

# Journal of Photochemistry and Photobiology

## WiWell® TiO<sub>2</sub>-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time --Manuscript Draft--

<b>Manuscript Number:</b>	JPAP-D-22-00076R2
<b>Full Title:</b>	WiWell® TiO <sub>2</sub> -photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time
<b>Short Title:</b>	TiO <sub>2</sub> -photocatalytic adhesive films to reduce microbial charge in indoor microenvironments
<b>Article Type:</b>	Communications
<b>Section/Category:</b>	Photobiology
<b>Keywords:</b>	membrane; tiO <sub>2</sub> -Ag doped; anti-microbial; sanitization
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<b>Corresponding Author's Institution:</b>	University of Verona
<b>Corresponding Author's Secondary Institution:</b>	
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<b>Order of Authors:</b>	Umberto Tirelli
	Luca Berto
	Salvatore Chirumbolo, Ph.D.
<b>Order of Authors Secondary Information:</b>	
<b>Abstract:</b>	<p><b>Abstract</b></p> <p>A nanosized TiO<sub>2</sub>-AgNPs doped photocatalytic adhesive membrane, recently patented as WiWell<sup>™</sup> (Wippyidea<sup>®</sup>), has been used in the indoor environments of public buses to decrease the microbial charge, evaluated by bio-luminometry, from people crowding and climatic conditions. While the simple cleaning process, with chemical sanitizers, reached a reduction of indoor microbial contamination as high as 40%, the use of the photocatalytic films lowered the bio-luminescence to values <math>\geq 94\%</math>, ensuring much safer indoor conditions for people travelling. This pilot study encourages further studies to support this technology and apply it everywhere.</p>
<b>Suggested Reviewers:</b>	Antonio Vella, Ph D Integrated University Hospital of Verona antonio.vella@univr.it Expert in microbiology
	Kewin Wu, Ph D National Taiwan University kevinwu@ntu.edu.tw Expert
<b>Opposed Reviewers:</b>	
<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>

Point by point reply to the reviewers' comments

Manuscript Number: JPAP-D-22-00076R1

WiWell® TiO<sub>2</sub>-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time

Reviewer 1: The manuscript can be accepted for the possible publication in this Journal.

**Authors' response: Many thanks for this comment**

Reviewer 2: The authors did a great improvement in the paper. However, some issues have to be reviewed in order to make the work suitable for publication. The work addresses a thin film containing nanoparticles of TiO<sub>2</sub> and Ag. There are a huge number of review papers in the literature concerning the safety use of nanoparticles. Therefore, although the authors had chosen to omit the sentences that had reported the absence of toxicity, it could be better to discuss the information pointing out the evidences of safe use of these nanoparticles in materials coating and also the evidences of about the nanotoxicity.

**Authors' response: A new paragraph dealing with this topic was added in the manuscript. The literature on the field generally attributes negligible toxicity to TiO<sub>2</sub> nanoparticles exposure from TiO<sub>2</sub>-Ag-NPs membrane, particularly because of the elevated presence of titanium in many common used products.**

The authors also highlighted the limited use of the coating in Italy but there is no mention about its use elsewhere. Is it used or not in other countries?

**Authors' response: Right. We removed the reference to Italy**

Some minor corrections are also needed:

1) Do not use abbreviations in the abstract

**Authors' response: Abbreviations in the abstract were removed, accordingly**

2) When the SD is already in the first significant digit, do not present the mean value with 2 significant digit. (Lines 48 and 61)

**Authors' response: Done**

3) Please check the symbols used to report wavelength and gap (lines 18 and 24).

**Authors' response: Done**



To the Editor in Chief

Journal of Photochemistry and Photobiology

Dear EIC,

We are submitting the second revised manuscript titled “WiWell® TiO<sub>2</sub>-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time” to be published in Journal of Photochemistry and Photobiology. A point by point reply to the Reviewers’ comments is appended herein. The paper represents a further contribution on the bioactivity of TiO<sub>2</sub>-Ag doped anti-microbial sanitizing membranes. While the simple cleaning process, with chemical sanitizers, reached a reduction of indoor microbial contamination as high as 40%, the use of the photocatalytic films lowered the bio-luminescence to values  $\geq 94\%$ , ensuring much safer indoor conditions for people travelling. This pilot study encourages further studies to support this technology and apply it everywhere.

The paper is original, is not pending any evaluation elsewhere and has never been submitted to the journal previously. We hope that this manuscript, in its revised form, is considered interesting for the readers of the journal and judged worth of publication in Journal of Photochemistry and Photobiology.

Looking forward to a kind reply from yours

Yours sincerely,

Salvatore Chirumbolo (on behalf of coauthors)



Point by point reply to the reviewers' comments

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**Authors' response: Done**

3) Please check the symbols used to report wavelength and gap (lines 18 and 24).



