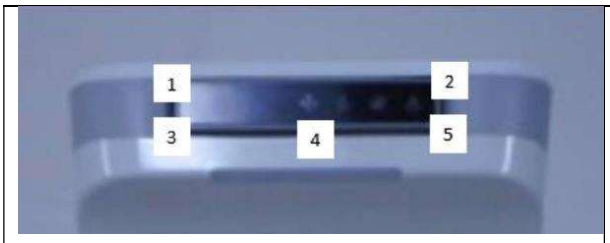
Wellisair: Emisiones de ozono

Informe (nº 103502990CRT-001): 30 de abril de 2018, Intertek Group plc (Nueva York)

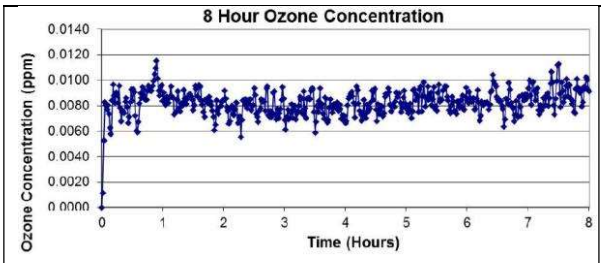
Alcance: verificar las emisiones de ozono de Wellisair de acuerdo con la normativa "Electrostatic Air Purifiers, UL867, sección 40, 2016". Para cumplir con los criterios de la norma, la emisión de ozono no puede exceder una concentración de 0,050 ppm.

Procedimiento: Wellisair fue ubicado en una cámara de ensayo y se realizaron medidas en cinco ubicaciones con el siguiente equipo: calibrador de ozono Teledyne 703E; monitor de ozono Teledyne 400E; transductor de temperatura Vaisala HMD-70Y y un caudalímetro ST75V. Después de comprobar los dos puntos con concentraciones más altas, las mediciones se repitieron con/sin solución y en modo día/noche durante 8 horas.

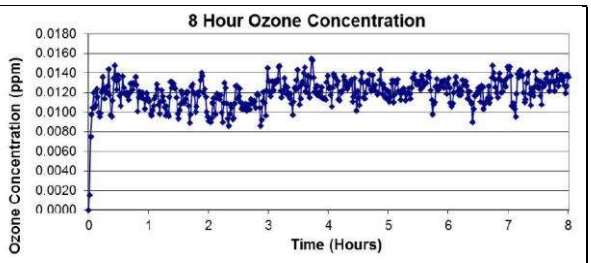


PEAK OZONE CONCENTRATIONS (ppm)				
Location	*Without Solution*		*With Solution*	
	Highest	*Lowest*	*Highest*	*Lowest*
1	0.0082	**0.0123**	**0.0113**	**0.0105**
2	**0.0102**	0.0110	0.0069	0.0085
3	0.0038	0.0031	0.0028	0.0038
4	0.0006	0.0008	0.0005	0.0003
5	0.0008	0.0012	0.0007	0.0011

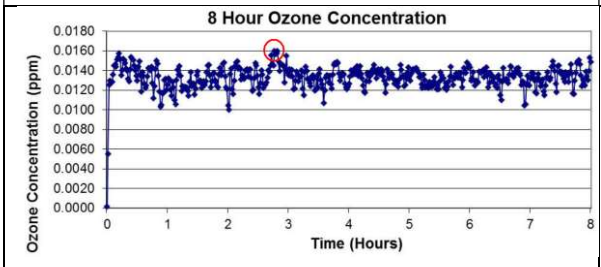
Resultados:



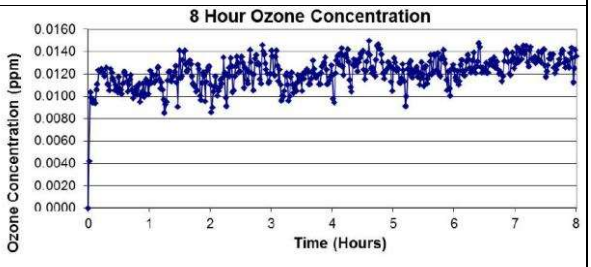
Modo día y solucion de peróxido sin abrir



Modo noche y solucion de peróxido sin abrir



Modo día y solucion de peróxido abierta



Modo noche y solucion de peróxido abierta

Conclusiones: Wellisair cumple con los criterios requeridos por la norma con una emisión máxima de 0,016ppm no superior a la concentración permitida de 0.050ppm. Además, no es necesario probar un segundo Wellisair según UL867, ya que las emisiones máximas de la primera muestra han sido inferiores a 0,030 ppm, lo que satisface la excepción de la sección 40.1.1.

