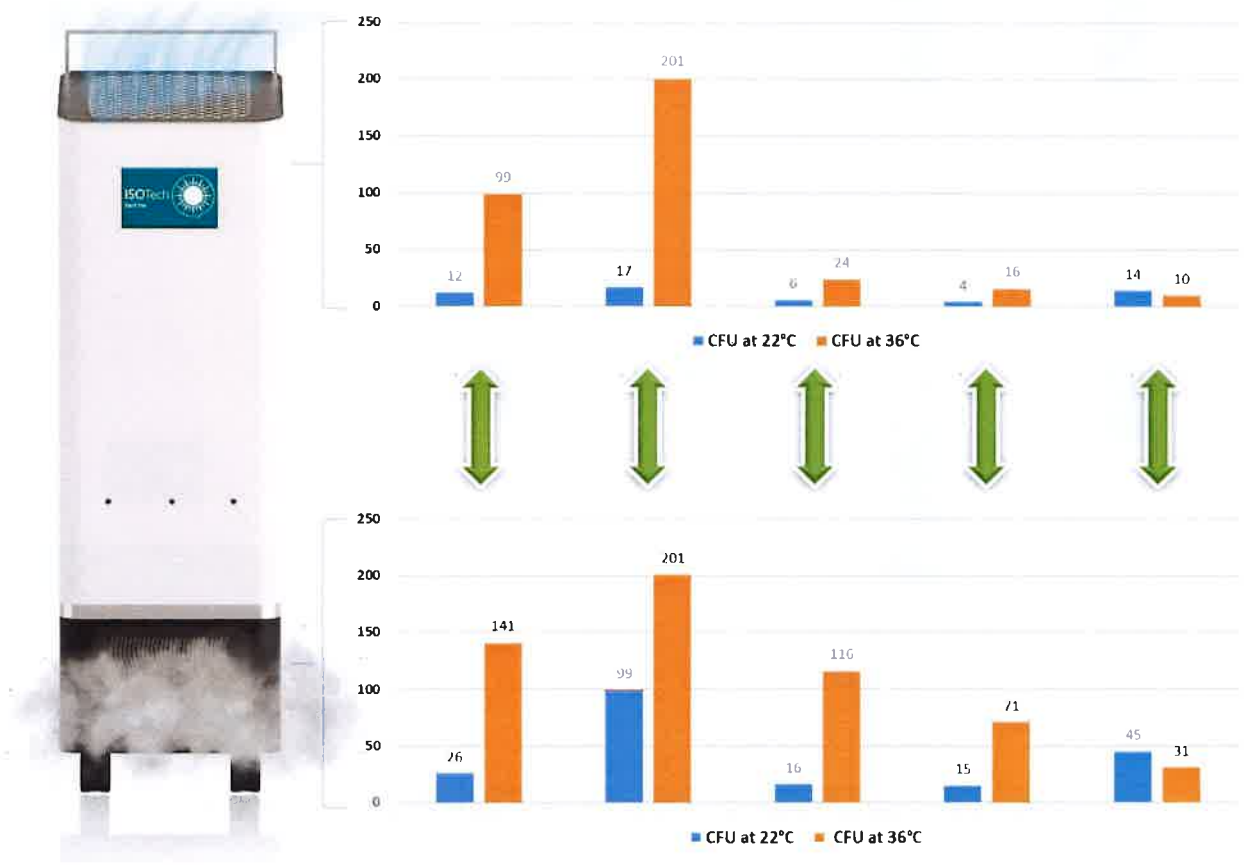
Number air sampling		CFU 22°C	CFU 36°C
1	Incomig air	26	141
	outcoming air	12	99
2	Incoming air	99	201
	outcoming air	17	32
3	Incoming air	16	116
	outcoming air	6	24
4	Incoming air	15	71
	outcoming air	4	16
5	Incoming air	45	31
	outcoming air	14	10



Contamination detected in the **upper output**

Contamination detected in the **lower output**

Figure 4. Preliminary stage: Day 1, graphical results.



Contamination detected in the **upper output**

Contamination detected in the **lower output**

Figure 5. Preliminary stage: Day 2, graphical results.

Analysis of particulate matter (>0.3, >0.5, >1.0, >3.0 >5.0 and >10 µm) in the pre/post air treatment was carried out using 10 consecutive measurements were with the Climet CI 550 particle counter near the bottom inlet and top outlet of the device. A statistically significant difference was verified between the means of all particles measured in the air entering and leaving the device ($p<0.001$). The results obtained are shown in Tables 2A and 2B. The average reduction percentages were 26%, 31%, 46%, 71%, 69%, and 64%, for the >0.3, >0.5, >1.0, >3.0 >5.0 and >10 µm particles, respectively

Table 2 A. Analysis of particulate matter in pre-treatment air

Measurement	Particles/m^3					
	>0,3 µm	>0,5 µm	>1,0 µm	>3,0 µm	>5,0 µm	>10 µm
1	2931689	883617	224433	9249	5650	2253
2	2968209	856094	196988	5145	3860	1244
3	2931184	828717	103480	4076	2959	874
4	3047958	919089	234489	9088	5515	2118
5	3032117	857810	193827	5246	2724	1210
6	2974302	848863	185486	5414	2959	1143
7	2931058	803929	174084	5549	3060	1278
8	2888403	772785	157200	3699	1782	706
9	2929502	782303	162985	5347	3329	1378
10	2965557	794008	167122	6390	3733	1715

Table 2 B. Analysis of particulate matter in post-treatment air

Measurement	Particles/m^3					
	>0,3 µm	>0,5 µm	>1,0 µm	>3,0 µm	>5,0 µm	>10 µm
1	2526814	611414	93499	1345	739	235
2	2530043	611885	92288	1177	975	336
3	2490793	613533	93432	1210	706	369
4	2392619	593488	96795	1345	941	302
5	2274937	580438	99923	2152	1513	706
6	2254488	595506	105742	2253	1648	807
7	2136301	572366	95854	1917	1008	470
8	1825465	524641	87748	1648	1143	571
9	1790890	532545	93567	1614	908	504
10	1681348	530796	106717	2421	1580	706

The photometric irradiance measurements ($\mu W/cm^2$) were taken on both sides of the device at the positions shown in Table 3 A and B and Figure 6. These are averages obtained from 10 points sampled in the range of maximum lamp distances, i.e. 16-20 cm..