UNIVERSITÀ  
di VERONA

Salvatore Chirumbolo-Department of Neurosciences, Biomedicine and Movement Sciences

**Authors' response: Done**

# ICMJE DISCLOSURE FORM

**Date:** 7/5/2022

**Your Name:** Luca Berto

**Manuscript Title:** WiWell® TiO2-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time

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**Date:** 7/5/2022

**Your Name:** Umberto Tirelli

**Manuscript Title:** WiWell® TiO2-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time

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ICMJE DISCLOSURE FORM

Date:7/5/2022

Your Name:Salvatore Chirumbolo

Manuscript Title:WiWell® TiO2-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time

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# WiWell® TiO<sub>2</sub>-photocatalytic adhesive films to reduce microbial charge in indoor microenvironments of public transportation and ensure biosafety in the COVID-19 time

Tirelli Umberto<sup>1</sup>, Luca Berto<sup>2</sup>, Salvatore Chirumbolo<sup>3</sup>

<sup>1</sup>Tirelli Clinical Group, Pordenone, Italy

<sup>2</sup>Material Chemical Expert Labs, Treviso, Italy

<sup>3</sup>University of Verona, Verona, Italy

## Correspondence

Prof Salvatore Chirumbolo

Department of Neurosciences, Biomedicine and Movement Sciences, Unit of Human Anatomy

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## Abstract

A nanosized TiO<sub>2</sub>-Ag nanoparticulate doped photocatalytic adhesive membrane, recently patented as WiWell™ (WippyIdea®), has been used in the indoor environment of public transportation to decrease the airborne microbiome and the microbial charge on handy surfaces, to ensure a safe context to people using and or crowding the place. While the simple cleaning process with chemical sanitizers reached a reduction of indoor microbial contamination as high as 40%, the use of the photocatalytic films lowered the microbial pollution, measured via ATP-bio-luminescence, to values  $\geq 94\%$ , ensuring much safer indoor conditions for people travelling. This pilot study, performed on the field, encourages further research to support this patented technology and apply it everywhere.

## INTRODUCTION

The need to develop human indoor sanitized microenvironments, particularly for cabs crowded by daily routine travelers such as public transportation, has become a crucial matter of debate in the COVID-19 era (1,2). For example, an indiscriminate use of disinfectants and chemical sanitizers may represent a serious concern for those indoor spaces usually occupied by children (3). Moreover, while disinfection of such spaces can be performed properly by expert personnel

in the complete absence of customers or using people, these latter are not always prompted in observing any personal warning to maintain a sanitized indoor environment while is crowded. To address this concern, besides to the introduction of affordable and safer chemical disinfectants, the use of eco-friendly nanotechnologies to sanitize indoor spaces, is an encouraging novelty in the field.

The existence of straightforward nanostructured coating systems, endowed with a photocatalytic-mediated anti-microbial activity, are characterizing the field of indoor sanitization particularly in those micro-environments such as transportation vehicles, notably crowded with numerous customers. Many of these systems are made by a layer of SiO<sub>2</sub> associated with a layer of mesoporous or dense TiO<sub>2</sub>-anatase and silver nanoparticles (Ag-NPs) as doping. And usually, the coating is synthesized using a sol-gel technology by merging Ag-NPs with SiO<sub>2</sub> and TiO<sub>2</sub> sols (4).

Polymerizing substances, such as polyethylene glycol (PEG-600) (5) or polyethersulfone (PES) (6) and immobilizing TiO<sub>2</sub>-Ag nanoparticles (NPs) with polyamide nanofiltration membranes are widely used to stabilize nanoparticles on a membrane and reduce greatly the possible leakage of metal nanoparticles in the environment (7).

Titanium dioxide (TiO<sub>2</sub>), the semiconductor used in photocatalytic procedures, is an environmentally-friendly choice for indoor disinfection in different technology devices. This compound, following studies conducted using electron paramagnetic resonance and a 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), which is a trapping molecule in water or in ethanol, is able to induce the formation of reactive oxygen species (ROS) following solar light excitation for at least 20 minutes (8,9). The ultraviolet components in the solar light are able to produce an initial rate of ROS (such as OH• radical) as low as  $1.0 \times 10^{-8}$  mol radical sec<sup>-1</sup> mg<sup>-1</sup>, an amount able to damage and kill both Gram-negative and Gram-positive bacterial species, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* (9). Therefore, in these systems, ROS reached the bioactive surface of the polymer-TiO<sub>2</sub> film and interacting with the neighboring microorganisms, inactivate them in few minutes of light exposure.

Despite the increasing number of studies about self-cleaning membranes, their application in public transportation, particularly in Italy, is still poorly considered.