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

Product Service

Attestation of Conformity

No. E8A 096006 0002 Rev. 00

Holder of Certificate:	**Wellis Co., Ltd.** W-801, SK V1 Center, 11, Dangsan-ro 41-gil, Yeongdeungpo-gu, Seoul 07217 REPUBLIC OF KOREA
Name of Object:	**Air cleaners** **(Air Disinfection Purifier)**
Model(s):	**WADU-02**
Description of Object:	Rated input voltage of adapter: 110-240 V a.c. Rated frequency: 50 / 60 Hz Rated output voltage of adapter: 12 V d.c.
Tested according to:	EN 55014-1:2006/A2:2011 EN 55014-1:2017 EN 55014-2:2015 EN 61000-3-2:2014 EN 61000-3-3:2013

This Attestation of Conformity is issued on a voluntary basis according to the Directive 2014/30/EU relating to electromagnetic compatibility. It confirms that the listed apparatus complies with all essential requirements of the directive and is based on the technical specifications applicable at the time of issuance. It refers only to the particular sample submitted for testing and certification. For details see: www.tuvsud.com/ps-cert

Test report no.: KR20-YEC0121

Date, 2020-06-03

(Byung-Soo Kang)

After preparation of the necessary technical documentation as well as the EU Declaration of conformity the required CE marking can be affixed on the product. That Declaration of conformity is issued under the sole responsibility of the manufacturer. Other relevant EU-directives have to be observed.

TÜV SÜD Product Service GmbH • Certification Body • Ridlerstraße 65 • 80339 Munich • Germany

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

Product Service

Attestation of Conformity

No. N8A 096006 0004 Rev. 00

Holder of Certificate: **Wellis Co., Ltd.**
W-801, SK V1 Center, 11, Dangsan-ro 41-gil, Yeongdeungpo-gu
Seoul 07217
REPUBLIC OF KOREA

Product: **Air cleaning appliances**
Air disinfection purifier

Model(s): **WADU-02**

Parameters:

Rated voltage:	AC/DC Adapter: 100-240 V~
	Air disinfection purifier: DC12 V
Rated frequency:	AC/DC Adapter: 50/60 Hz
Rated current:	AC/DC Adapter: 0,5 A
Protection class:	AC/DC Adapter: Class II
	Air disinfection purifier: Class III

Tested according to:
EN 60335-1:2012/A2:2019
EN 60335-2-65:2003/A11:2012
EN 62233:2008

This Attestation of Conformity is issued on a voluntary basis according to the Low Voltage Directive 2014/35/EU relating to electrical equipment designed for use within certain voltage limits. It confirms that the listed equipment complies with the principal protection requirements of the directive and is based on the technical specifications applicable at the time of issuance. It refers only to the particular sample submitted for testing and certification. For details see: www.tuvsud.com/ps-cert

Test report no.: 077-2266820-000

Date, 2020-07-27

(Brian Cha)

After preparation of the necessary technical documentation as well as the EU declaration of conformity the required CE marking can be affixed on the product. The declaration of conformity is issued under the sole responsibility of the manufacturer. Other relevant EU-directives have to be observed.

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Evidence of OH· radicals disinfecting indoor air and surfaces in a harmless for humans method

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Abstract— *The development of human societies around the world has generated a very serious environmental damage that threatens human health and the survival of animals and plants due to a higher incidence of infectious diseases.*

This awareness led to the designing of an advanced harmless environmental sanitation method for the elimination of pathogenic microorganisms and volatile organic compounds (VOC's) in large air spaces and surfaces. Advanced oxidation processes (AOP) based on hydroxyl radicals ($OH^{\bullet}$) in sufficient concentrations perform biocidal functions on pathogenic microorganisms and degrade airborne organic compounds to mineral forms of harmless organic compounds.