Table 3 A. Photometric mesuraments: Side 1

Area	Radiant flux (μW)	Irradiance (μW/cm²)
A1	707	5920
B1	897	7500
C1	785	6600

Table 3 B. Photometric mesuraments: Side 2

Area	Radiant flux (μW)	Irradiance (μW/cm²)
A2	658	5520
B2	877	7340
C2	741	6220

MEASUREMENT CARRIED OUT AT 20 CM DISTANCE FROM THE LAMPS

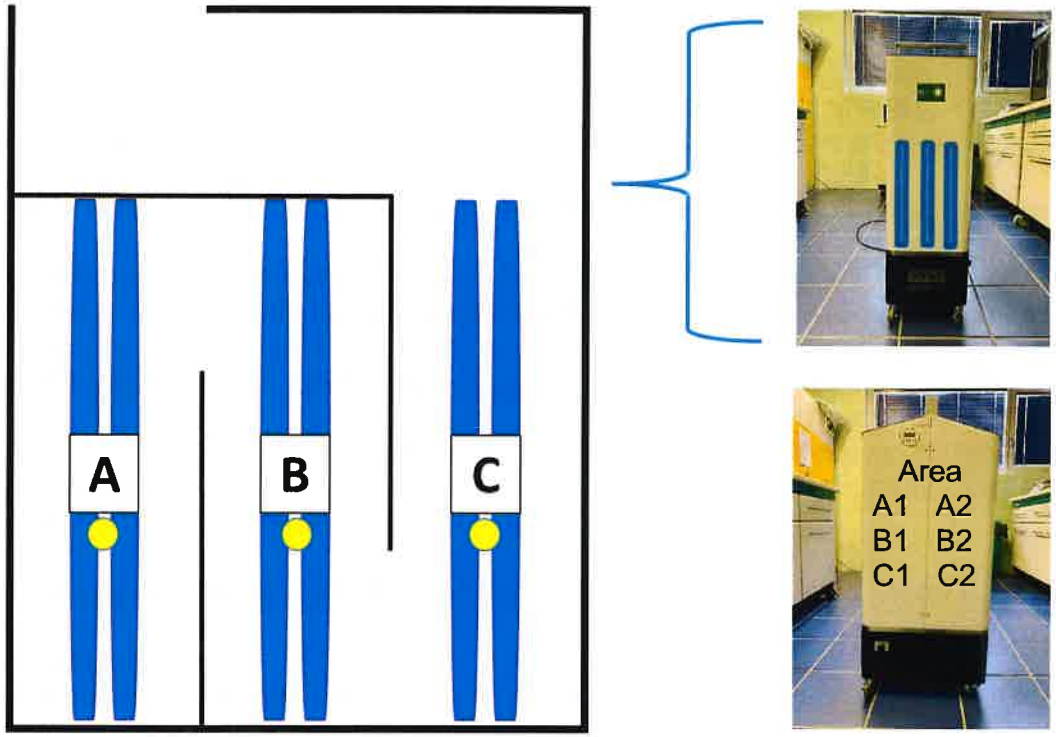


Figure 6. Preliminary stage: Photometric measurements of the lamps place into the device.

Based on the data in Table 3, it can be calculated that the average irradiance in the cross section of the air duct is 6.5 mW/cm^2.
Considering that, for each of the two sections of the T12:

- the output airflow, Q = 105 m^3/h = 0.029 m^3/s;
- the cross-sectional area, S, of the air duct (excluding the part occupied by the lamp), is approximately S = 160 cm^2 = 0.016 m^2;
- the length of each lamp is 45 cm = 0.45 m.

Then, the mean air velocity in the duct, Vm=Q/S, is about 1.8 m^2/s.
Considering the total length of the three pairs of lamps in series to be 1.35 m, the average air dwell time (length/speed) near the lamps is about 0.75 seconds. The result is that the average dose in the area of lowest irradiance (16-20 cm from the lamp) is 6.5x0.75 = 4.875 mJ/cm^2.
In the most critical range measured the average distance from the air lamp is 18 cm. The average distance from the lamp in the remaining range of 80% air volume in the duct (at 0-16 cm from the lamp) is clearly 8 cm. Therefore, taking into account the quadratic law with the distance at which the light energy propagates, the multiplication factor of the average dose in this range is approximately 5. Although conservatively estimated at 4 due to reflections, we still have an average dose in the range of the nearest 80% air volume of about 20 mJ/cm^2.
Since the distances from the lamp have a high range of variation (0-20 cm) the range of variation of the dose will also be high, estimated between 4 mJ/cm^2 (farthest point) and over 100 mJ/cm^2 (near the lamp).
These doses, in the average, are however compatible with reductions in the microbial load of almost all species (bacteria, fungi and viruses), varying between 2 and 4 log10 (99%-99.99%), in the air volume of each passage (Table 4 A and B).

Table 4 A. Inactivation of different bacteria/fungi by UVC irradiation

Bacterial/fungal species	UVC Dose (mJ/cm²)	Wavelength (nm)	Source	Inactivation efficacy (Log10)	Inactivation efficacy (%)	References
E. Coli	9	255	LED	2.7	99.8	Song K et al., 2016 (2)
E. Coli	1.93	265	LED	4	99.99	Yin R. et al., 2013 (3)
E. Coli	1.17	265	Lamp	7	99.99999	Vermeulen N. et al.,2007 (4)
A. baumanii	1.4-16.8	254 ± 2	Lamp	1	90	Dai T. et al., 2012 (5)
S. aureus MRSA	0.159	254	Lamp	1	90	Rao BK et al., 2011 (6)
S. aureus MRSA	0.318	254	Lamp	5	99.999	Rao BK et al., 2011 (6)
S. aureus MSSA	0.159	254	Lamp	1	90	Rao BK et al., 2001 (6)
S. aureus MSSA	0.318	254	Lamp	2	99	Rao BK et al., 2011 (6)
Staph. MSCONS	0.159	254	Lamp	1	90	Rao BK et al., 2011 (6)
S. pyogenes	0.159	254	Lamp	5	99.999	Rao BK et al., 2011 (6)
S. pyogenes	1.93	265	LED	4	99.99	Yin R. et al., 2013 (3)
Enterococcus spp	0.318	254	Lamp	2	99	Rao BK et al., 2011 (6)
S. epidermidis	25	254	Lamp	7	99.99999	Terpstra F.G et al., 2008 (7)
S. epidermidis	24	254	Lamp	2.6	99.75	Caillet-F.P. et al.,2004 (8)
S. epidermidis	0.8-1.1	253.5	Lamp	1	90	Helmke A. et al., 2011 (9)
P. aeruginosa	0.64-2.04	254 ± 2	Lamp	1	90	Dai T. et al., 2012 (10)
B. subtilis	4-6	254	Lamp	1	90	Coohill T. P. et al., 2008 (11)
C. perfrigens	48-64	254	Lamp	3	99.9	Hijnen W. et al., 2006 (12)
C. albicans	19.2	254	Lamp	2	99	Dai T. et al., 2011 (13)
L. pneumophila	8	254	Lamp	1	90	Hijnen W et al., 2006 (12)
L. pneumophila	15	254	Lamp	2	99	Hijnen W. et al., 2006 (12)
M. tuberculosis	7.4	254	Lamp	1	90	Boshoff H. et al., 2003 (14)
M. tubercolosis	4.8	254	Lamp	1	90	Lindsley W. et al., 2015 (15)
Salmonella typhi	12	254	Lamp	2	99	Hijnen W. et al., 2006 (12)
Salmonella	22.3	254	Lamp	3.22	99.94	Lim W. et al., 2016 (16)

Table 4 B. Inactivation of different virus by UVC irradiation

Virus species	UVC Dose (mJ/cm²)	Wavelength (nm)	Source	Inactivation efficacy (Log10)	Inactivation efficacy (%)	References
Adenovirus	186	265	LED	4	99.99	Keshavarzfathy M. et al., 2021 (17)
Adenovirus type 2	64-68	260	LED	3	99.9	Beck S. et al., 2017 (18)
Coxsackie A10	8	260	LED	2	99	Woo H. et al., 2019 (19)
Coxsackie B5	17	254	Lamp	2	99	Hijnen W et al., 2006 (12)
Ebola	50	254	Lamp	2.4	99.6	Eickman M. et al., 2018 (20)
Echovirus 30	13	260	LED	2	99	Woo H. et al., 2019 (19)
Enterovirus	10	260	LED	2	99	Woo H. et al., 2019 (19)
Epatite A	18	254	Lamp	4	99.99	Wang J. Et al., 2004 (21)
Epatite A	11	254	Lamp	2	99	Hijnen W. et al., 2006 (12)
Epatite C	54	253.7	Lamp	5	99.999	Song H. et al., 2010 (22)
EBV	16.2	254	Lamp	1	90	Kowalski W., 2009 (23)
HSV type 1	19.3	254	Lamp	4	99.99	Nossik N. et al., 2017(24)
Influenza A	28	280	LED	1	90	Nishisaka R. et al., 2018 (25)
Measles Virus	10-30	254	Lamp	3.5-4.5	99.97-99.997	Vaidya V. et al., 2018 (26)
MERS-CoV	50	254	Lamp	2.9	99.87	Eickman M. et al., 2018 (20)
Poliovirus type 1	8	260	LED	2	99	Woo H. et al., 2019 (19)
Poliovirus type 1	7	254	Lamp	1	90	Hijnen W. et al., 2006 (12)
Rotavirus	25	254	Lamp	3	99.9	Heßling M. et al., 2020 (27)
Rotavirus	22.5	254	Lamp	2	99	Araud E. et al., 2020 (28)
Rotavirus SA-11	10	254	Lamp	1	90	Hijnen W. et al., 2006 (12)
SARS-CoV-2	37,5	280±5	LED	3	99.9	Inagaki H. et al.,2020 (29)
SARS-CoV-2	3.7	254	Lamp	3	99.9	Bianco A. et al., 2020 (30)
SARS-CoV-2	5	254	Lamp	2	99	Beggs C.B. et al., 2020 (31)
SARS-CoV-2	0,7	254	Lamp	1	90	Sagripanti J.L. et al., 2020 (32)
SARS-CoV-2	3	222	Lamp	2.52	99.7	Kitagawa H. et al., 2021 (33)
CoV α HCoV-229E	1.7	222	Lamp	3	99.9	Buonanno M. et al., 2020 (34)
CoV β HCoV-OC43	1.2	222	Lamp	3	99.9	Buonanno M. et al., 2020 (34)