The technology is widely affordable, cost-effective, harmless, eco-friendly and endowed with a good safety profile, it is reusable and stable from a chemical standpoint, without risk of byproducts that can prove themselves harmful for the human health. However, the efficiency of these nanostructured coating systems as catalysts, are yet under research, in order to reach

1 further improvements, usually by doping with metals and non-metals and achieve an optimal  
2 self-cleaning property (10). The whole process can be improved by studying other impactful  
3 aspects such as the source of ultraviolet irradiation or the reactor designs and configurations,  
4 which can enhance its efficiency. Its application is greatly widespread to date and may promote  
5 important developments in such contexts dealing with environmental health: indoor air, water  
6 sources (from drinking, to effluents treatment and wastewater), plant protection, pharmaceutical  
7 and food industry, biological and medical settings, such as laboratories and hospitals and public  
8 transportation (11-13).

14 Aside from our studies, this technology, once patented, is still prevalently conceived for  
15 seawater filtration and wastewater treatment, very rarely for indoor environments hosted by  
16 humans (14).

20 To date, self-cleaning TiO<sub>2</sub> membranes are widely spreading in domestic indoor environments  
21 thanks to our recent innovative investigations, which allowed us to hold to date the leadership  
22 in the market about these devices used in public indoor spaces, despite still in a prototype phase.  
23 Recent data showed that photocatalysis offers powerful solutions to disinfect human indoor  
24 environments, even for SARS-CoV2 (15-17), however the experimental system we are  
25 describing here, by mainly evaluating an ATP-related bioluminescence, is at present unable to  
26 fully ensure any people-transfer systems such as public transportation about the complete viral  
27 sanitization of indoor environments. Yet, sanitization from bacterial and yeast presence on  
28 handling surfaces in an indoor environment, by reducing air microbiome lowers also the  
29 incidence of SARS-CoV2 indoor spreading (18).

38 This ability is particularly useful to sanitize indoor microenvironments, such as inside school-  
39 buses, usually highly attended by pupils and scholars, as well as, public people transports, with  
40 high efficiency and making them microbial-free places.

43 As no sound documentation has been reported so far describing the application of an affordable  
44 and patented self-cleaning membrane on public transportation in our country, in this study we  
45 performed (on the field) a preliminary evaluation of the ability of specific high-performance  
46 composite photocatalytic membranes (as adhesive films) based on titanium dioxide (WiWell®)  
47 and known also as WiGlass™, via a bio-luminometry assay and using a Reed-Muench method.  
48 Despite the widespread knowledge about the TiO<sub>2</sub>-photocatalytic adhesive films, their use in  
49 Italy is limited to industrial water filtration, never in indoor environments, so this study  
50 represents the first research on the field about the application of a patented self-cleaning  
51 membrane on public transportation.

## MATERIALS AND METHODS

### *Sample collection on the sample spots*

Samples were collected by one of us (LB), joined with three assistants, using a sterile swab in at least three different replicates on different points (Figure 1) (central or medial, lateral left and lateral right side) of the following surfaces usually in close contact with humans: a) stop call button (medial pressing area); b) passenger seat (handy frontal areas and seat); c) driver proximity; d) steering wheel; e) window-close passenger seat; f) entrance and exit doors neighboring areas. Sampling was performed at the same scheduled time, following 8-9 hours from chemical cleaning of the surfaces. In each seasonal time the same transportation mean was sampled with the same scheme for at least four times.

Microbiological monitoring, a parameter of the air quality, is generally performed to assess the microbial concentration in the air and on surfaces in human indoor environments. This procedure also makes it possible to verify the effectiveness of the containment measure with photocatalytic membranes which is promptly undertaken and the correctness of the procedures implemented in order to eliminate or minimize microbial exposure.

The choice of the type of sampling and of the matrices to be analyzed requires a careful preliminary study of the types of microorganisms presumably present according to the work activities and people attendance performed and the possible routes of diffusion and infection (19). Usually the bacterial population targeted is the mesophilic bacteria of human origin with the genus *Staphylococcus* as the reference standard, as modified by air pollution (20).

### *Type of monitoring and control process*

We performed a real time sampling and monitoring of the bacterial presence in situ (i.e. on the field) in three different seasonal times and different sunlight exposition (in terms of different meteorological day by day conditions) in order to reduce statistical confounders due to differences in lightness, temperature, relative humidity and people crowding with school and/or job attendance (21). This pilot study engaged three different transport areas in Veneto (Treviso, Venice) and Friuli (Pordenone) (North-Eastern Italy). The areas considered, encompassing about 10-20 km each of bus transport ( $\geq 60$  min), to standardize a comparable amount of indoor permanence, are indicated in Figure 2.

The seasonal time included April, June and September, as hot spots of research sampling, with various degrees of people crowding and sunlight daylight. Microbiological controls as CFU/ml in Mueller-Hinton agar (each for every seasonal time) were performed and assessed the absence of microbial growth, when the bio-luminometer reached its lowest values in RLU.