It is a technology recognized as clean and safe and is generally carried out through solar radiation as a process initiator with photocatalyst material. The problem presented in the photocatalysis methods is its low speed, the generation of toxic degradation intermediates, deactivation of the material and the need for UV irradiation.

The increased airborne spread of pathogenic microorganisms has raised serious concerns about its threat to environmental security. However, there is no effective method to quickly eliminate these harmful microorganisms in a large air space. Compared to conventional disinfectants, $OH^{\bullet}$ radical-based oxidation processes have excellent advantages.

Keywords— advanced oxidation process, disinfection, hydroxyls radicals ($OH^{\bullet}$), infectious diseases, ozone, terpenes, VOC's.

I. INTRODUCTION

The development of human societies around the world has generated a very serious environmental damage that threatens human health and the survival of animals and plants due to a higher incidence of infectious diseases.

The atmosphere does not have a native microbiota but is a rapid and global means of dispersal for many types of microorganisms. The history of aerobiology has demonstrated until the last century the fundamental role that respiratory air pollution plays in the development of epidemics such as cholera, influenza or Legionella [1]. This respiratory air pollution together with the ease of mobility of human beings around the world has generated in this new century extremely serious respiratory syndromes for survival.

Between November 2002 and July 2003, severe acute respiratory syndrome (SARS) spread rapidly from China to 37 other countries around the world, causing 775 human deaths with an economic loss of $ 40 billion [2].

In early 2009, a new strain of H1N1 of porcine origin spread worldwide from Mexico. H1N1 was declared a flu pandemic by the World Health Organization (WHO), causing around 17,000 human deaths in early 2010[3].

In 2012 a new episode of coronavirus emerged, the MERS-CoV (Middle East Respiratory Syndrome coronavirus). The emergence of SARS-CoV in 2002 and MERS-CoV in 2012 has changed the perspective of the Coronoviridae family since the pneumonias they have caused (SARS and MERS) have mortality rates of 10% and 30% respectively, which are elevated compared to the rest of the viruses in the family [4].

In December 2019 (after 17 years) a third new coronavirus, named SARS-CoV2 (Severe acute respiratory syndrome coronavirus 2), emerged in Wuhan Hubei province, China [5]. In February 2020, it was renamed as COVID-19 and declared pandemic by the World Organization of Health (WHO). Therefore, it is very important to develop a fast and efficient method for the elimination of pathogenic microorganisms in large air spaces.

This awareness led to the designing of an advanced harmless environmental sanitation method for the elimination of pathogenic microorganisms and volatile organic compounds (VOC's) in large air spaces and surfaces. Advanced oxidation

processes (AOP) based on hydroxyl radicals ($OH^{\bullet}$) in sufficient concentrations perform biocidal functions on pathogenic microorganisms and degrade airborne organic compounds to mineral forms of harmless organic compounds [6].

The results of different studies show that $OH^{\bullet}$ radicals rapidly destroy different microorganisms with a concentration of 0.8 mg/L and a spray density of 21 $\mu L/m^2$ in 4 seconds [7]. Vital and essential cellular morphological changes in pathogenic microorganisms are also observed under a microscope when exposed to a fatal dose of $OH^{\bullet}$ radicals.

It is a technology recognized as clean and safe and is generally carried out through solar radiation as a process initiator with photocatalyst material. The problem presented in the photocatalysis methods is its low speed, the generation of toxic degradation intermediates, deactivation of the material and the need for UV irradiation.

The increased airborne spread of pathogenic microorganisms has raised serious concerns about its threat to environmental security. However, there is no effective method to quickly eliminate these harmful microorganisms in a large airspace. Compared to conventional disinfectants, $OH^{\bullet}$ radical-based oxidation processes have excellent advantages.

Currently, chlorine, alkali, and alkali-alcohol-amine are the three main types of chemical disinfectants that are widely used to eliminate microbial contamination, but they have some drawbacks. A chemical disinfectant can only selectively kill one or similar types of pathogenic microorganisms; its processing time is long, in a range of 0.5 - 1 hour due to the low chemical reaction rate and a very high lethal dosage value, which could reach 9% (v / v);the remaining chlorine intermediates imply severe secondary contamination. Finally, its lethal processing is limited to the surface of the objects, making it impossible to apply in large air spaces [8,9].