Experimental stage:
The results obtained from the experiments conducted in 5 days in the office are described in Tables 5A to 5E and in Figures 7 to 13 (see ANNEX for details, separate document). 176 Petri dishes and corresponding were collected.

Table 5 A. Experimental stage: Day 1 results (Device OFF)

Number of air sampling	Device	Time	CFU 22°C	CFU 36°C	Doors and windows closed	People inside the office	Note (people behavior)
T0 (1)	OFF	11:41	257	151	•*	4	people chattering
T0 (2)	OFF	11:50	225	272	•	4	people chattering
1	OFF	11:58	257	194	•	4	people chattering
2	OFF	12:06	199	116	•	4	people chattering
3	OFF	12:14	135	124		4	people chattering
4	OFF	12:22	212	254	•	4	people chattering
5	OFF	12:30	227	141		4	people chattering
6	OFF	12:38	198	151		4	people chattering
7	OFF	12:46	113	133		4	people chattering
8	OFF	12:54	136	121		4	people chattering
9	OFF	13:02	110	144		4	people chattering
10	OFF	13:10	225	136		4	people are silent
11	OFF	13:18	134	157		4	people are silent
12	OFF	13:26	114	120		4	people chattering
13	OFF	13:34	146	219		4	people chattering

** At the time indicated, the office is completely closed.*

Table 5 B. Experimental stage: Day 2 results (Device OFF)

Number of air sampling	Device	Time	CFU 22°C	CFU 36°C	Doors and windows closed	People inside the office	Note (people behavior)
T0 (1)	OFF	11:00	66	110		3	*people chattering*
T0 (2)	OFF	11:08	79	100		3	*people chattering*
1	OFF	11:16	58	101	•*	3	*people chattering*
2	OFF	11:24	95	92	•	3	*people chattering*
3	OFF	11:32	138	91	•	3	*people chattering*
4	OFF	11:40	143	172	•	3	*people chattering*
5	OFF	11:48	141	101	•	3	*people chattering*
6	OFF	11:56	168	65	•	3	*people chattering*
7	OFF	12:04	86	68	•	3	*people chattering*
8	OFF	12:12	64	85	•	3	*people chattering*
9	OFF	12:20	66	243	•	3	*people chattering*
10	OFF	12:28	61	86	•	3	*people chattering*
11	OFF	12:36	52	88	•	3	*people chattering*
12	OFF	12:44	64	85	•	3	*people chattering*
13	OFF	12:52	40	77	•	3	*people chattering*

** At the time indicated, the office is completely closed.*

Table 5 C. Experimental stage: Day 3 results (Device OFF)

Number of air sampling	Device	Time	CFU 22°C	CFU 36°C	Doors and windows closed	People inside the office	Note (people behavior)
T0 (1)	OFF	12:00	250	173	•*	4	*people chattering*
T0 (2)	OFF	12:08	120	205	•	4	*people chattering*
1	OFF	12:16	153	173	•	4	*people chattering*
2	OFF	12:24	110	188	•	4	*people chattering*
3	OFF	12:32	103	147	•	4	*people chattering*
4	OFF	12:40	96	158	•	4	*people chattering*
5	OFF	12:48	158	112	•	4	*people chattering*
6	OFF	12:56	190	138	•	4	*people chattering*
7	OFF	13:04	215	275	•	4	*people chattering*
8	OFF	13:12	108	244	•	4	*people chattering*
9	OFF	13:20	156	255	•	4	*people chattering*
10	OFF	13:28	164	160	•	*3*	*people chattering*
11	OFF	13:36	153	136	•	*3*	*people chattering*

* *At the time indicated, the office is completely closed.*

Table 5 D. Experimental stage: Day 4 results (Device ON)

Number of air sampling	Device	Time	CFU 22°C	CFU 36°C	Doors and windows closed	People inside the office	Note (people behavior)
T0 (1)	OFF	10:27	294	406	•*	5	*people chattering*
T0 (2)	OFF	10:35	257	332	•	5	*people chattering*
1	ON	10:43	150	508	•	5	*people chattering*
2	ON	10:51	164	302	•	5	*people chattering*
3	ON	10:59	183	303	•	5	*people chattering*
4	ON	11:07	159	267	•	5	*people chattering*
5	ON	11:15	223	441	•	5	*people chattering*
6	ON	11:23	182	332	•	5	*people chattering*
7	ON	11:31	158	332	•	5	*people chattering*
8	ON	11:39	155	220	•	5	*people chattering*
9	ON	11:47	153	222	•	5	*people chattering*
10	ON	11:55	164	279	•	5	*people chattering*
11	ON	12:03	113	262	•	5	*people chattering*
12	ON	12:11	121	268	•	5	*people are silent*
13	ON	12:19	208	273	•	5	*people are silent*
14	ON	12:27	211	304	•	5	*people are silent*
15	ON	12:35	164	249	•	5	*people are silent*
16	ON	12:43	154	230	•	5	*people are silent*
17	ON	12:51	164	257	•	5	*people are silent*
18	ON	12:59	155	216	•	5	*people are silent*
19	ON	13:07	179	209	•	5	*people chattering*
20	ON	13:15	134	178	•	5	*people chattering*

** At the time indicated, the office is completely closed.*

Table 5 E. Experimental stage: Day 5 results (Device ON)

Number of air sampling	Device	Time	CFU 22°C	CFU 36°C	Doors and windows closed	People inside the office	Note (people behavior)
T0 (1)	OFF	10:40	225	177	•*	5	*people chattering*
T0 (2)	OFF	10:48	189	229	•	5	*people chattering*
1	ON	10:56	163	240	•	5	*people are silent*
2	ON	11:04	101	163	•	5	*people are silent*
3	ON	11:12	28	121	•	5	*people are silent*
4	ON	11:20	106	163	•	5	*people are silent*
5	ON	11:28	129	120	•	5	*people chattering*
6	ON	11:36	107	151	•	5	*people chattering*
7	ON	11:44	69	165	•	5	*people chattering*
8	ON	11:52	83	125	•	5	*people chattering*
9	ON	12:00	68	139	•	5	*people chattering*
10	ON	12:08	74	60	•	5	*people chattering*
11	ON	12:16	60	124	•	5	*people chattering*
12	ON	12:24	65	112	•	5	*people are silent*
13	ON	12:32	73	99	•	5	*people are silent*
14	OFF	12:40	116	300	•	5	*people are silent*
15	OFF	12:48	204	263	•	5	*people are silent*
16	OFF	12:56	205	313	•	5	*people are silent*
17	OFF	13:04	152	245	•	5	*people are silent*
18	OFF	13:12	143	216	•	5	*people are silent*
19	OFF	13:20	171	218	•	5	*people chattering*
20	OFF	13:28	176	232	•	5	*people chattering*
21	OFF	13:36	202	288	•	5	*people chattering*