Experiments were all carried out on daily sunlight, from 1,000 lux (typical overcast day in midday) to 120,000 lux (brightest sunlight) at the same time (3.00 p.m.) (22).

#### *Leakage of nanoparticles from TiO<sub>2</sub>-Ag-NPs membrane and environmental toxicology profiles*

*In vivo* studies on laboratory animals reported that TiO<sub>2</sub> NPs did not cause significant effects of cell damage at dose ranges of 10-50 µg/ml (effects appear at 100-250 µg/ml), whereas Ag NPs are toxic at 5-50 µg/ml (21). Moreover, *in vitro* studies showed that IC<sub>50</sub> of TiO<sub>2</sub> NPs at 24 hours exposure was 211.3 µg/ml ±15.2 SD and 5408.8 µg/ml ±45.9 SD for the chondrosarcoma cell line SW 1353 and the osteosarcoma U-2-OS cell line, respectively (23).

However, despite the alarming warning about the biotoxicity of TiO<sub>2</sub> nanoparticles still might represent an indoor concern, recent studies have deeply highlighted the issue (24). Recent data report that only a chronic and overload exposure of this matter may develop severe forms of organ damage, particularly for lungs, in laboratory animals. In particular, in workers heavily exposed to TiO<sub>2</sub>, a lack of statistical correlation exists between prolonged exposure and cancerous or non-cancerous chronic lung pathologies (25). TiO<sub>2</sub> is present in cosmetics and sunscreens but studies on epidermis reported lack of penetration of particles via the skin and moreover even oral exposure of TiO<sub>2</sub> particles with food indicated a negligible uptake by the gastrointestinal tract of particles into the bloodstream (25). Finally, toxicity studies on rats have shown that very low toxicity effects are attributed to TiO<sub>2</sub> particles, with No Observed Adverse Effects Level (NOAELs) of 1,000 mg/kg bw/day (25).

Our lab calculation reached the estimation that, in order to achieve an indoor pollution of TiO<sub>2</sub> NPs close to the sub-chronic exposure able to induce serious health damages, the TiO<sub>2</sub>-Ag-NP membrane should be damaged for at least 30%, an occurrence that is quite impossible to get, as minimal ruptures are visible as detached adhesive film, which should compel the operator to replace the membrane itself on the place at the earliest.

#### *Photocatalysis quality check*

For TiO<sub>2</sub>, experimentally ultraviolet (UV) light with energy greater than or equal to the Energy Gap (EG) is required for the formation of charge carriers. Previously evaluations were accomplished to quantify the photocatalytic performance of the WiWell® TiO<sub>2</sub>-membrane.

A Delta Ohm, model HD 9021 quantum photo-radiometer, which is an instrument for laboratory tests to measure the radiations emitted by UV lamps and those absorbed by the photocatalytic process, is usually employed for this purpose. The device is equipped with different probes, which allow to measure different aspects of the light, according to the needs the most suitable probe will be used. The probes allow to measure: a) illuminance (lux); b) the irradiance (W/m<sup>2</sup>); c) the luminance (cd

/m<sup>2</sup>) (cd = candles). Using the probe type HD 9021 UVA, a radiometric probe for measuring the power of radiation, irradiance, including in the UV-A range (with a wavelength of 315-400 nm), with a peak at 365 nm, the measurement range goes from 10 nW/cm<sup>2</sup> to 200 mW/cm<sup>2</sup> expressed as the power of the electromagnetic radiation that strikes the surface per unit area.

TiO<sub>2</sub> is a semiconductor with an energy gap equal to  $EG = 3-3.3$  eV, the energy required varies with the allotropic form used. Anyway, if TiO<sub>2</sub> is irradiated with photons of energy greater than EG (i.e. wavelength,  $\lambda \leq 390$  nm), an electron is able to overcome the energy gap and be promoted from the valence band to that of charge conduction. In this sense, the UV-A component of the natural daily sunlight is able to activate the photocatalysis of the WiWell® TiO<sub>2</sub> membrane.

The gaps in the valence band, scattered on the surface of the photocatalyst, react with the absorbed water molecules forming the hydroxyl radical ( $\bullet OH$ ) or directly with any adsorbed organic compounds. Both the hole and the hydroxyl radical can oxidize the organic molecules near the surface of the photocatalyst. The electrons promoted in the conduction band can participate in reduction processes, generally by reacting with molecular oxygen dissolved in solution, or in air, producing the superoxide radical ( $\bullet O_2^-$ ).

The highest efficiency of the photocatalytic membrane (> 99%) is reached upon > 109,000 lux sunlight, i.e. during brightest sunlight (120,000 lux) or bright sunlight (111,000 lux) but the efficiency of bacterial removal (> 99.99%) is reached following 90 minutes of activation at 2,000 lux, i.e. even during a complete overcast midday, as over 90% of UV-A rays are able to pass through clouds and glass windows.