Compared to previous chemical disinfectants, advanced $OH^{\bullet}$ radical-based oxidation technology has several advantages: 1) Absence of selectivity, they can kill any pathogenic microorganism in low lethal doses due to its strong oxidative character, with an oxidation potential of 2.8 V, slightly less than fluorines (3.03 V). 2) The processing time of $OH^{\bullet}$ radicalsis very short, several seconds, because the chemical reaction rate of $OH^{\bullet}$ radicals is greater than 10^9 L $mol^{-1}second^{-1}$, which is 10^7 times greater than other oxidants' such as O_3, H_2O_2, Cl_2, etc. 3) As a green oxidant, $OH^{\bullet}$ radicals decompose into H_2O and O_2 without any residual oxidants after their biochemical reactions [10,11].

II. MATERIALS AND METHODS

2.1 Formation of $OH^{\bullet}$ radicals

Oxygen is an essential molecule for life, but due to its high reactivity it also becomes a toxic element that gives rise to the so-called *oxygen paradox*. Oxygen is basically an oxidizing molecule. The following concentration of pollutants is generally found in "clean" outdoor air (without sources of pollution): carbon dioxide, 320 ppm; ozone, 0.02 ppm, carbon monoxide, 0.12 ppm, nitric oxide, 0.003 ppm, and nitrogen dioxide, 0.001 ppm. However, these values increase significantly in urban air [12].

The $OH^{\bullet}$ radical is the most important natural oxidant in tropospheric chemistry, often called the "detergent" in the atmosphere since it reacts with many pollutants, initiating the process of cleaning them up. It also plays an important role in the elimination of greenhouse gases such as carbon dioxide, methane or ozone. Using Advanced Oxidation Processes (AOP) is attractive, among other reasons, because the contaminant is destroyed, not concentrated or transferred to the environment, a total or almost total mineralization of organic pollutants is achieved. Therefore they can be applied in the destruction of the vast majority of organic compounds, especially in non-biodegradable compounds such as organochlorines, PCBs, PAHs, etc. It is a clean and safe technology and in some processes, solar radiation can be used as the initiator.

The main problem for rapidly eliminating pathogenic microorganisms in large air spaces is how to produce the $OH^{\bullet}$ radicals with high concentration and large production. Currently, the main methods are Fenton catalysis, photocatalysis and ozone as well as their collaborative effects [13-17]. However, these technologies have some serious disadvantages: 1) The $OH^{\bullet}$ radicals rate of production is low and they are obtained at low concentration, so that the whole biochemical reaction time is long, in the range of 15 - 360 min. 2) The above-mentioned technologies are only applied to small scale experiments or applications. 3) A large number of chemical reagents such as H_2O_2, TiO_2 or $Fe^{2}+$, are necessary in the process of $OH^{\bullet}$ production, resulting in high cost and a safety problem. 4) In order to increase the $OH^{\bullet}$ radicals production, several kinds of technologies are collaborated together resulting in large-volume accessory equipment such as the bubble tower or rotating packed bed.

In previous studies, the production of a large number of $OH^{\bullet}$ radicals has been reported by ionization and dissociation of O_2 in air and H_2O in the gaseous state, using a physical method of strong electric field discharge. In this way, $OH^{\bullet}$ radicals have been successfully used in the treatment of ship's ballast water and red-tide in the ocean [18,19].

Titanium oxide is the current reference as a photocatalyst material given its high activity, relative stability, low cost and low toxicity. However, there are problems to be solved such as the low rate of photocatalysis, generation of toxic degradation intermediates, deactivation of the material and the need for UV irradiation as its band gap is not coupled with sunlight [20].

2.2 Development of a new advanced oxidation process for the decontamination of air and surfaces.

Given the described scenario, a challenge for safe and effective technological development is generated in the decontamination of air and surfaces. The technological objective is based on the milestone of achieving a method capable of producing $OH^{\bullet}$ radicals in sufficient quantities by means of an innovative system that ensures their efficacy and safety for human beings. The Wadu02® system is a device by which are active oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or a terpene such as d-limonene, evaporates and reacts with an internal ozone emission below a concentration of 0.050 ppm (0.1 mg / m^3). This ozone exposure limit, established in the regulations issued by the WHO in the environmental limit values (VLA) of the year 2000 for the general public in exposures of up to 8 hours [21], is taken as an international benchmark of safety in ozone emission to obtain a constant and non-damaging production of $OH^{\bullet}$ radicals.