* *At the time indicated, the office is completely closed.*

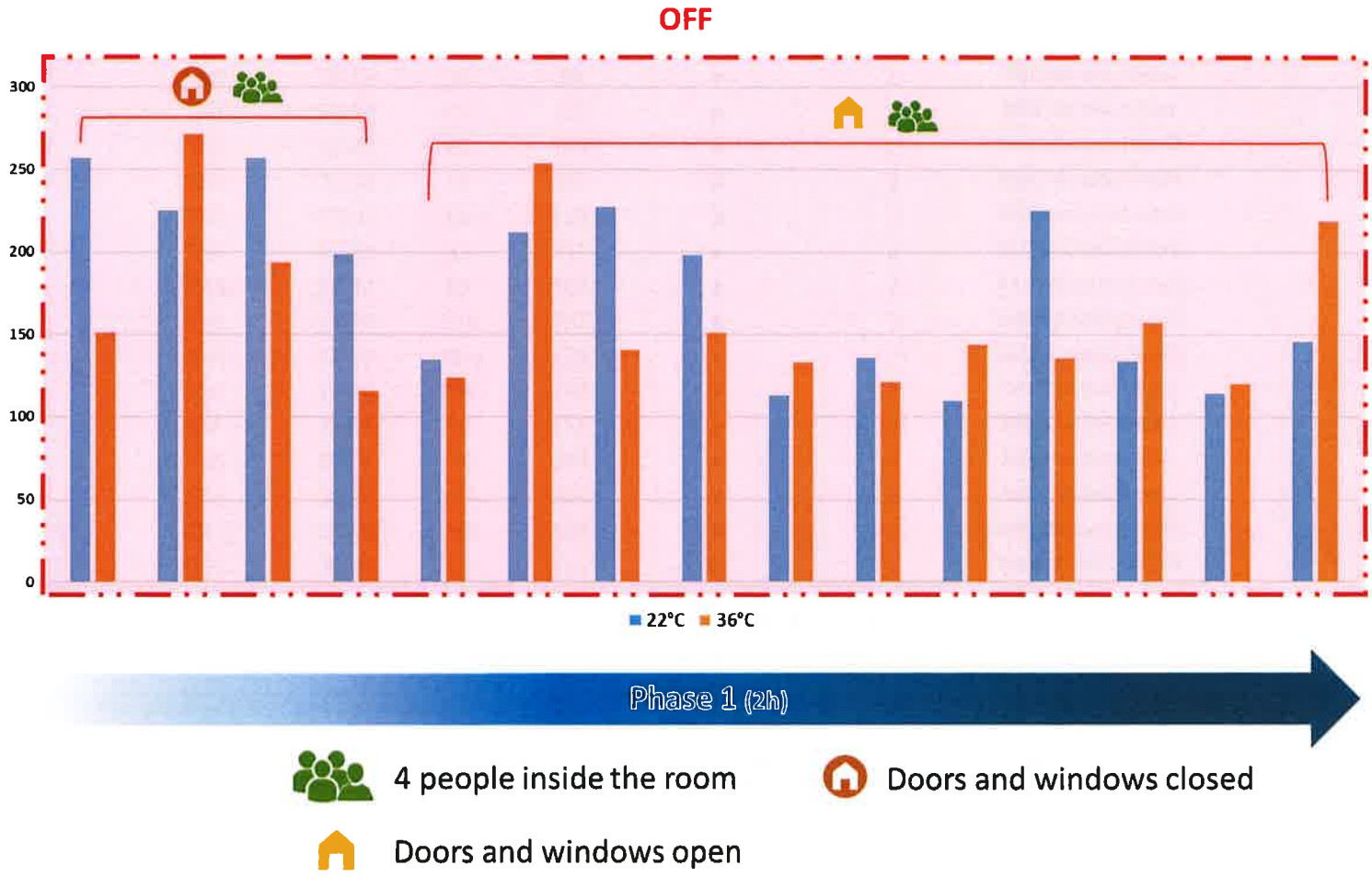


Figure 7. Experimental stage: Day 1 graphical results (Device OFF)

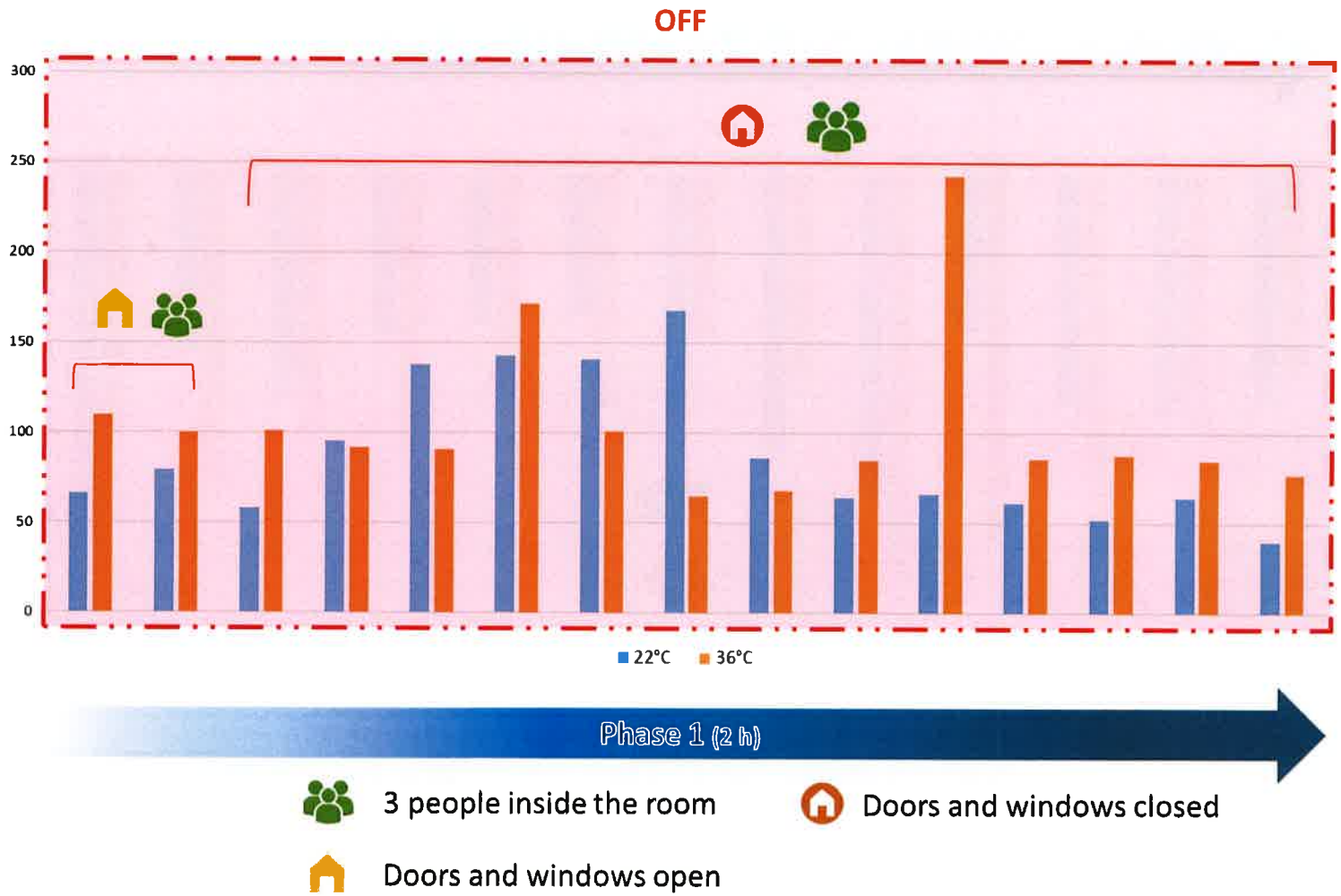


Figure 8. Experimental stage: Day 2 graphical results (Device OFF)