### *Membrane hallmarks and safety profile*

WiWell® adhesive films are composed by a mixture of titanium dioxide, colloidal silver and other nanosized components as a nanoparticulate matrix, able to lead to a self-cleaning process by exploiting and stimulating a photocatalytic process, which is fully harmless to human health. The expected half-life of this photocatalytic device is  $24 \pm 2$  months.

In general, TiO<sub>2</sub> is considered as both an inert and safe nanomaterial, despite some controversial issue about the biotoxicity of titanium oxide nanoparticles has been recently raised (26,27). Neurotoxicity from TiO<sub>2</sub>, at least in experimental animals, has been observed for concentrations as low as 2.5 mg/kg body weight (bulk TiO<sub>2</sub>) (28), despite the EC<sub>50</sub> of TiO<sub>2</sub> nanosized formulation is around 5.83 mg/L (29). However, toxicological tests on *S. cerevisiae* showed no titanium nanoparticulate toxicity even at 20,000 mg/L (30). As outlined before, the toxicity of TiO<sub>2</sub> NPs should be negligible for our membranes. These values are extremely far from TiO<sub>2</sub> nanoparticulate leakage in the indoor

environment from the WiWell® membrane, which is replaced routinely each week/month or each 6 months, depending on its use frequency.

In our study, these adhesive membranes were applied to indoor surfaces of windows, walls and inner objects, such as backseats, and exposed to daily sunlight, for at least 6 hours.

### *Bioluminescence testing*

In order to verify the indoor sanitization via the activation of photocatalysis, we proceeded with sampling through swabs for quantitative verification of living organic particles (mainly bacteria) on the surfaces where the product was applied, as previously described. To perform this check we used a bio-luminometer (Ensure™ Touch, Hygiena® Ultrasnap model), and an immediate response of the result was reported in a real time process. The monitoring system guarantees high sensitivity (it detects up to 0.1 femtomoles of ATP) and results in only 15 seconds of process time.

Considering that the cut off used in our investigation to consider as a microbe-free a surface subjected to cleaning process is RLU (Relative Luminescence Units)  $\leq 20$ , a surface with RLU from 21 to 59 is simply cleaned, whereas  $RLU \geq 60$  the surface is not cleaned or dirty.

All the above tests report the quantitative counting of organic particles present on surfaces, and through the comparison with control (aforementioned reference data), resulting from the investigation, it is possible to proceed to the verification of the sanitization intervention. Direct suspensions in air were not analyzed. ATP or adenosine triphosphate is an energy molecule found in all living things, making it as a perfect biomarker of microbiological contamination, i.e. if a surface is clean (sanitized) or not. With a Hygiena® monitoring system, ATP is brought into contact with the patented reagent, which is stable in the buffered solutions of the testing device (bio-luminometer). Light is then emitted in direct proportion to the amount of ATP present in the sample (swab inserted into the bio-luminometer) providing information on the level of contamination (quantity) in seconds. Ultrasnap™ model swabs are used, a simple collection method with a stable reagent insertion that provides a fast response with precision. The bio-luminometer is provided with a CE certification and is calibrated by a specialized and authorized chemistry expert at least once a year; the swabs are purchased and supplied with batch certification and, being disposable (not reusable), do not require periodic checks.

### *Statistics*

Statistics with mean  $\pm$  standard deviation (SD) used an ANOVA (Tukey's post hoc) for evaluating significance at  $p < 0.05$  and box plot for graphics (Sigma plot 14.0).

## RESULTS AND DISCUSSION

Figure 3 shows the RLU values in three different days on a parked bus undergoing a routine cleaning process with sanitizing chemicals early in the morning. A significant difference can occur, depending on the cleaning modality, time, climatic conditions and so on. This variability was observed also upon working bus (sampling performed at 3 p.m., the bus work-in scheduled at 9 a.m.). Highest RLU values were observed in steering wheel (218 RLU, 594 RLU) and passenger seat (1214 RLU, 566 RLU, 294 RLU).

When the photocatalytic films were applied, the RLU values dropped down drastically within the sanitization cut offs (Figure 3), aside from any different outdoor and/or indoor climatic condition, number of people, length of travelling.

Figure 4 summarizes five different cases of cumulated data collected in different season times and different bus routes. The reduction of RLU is indicated in Table 1.

Indoor sanitization via the WiWell® films allowed a reduction of RLU widely  $\leq 20$  RLU, within the scheduled time (9 a.m.-3 p.m.) of usually working buses, reaching a reduction percentage of 94.7% ( $\approx 95\%$ ) as median, much higher than chemical sanitization ( $\approx 40\%$ ). RT-PCR molecular swabs on surfaces at the peak ( $3.8 \pm 2.1$  SD days) for testing SARS-CoV2 at the University labs gave negative results ( $\leq 150$  copies/ml) (31).