Wadu02®'s ozone emissions were evaluated through testing of household electrostatic air cleaners in an external laboratory [22] under the Electrostatic air cleaners standard, SUN - UL 867 and tested with a Teledyne ozone calibrator and monitor, at temperature and humidity controlled by Vaisala transducer and flow meter. The ozone emission of the Wadu02® device was certified in active mode and night mode in parameters less than 0.020ppm (0.012 - 0.015 ppm without filters and 0.015 - 0.016 ppm with filters respectively). Results were lower than all international standards regarding safety in prolonged exposures to ozone.

The production of $OH^{\bullet}$ radicals through the oxidation of H_2O_2 was evaluated under controlled conditions according to the oxidative functionality of the Wadu02® device and compared to liquid hydrogen peroxides in the purity ranges of 0.25% to 0.75%, aided by the colorimetric reaction performed on a potassium iodide test strip. The results indicate that the average oxidative capacity of H_2O_2 at 0.5% purity is equivalent to the oxidative capacity offered by the Wadu02® model devices, with a maximum production of 0.9 mg/m^3 (0.64 ppm), which is approximately 64.2% of the current workplace exposure limit (WEL) adjusted to 1.4 mg / m3 (1ppm)[23,24].

The threshold concentration for the acute irritant effects of hydrogen peroxide gas in the respiratory tract is 10 mg/m^3 (equivalent to 7 ppm) in humans, while the corresponding values for the skin are 20mg/m^3. Regarding its prolonged exposure, hydrogen peroxide has not been found to cause teratogenic or carcinogenic effects in humans. Mutagenic or chromosomal effects have also not been observed.

It was also verified as an alternative to the high natural reactivity of hydrogen peroxide, the substitution of the cartridge load with aromatic essences extracted from flowers and plants due to the biocidal role that terpenes have for their antiviral and antibacterial properties. The process of advanced oxidation was analyzed under the same conditions of low ozone emission (less than 0.02ppm) with the Wadu02® model, to compare the proven efficacy of hydrogen peroxide.

Limonene is one of the most abundant monoterpenes in nature present in essential oils extracted from the peel of citrus fruits, including the essential oils of orange and tangerine. This monoterpene is susceptible to oxidation to generate compounds with higher added value [25].

Terpenes are hydrocarbons present in essential oils that consist of more than one unit of isoprene with five carbons. Monoterpenes, most terpenes, along with sesquiterpenes and diterpenes, comprise the majority of essential oils. Due to the low molecular weight and high volatility of monoterpenes and sesquiterpenes, the use of essential oils in indoor environments can increase the levels of volatile organic compounds (VOCs) [26].

Terpenes contain one or more C=C double bonds, which interact easily with strong oxidants such as ozone, hydroxyl radicals [27-29] and nitrate radicals. Ozone is a common indoor pollutant, the general levels of which are distributed approximately 20 to 40 ppb [30,31]. The use of office machines, such as copy machines, printers and fax machines, also elevates indoor ozone concentrations [32]. The VOCs emitted through the evaporation of indoor terpene-based products may interact with ozone and generate secondary air pollutants, mostly formaldehyde and suspended particulates [33-37]. Secondary organic aerosols generated by the interaction of terpenes and ozone consist of fine and ultrafine particles [38-40]. Consequently, prior

evaluations of total limonene consumption were performed on the Wadu02® device to obtain controlled and safe evaporation.

The total consumption of d-limonene in the Wadu02® products was determined to be of the order of 0.4 g/24 h. According to the functionality of this device and the average evaporation of the measurements recorded in the laboratory, Wadu02® products emit a cloud containing d-limonene with a concentration of approximately 1.84 ppm, which in a room of $60m^2$ ($180m^3$) can give rise to a maximum concentration with a value less than 2ppb. This concentration is significantly lower than the Swedish and German OEL levels [41] (occupational exposure limits) which are 27ppm and 10ppm, respectively.

2.3 Toxicology

For the evaluation and analysis of the amounts of formaldehyde, which can be generated directly from the reaction of ozone with the structural units of C=C bonds, reports indicating that the proportions of formaldehyde formed by this mechanism during ozone-initiated reactions with terpenes represent only a small percentage of reactions to ozone were evaluated [33,34].