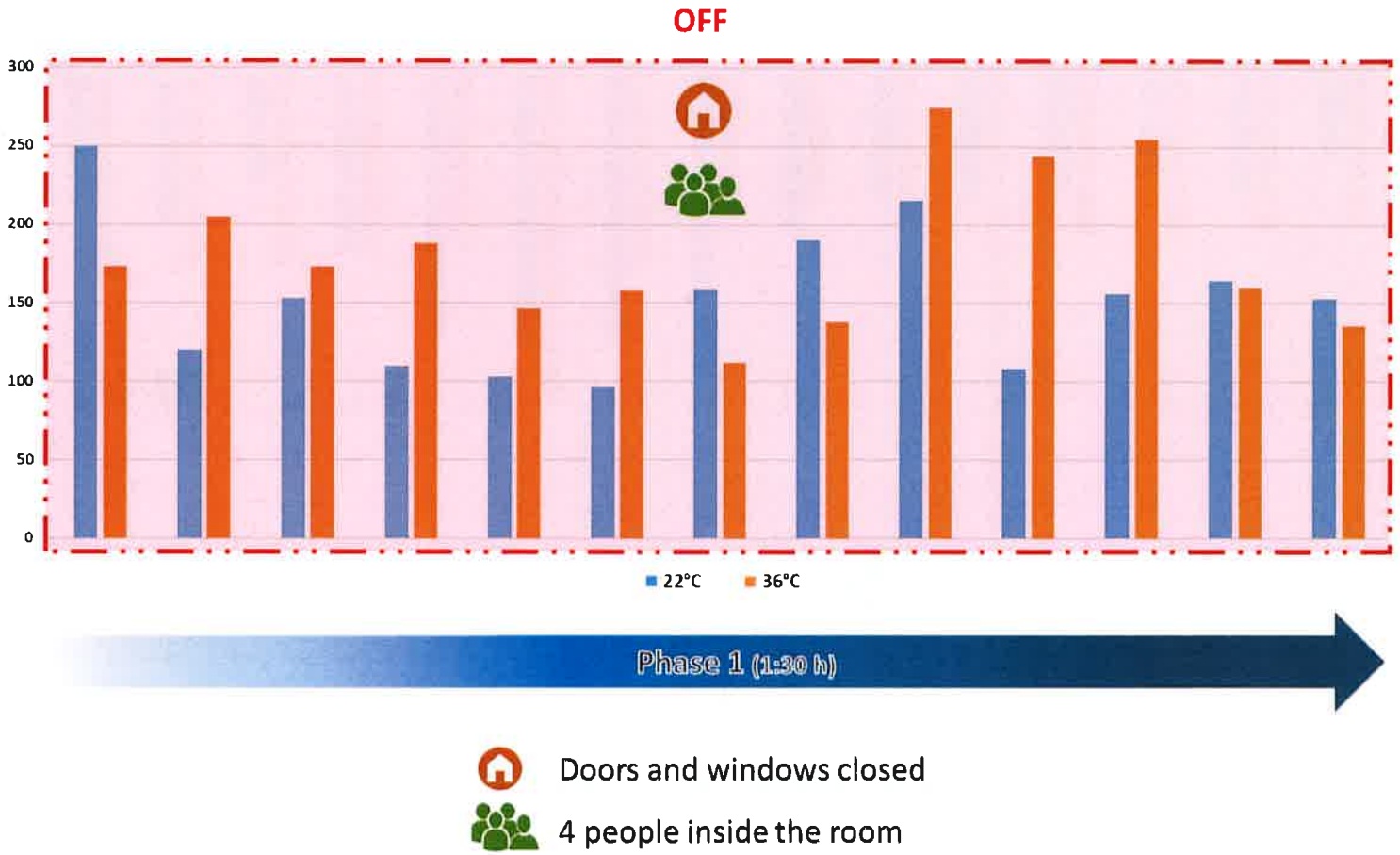


Figure 9. Experimental stage: Day 3 graphical results (Device OFF)

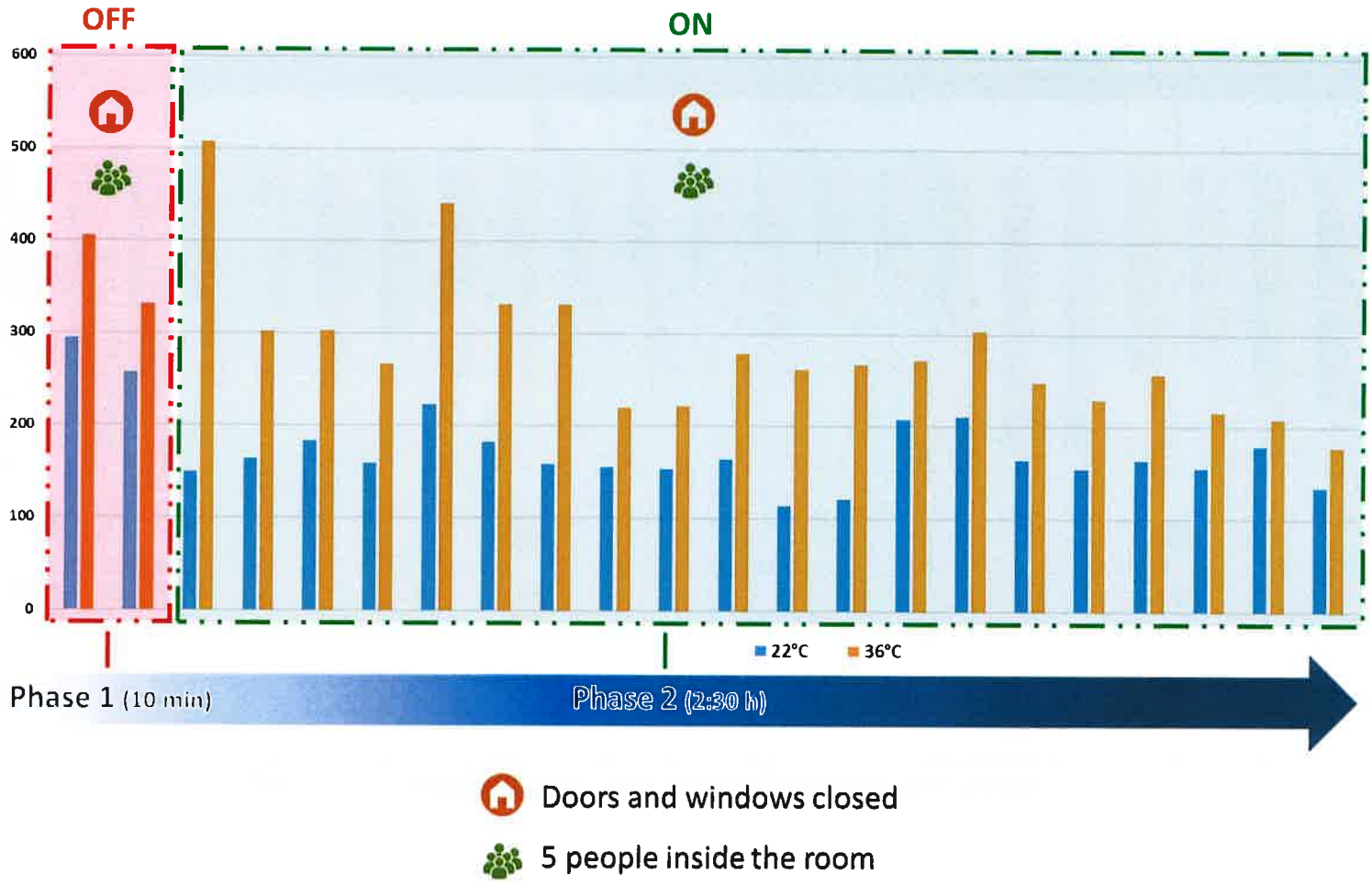


Figure 10. Experimental stage: Day 4 graphical results (Device OFF-ON)