Our results, using surface-spot swab sampling, carried out directly on the surfaces of the films, can confirm the activation of photocatalysis which resulted in an increase in the level of sanitization of the inner surfaces, which are an important contributing cause in the spread of microbes when not enough and correctly sanitized and frequently used (12). The reduction working of  $\text{TiO}_2$  WiWell® membranes lasted for more than a week, in our experimental conditions ( $9.7 \pm 1.2$  SD days).

Only in a specific spot, “spot two”, higher RLU levels than normal have been detected. The reason for this, with high probability, is that this spot is located in a hidden and shadowed position so the photocatalysis activation is delayed, so resulting in some outlier in our collected data. Given the results of the swab samples, made directly on the surfaces of the films, we can confirm the activation of photocatalysis of WiWell®  $\text{TiO}_2$  films, earning an increased level of sanitization of the internal surfaces and ensuring a microbial-free indoor environment.

## CONCLUSIONS

The WiWell® (WiGlass™) TiO<sub>2</sub> membrane exhibited particularly effectiveness in reducing microbial contamination, ensuring microbe-free indoor environments and allowing people to travel safe and healthy in North Eastern Italian public buses.

### Figure legends

Figure 1. Inside schematic plan of the sampling spots in a typical public transportation mean

Figure 2. Geographical area of the research study

Figure 3. Blank and cleaned samples in the research study described. Statistics comparisons via ANOVA /Tukey's post hoc) give  $p < 0.01$  (\*\*) or  $p < 0.001$  (\*\*\*). Red asterisks: outliers.

Figure 4. Data plotted about the use of the WiWell® membrane. Case 1: Data from the first two weeks April 2019 (daily sunlight mostly overcast and rainy); Case 2: First week June 2019 (bright sunlight), Case 3: Last week June 2019 (bright sunlight); Case 4: First week September 2019 (bright sunlight/overcast); Case 5: Last week September 2019 (overcast/bright sunlight). Red asterisks: outliers.