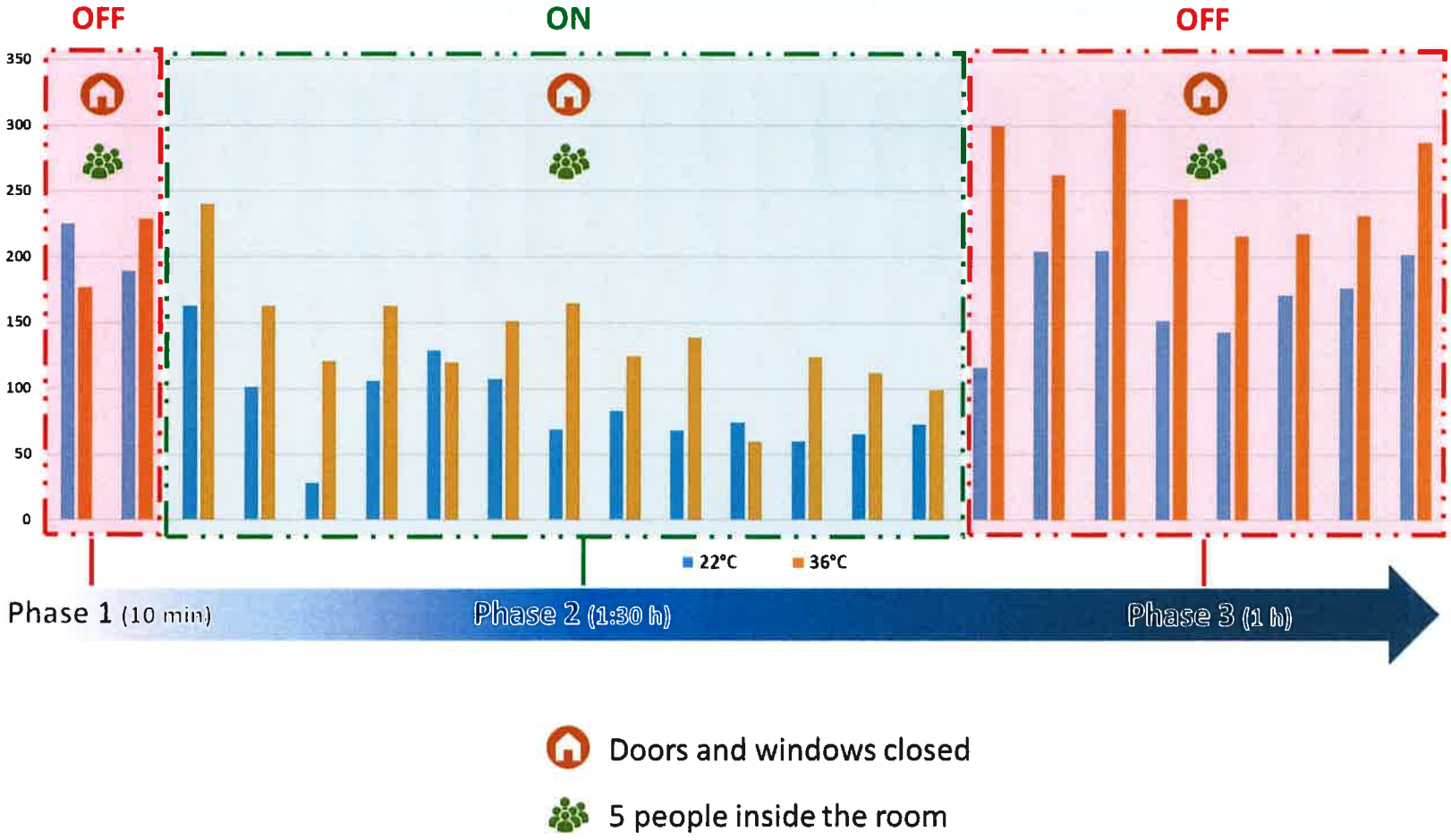


Figure 11. Experimental stage: Day 5 graphical results (Device OFF-ON-OFF)

On the first, second and third day of samplings, when the device was always switched OFF, with microbial incubation at 22 °C, we obtained an average microbial load of 186.8 CFU (95% CI 154 – 219), 93.6 (69.6 – 117.6) and 152 (124 – 180.0) per 0.5 m^3 of air sampled, respectively. Samples incubated at 36 °C had an average microbial load of 161.1 CFU (131 – 191.0), 107.8 (78.7 – 137.0) and 181 (151.7 – 212.0) per 0.5 m^3 of air sampled. It should be noted that the lower microbial load found on the second day is compatible with the fact that only 3 occupants were in the office instead of 4 on the first and third days.

The mean CFU count, at 22°C, during day 4, when the device was switched ON, was 164 (151.1 – 177.6) per 0.5 m^3 of air sampled; it was significantly lower than values recorded when the device was OFF, that is 294 and 257 CFU ($P<0.001$). Similarly, the mean CFU count, at 36°C, when the device was switched ON, was 282.2 (246.0 – 319.1) per 0.5 m^3; it was significantly lower ($p<0.001$) than the previous three samplings, when the device was OFF and just switched ON.

On the fourth day, when the device was switched ON, we had a significant decrease in CFU after about 8 minutes and the level of reduction remained constant, with only one exception, for all the other samplings.

On day 5, when the device was OFF, the CFU at 22°C were significantly higher (225 and 189 per 0.5 m^3) than the mean value, 118.8 (94.5 – 143) 0.5 m^3, when the device was switched ON.

After the sixth sampling, with the device switched ON, we could appreciate that the average level of microbial contamination decreased significantly ($P=0.001$) becoming 70.3 (63.5 – 77.1) per 0.5 m^3 of air sampled. In addition, when the device was switched OFF we had a significant increase in CFU. The mean contamination value passed from 86.6 (65.8 – 107.4) to 171.1 (143.9 – 198.3) CFU per 0.5 m^3 of sampled air. The mean reduction in CFU was 84.5 (52.8 – 143.1) per 0.5 m^3.
Similar increase was experienced with samples incubated at 36°C; we passed from 137.1 (111 – 162.8) CFU, when the device was ON, to 259.4 (228.0 – 290.8) CFU per 0.5 m^3 of air sampled, when the device was switched OFF, showing an average reduction of 122.3 (83.9 – 160.7) CFU per 0.5 m^3.

Figures 12 and 13 show the CFU at both 22°C and 36°C incubation during the day 5 air sampling procedures.
To assess device activity during the sampling procedures, in which the device went from OFF (initial phase, 2 points, 10 minutes) to ON (middle phase, 13 points, 1.5 hours) and back to OFF (final phase, 8 points, 1 hour), we interpolated the data when the device was ON (phase 2) with a non-parametric iterative least-squares method and we calculated the mean level of contamination in the last 8 samplings, when the device was turned OFF (phase 3). In spite of the strong and continuous environmental contamination caused by the simultaneous presence of 5 people in a room of only 65 m^3, and by the doors and windows closed during the whole experiment, during the operation phase of the device, we can notice that after 8 minutes (one sampling point) the system acts significantly on the reduction of environmental contamination, reaching a drop of about 50% at 36°C and 70% at 22°C. In addition, an increase in CFUs of approximately 150% (for both temperatures), between the final time of the device ON and the average value of points at the turned OFF device, can be observed.