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Figure 1

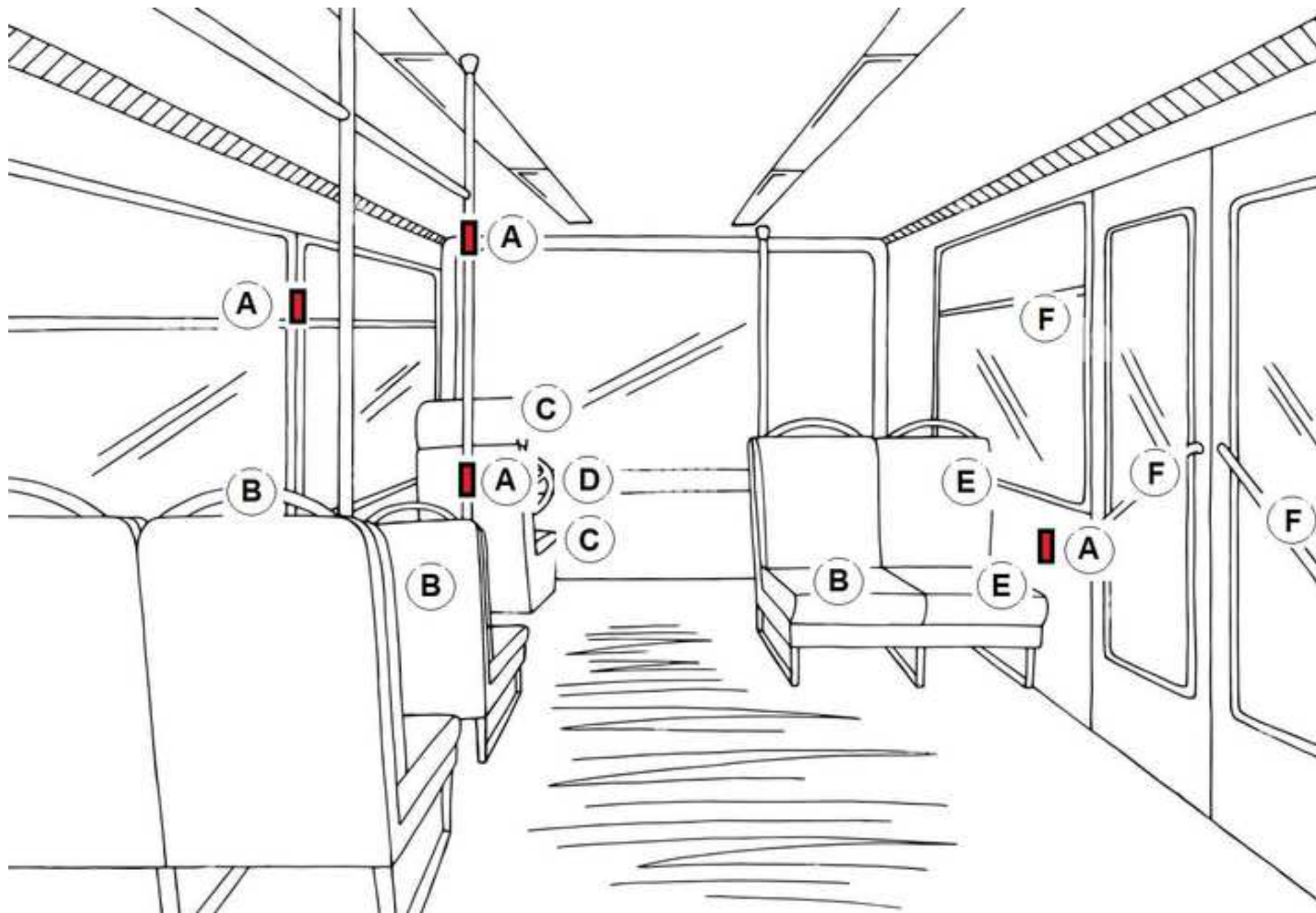


Figure 2

[Click here to access/download;Figure;JPPH-Figure 2.jpg](#)



Figure 3

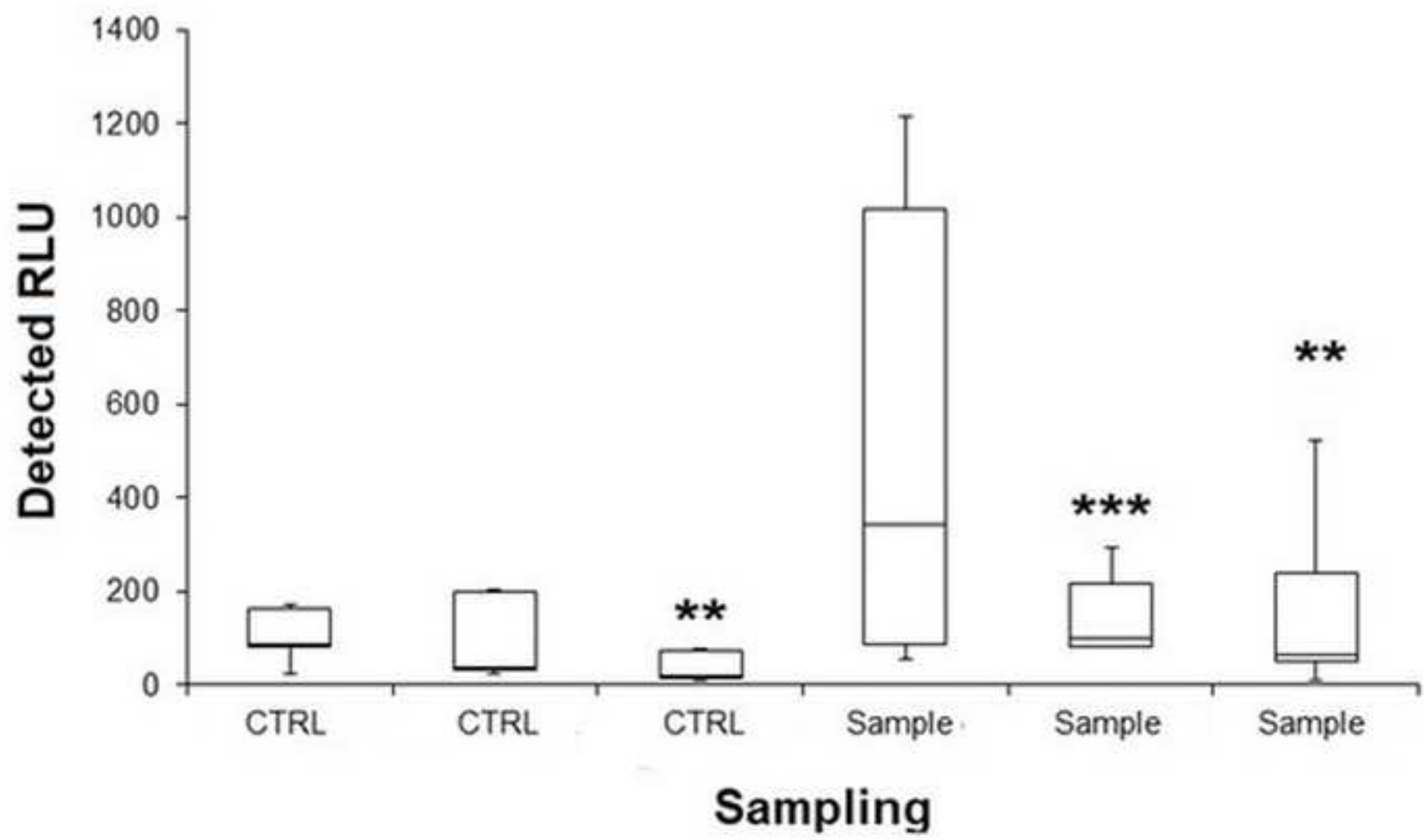


Figure 4

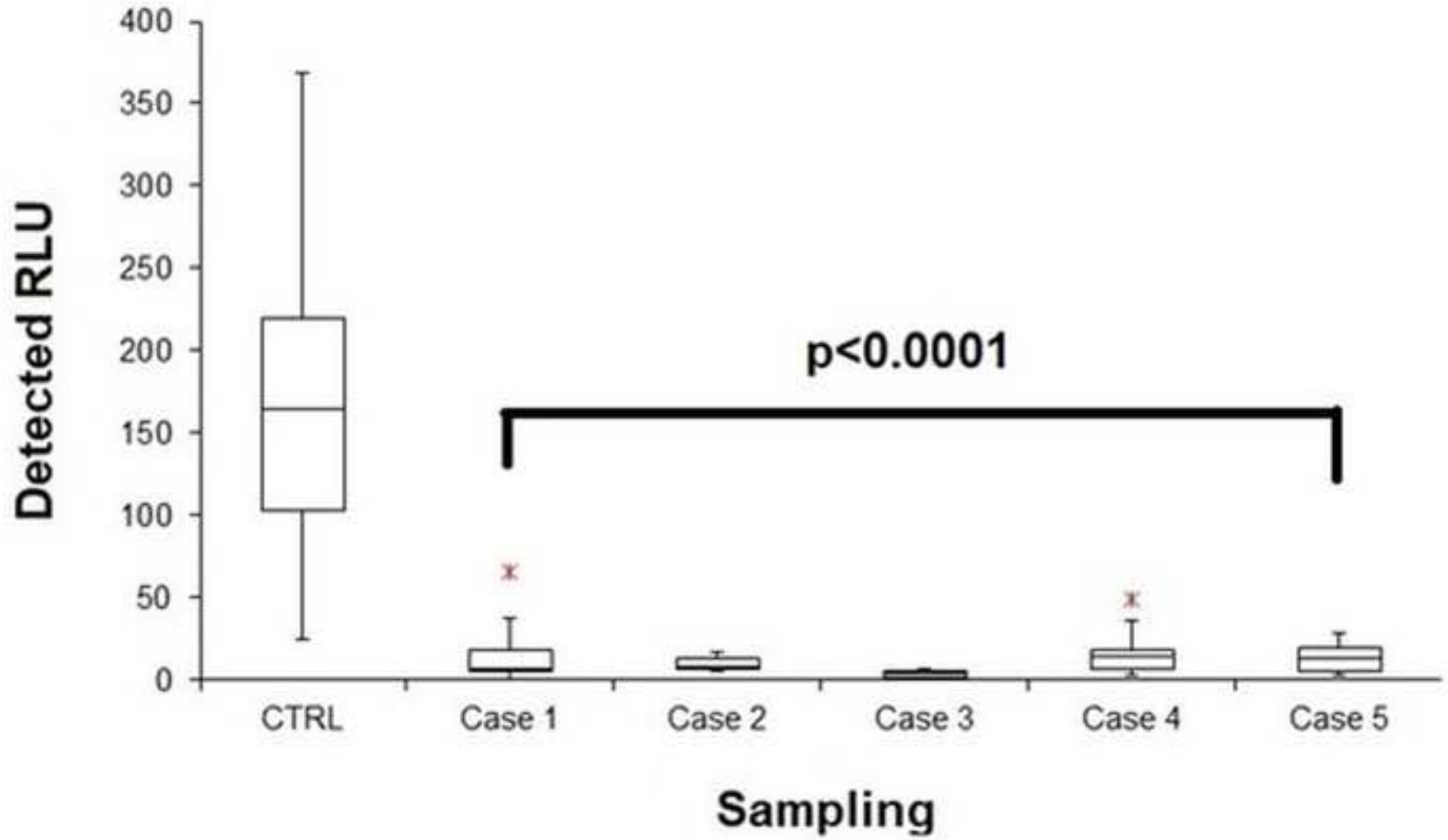


Table 1. WiWell performance in RLU

Labels	CTRL	Case 1	Case 2	Case 3	Case 4	Case 5
Min	25	0	5	0	3	3
Q <sub>1</sub>	102,5	5	6	1,5	6,75	4,75
Median	165	6	7	4	14	12,5
Q <sub>3</sub>	219,25	18	13	5	18,25	19,5
Max	369	65	17	6	49	28
IQR	116,75	13	7	3,5	11,5	14,75
Upper Outliers	0	1	0	0	1	0
Lower Outliers	0	0	0	0	0	0