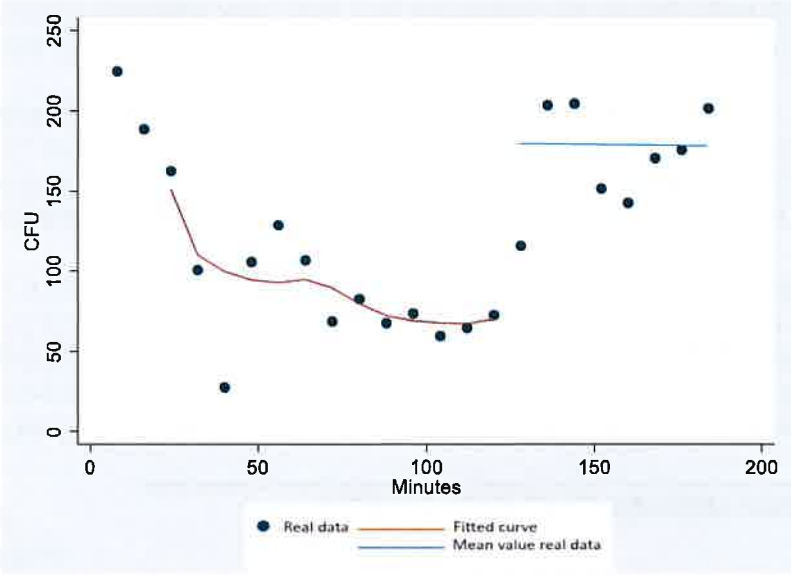


Figure 12. CFU at 22 C°, real data and fitted curve- Day 5

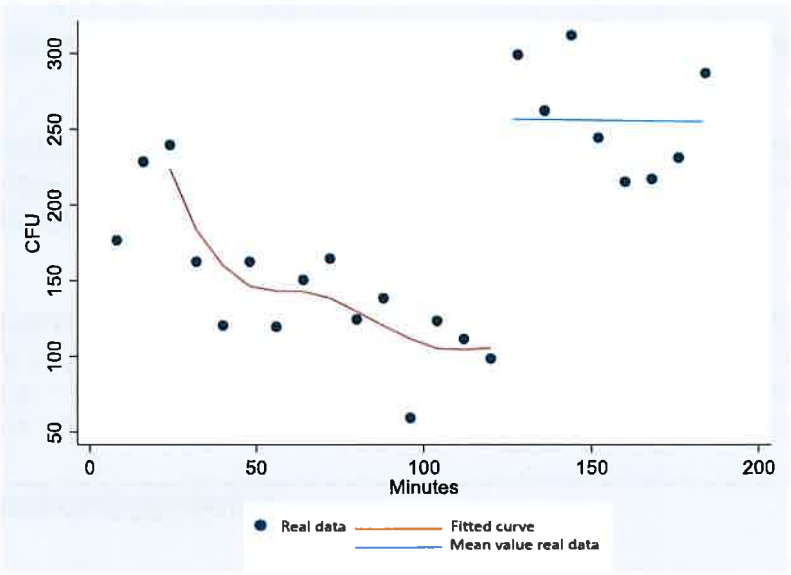


Figure 13. CFU at 36 C°, real data and fitted curve- Day 5

DISCUSSION AND CONCLUSIONS

In both the preliminary and experimental phases, the Cleaning air T12 device was able to significantly reduce and control microbial contamination of the air.

In a controlled environment, i.e. inside the chamber of irradiation and uniform contamination of the initial air, the experimental measurements of irradiance in the air duct, show how the device, at each air passage, is able to abate between 99% and 99.99% of the microbial load related to many microbial species (bacteria, viruses, fungi, molds, etc..) present in a real context.

In particular, the latest experiments conducted in real environment in the office of our Department clearly show how, during its operation, the environmental contamination is gradually reduced and controlled over time, despite the presence of 5 people in a completely closed and isolated environment.
The experimental tests show how, as soon as the device is turned OFF after at least half an hour of operation, the healthiness of the air drops dramatically within 10 minutes, bringing the levels of microbial contamination (induced by the presence of the operators in the room) to levels even higher than 150%.

The data obtained verify the effectiveness of the device in progressively reducing and controlling environmental air contamination induced by medium to high human presence.
Of course, changes in environmental and experimental conditions (different number of people, room size, device run times, etc.) could produce different results. However, the experiments we carried out are a good representation of a real context of work, study and, in general, of human frequentation of a closed environment.

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Prof. Gabriele Messina

ANNEX

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ANNEX 1

Report (version 1.0)

TEST ON REAL SETTING WITH CLEARING AIR T12 BY ISOGROUP ITALIA srl

Notes:

- *the resolution of the images may be low and it may appear that the colonies do not correspond correctly to the attributed count on the plates.*
- *In some plates some imperfections in the agar (e.g. micro bubbles, condensation) could be mistaken for colonies. The number of colonies is always indicated on the plates.*

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TEST ON CLEARING AIR T12 BY ISOGROUP ITALIA srl

Operative Protocol - Air sampling method (preliminary tests):

- Set microbiological air samplers (Sas Microflow α Aquaria) with the following parameters: aspiration volume of 0.5 m^3 and aspiration speed of 120 l/min.
- Insert the Petri dishes (60 Ø mm) in the sampling devices.
- Start the devices and seal the test room.
- After the aspiration, before and after the exposition at Cleaning air T12, remove the Petri dishes and incubate at 36 °C and 22 °C for 72h.

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SCHEMATIC PROCEDURE

Air sampling method

Plates Count Agar inserted in the device

Set different parameters and started Sas Microflow α Aquaria

The air in the room is aspirated before and after the exposure to the Cleaning Air T12

Incubate the plates at 36°C and 22°C for 72h

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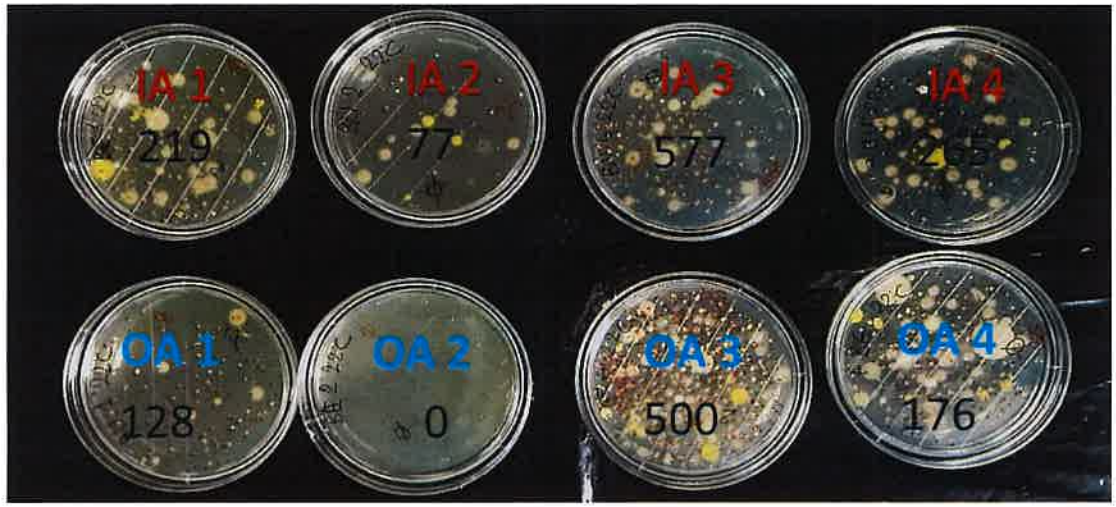
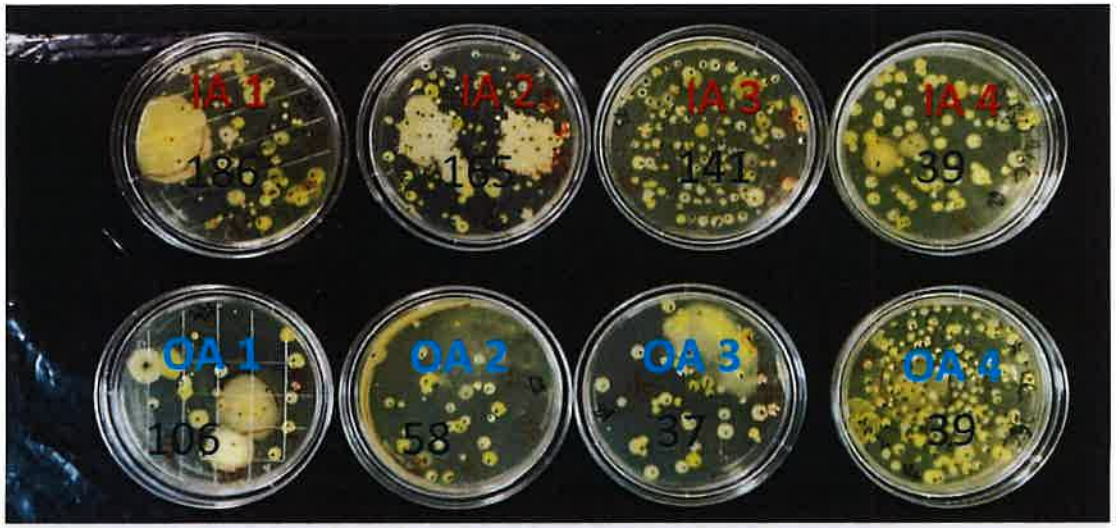
Preliminary stage

22°C



36°C



- *IA: incoming air*
- *OA: outgoing air*

TEST ON REAL SETTING WITH CLEARING AIR T12 BY ISOGROUP ITALIA srl

Test on real setting: study design

Tests were conducted for 5 days in a office setting (65 m^3) in Department of Molecular and Developmental Medicine in the University of Siena, with a different number of people inside the room:

- 1 – 3 days: device turned off
- 4 and 5 days: device turned on and off

In each test, the first sampling was **T0**, corresponding to the initial concentration (device turned off);

Air samples were collected with Sas Microflow α Aquaria: 0,5 m^3 for each sample, speed 120L/min;

Petri dishes were incubated at 36°C and 22°C.

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Test on real setting – day 1: Device OFF (22°C)

- *SMP: sample – device ON*
- *T0: device off during the sample*

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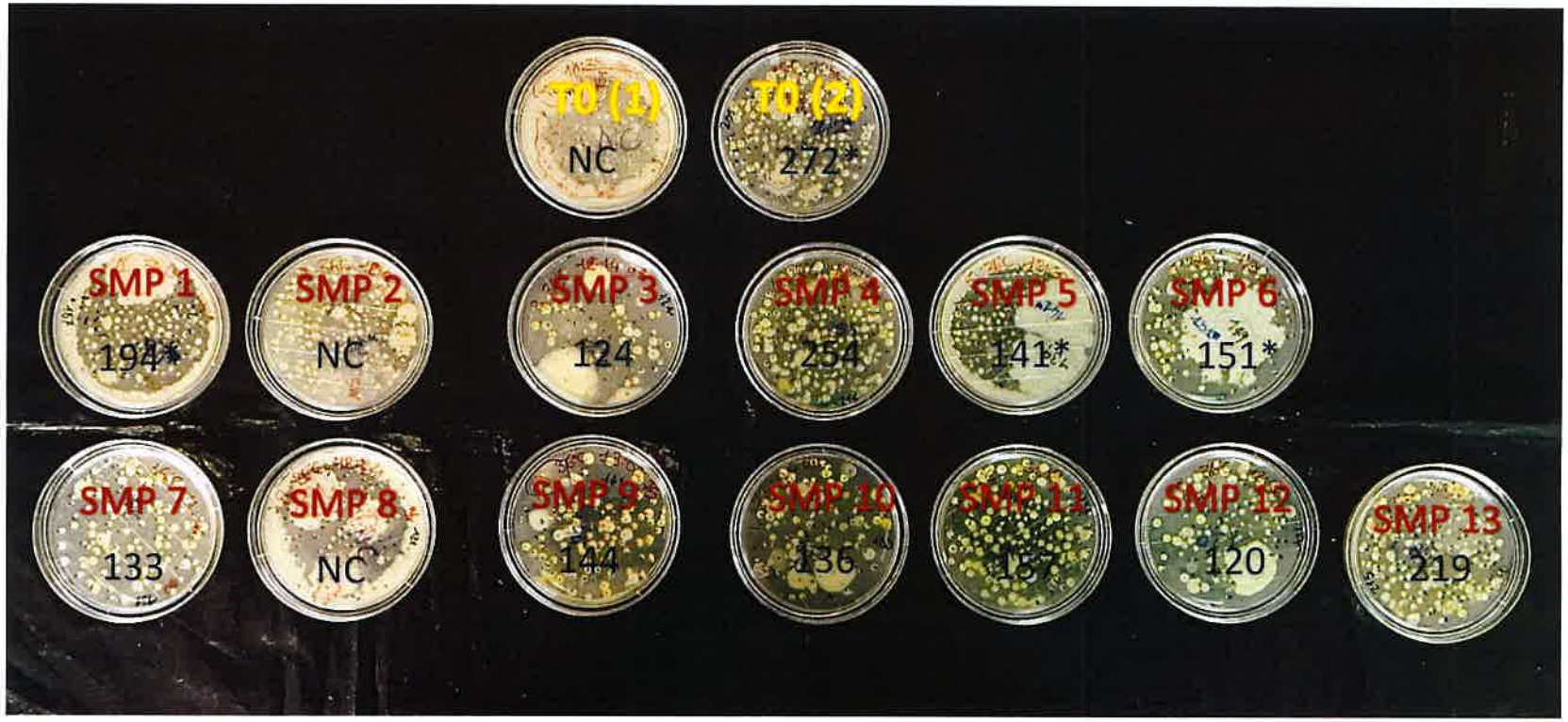
Test on real setting - day 1: Device OFF (36°C)



- ***SMP: sample – device ON***
- ***T0: device off during the sample***

**: plate completely covered by environmental contaminant*

- *NC: Not countable*

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Test on real setting – day 2: Device OFF (22°C)

- ***SMP: sample – device ON***
- ***T0: device off during the sample***

**: plate completely covered by environmental contaminant*

- *NC: Not countable*

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Test on real setting – day 2: Device OFF (36°C)

- *SMP: sample - device ON*
- *T0: device off during the sample*
- *NC: Not countable*

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Test on real setting – day 3: Device OFF (22°C)

- ***SMP: sample - device ON***
- ***T0: device off during the sample***

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Test on real setting – day 3: Device OFF (36°C)

- ***SMP: sample - device ON***
- ***T0: device off during the sample***
- *NC: Not countable*

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Test on real setting – day 4: Device ON (22°C)

- *SMP: sample - device ON*
- *T0: device off during the sample*

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Test on real setting – day 4: Device ON (36°C)

- *SMP: sample - device ON*
- *T0: device off during the sample*

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Test on real setting – day 5: Device ON/OFF (22°C)

- ***SMP: sample – device ON***
- ***SMP: sample – device OFF***
- ***T0: device off during the sample***

**: plate completely covered by environmental contaminant*

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Test on real setting – day 5: Device ON/OFF (36°C)

- ***SMP: sample – device ON***
- ***SMP: sample – device OFF***
- ***T0: device off during the sample***

**: plate completely covered by environmental contaminant*

- *NC: Not countable*

TEST ON REAL SETTING WITH CLEARING AIR T12 BY ISOGROUP ITALIA srl