The main mechanism that forms formaldehyde is initiated by the reaction of ozone with the functional group C = C to generate ozonide. Subsequently, the ozonide decomposes into a carbonyl and an energy-rich (bi-radical) Criegee intermediate. Both products participate in various additional oxidation reactions to form highly reactive species such as hydroxyl radicals and stable products. These stable products can be ketones and carboxylic acids if the process has taken place in an oxidizing medium, or aldehydes and ketones if the process has taken place in a reducing medium.

The formation of stable carbonyls with low molecular weights, including formaldehyde, acetaldehyde, acetone and propionaldehyde, were observed during the gas-phase reactions of ozone with terpenes [31,33,36]. Reactive hydroxyl radicals generated from ozone reactions with terpenes played a vital role in forming indoor formaldehyde.

Several studies have indicated that indoor $OH^{\bullet}$ radicals concentrations generated by ozone reactions with unsaturated compounds were higher than those outdoors at midday or night [42-44]. $OH^{\bullet}$ radicals were responsible for 56~70% of indoor formaldehyde in reactions between ozone and 23 VOCs and ozone and terpenes [45]. Therefore, a new security objective is the evaluation of the reactions of $OH^{\bullet}$ radicals by means of terpenes and the possible contribution to obtain high levels of formaldehyde indoors and potential effects on indoor air quality [46].

2.4 Security test

Once verified that the total consumption of limonene and hydrogen peroxide does not exceed limits considered teratogenic and carcinogenic in humans and that the emission of ozone is less than what is established in international regulations, the effectiveness in reducing formaldehydes was evaluated. The advanced oxidation of limonene with Wadu02® was assessed using the SPS-KACA002-132: 2016 test method under controlled temperature and humidity conditions (21 ± 1) ºC (45 ± 5)% RH with d-limonene in the cartridge and with d- limonene in a gel [47]. The results indicate that the reduction of formaldehyde in ozonolysis reactions with emissions less than 0.020 ppm and with low emission concentrations of d-limonene with an evaporation of 0.4 g / 24 h equivalent to 1.84 ppm is significant and reaches values of 19% with gel and 41% with liquid limonene cartridge.

These results show that despite the high reactivity of d-limonene with ozone for the formation of formaldehyde, the controlled emission of ozone below 0.02ppm and the evaporation of limonene below 2ppb in a space of $60m^2$ is a safe and harmless reaction.

To validate this hypothesis, a series of experimental tests were carried out to determine the reduction of particles and air pollutants emitted by the burning of an incense stick during a 2-hour exposure to a Wadu02® air purifier, using loaded cartridges with d-limonene and H_2O_2 in a 225.72 m3 (6.6 X 6 X 5.7) volume-controlled chamber [48].

Five air quality measurements were performed under different conditions. The first lecture was determined by the initial air quality in the room, without any exposition to incense or air purifiers. The second lecture was taken after 2 hours, since half of an incense stick was burned. The third lecture was determined after the initial air quality was reached and half of an incense stick was burned, with the presence of hydrogen peroxide cartridge air purifier for two hours. The fourth lecture was taken by the same conditions as the third one but, in this case, with a D-limonene cartridge air purifier. Finally, the fifth lecture was determined by the same conditions as third and fourth measurements but, this time, with the presence of both air purifiers (D-limonene and hydrogen peroxide cartridges).

FIGURE 1. Air quality values before and after contaminating the room with the combustion of an incense stick. Comparison of the safety and efficacy of Wellis in the elimination of VOCs and formaldehyde with hydrogen peroxide and limonene.

The study shows that under these conditions, burning an incense stick generates poor air quality an average of 30 minutes after the start of combustion with a tendency to regularize after one hour and to return to the initial conditions after two hours, as the particles emitted dispersed in the air space of the room. However, formaldehyde and VOC's readings are higher than the control reading, reflecting the risk of prolonged exposure.

The results in the presence of Wadu02® purifiers regardless of the cartridge content (d-limonene or H_2O_2) maintain the initial air quality from the first half-hour of exposure, significantly reducing the values of particle matters, formaldehyde and VOCs. The efficacy in terms of the reduction of formaldehyde and VOCs, according to the use of d-limonene or H_2O_2 to carry out the emission of $OH^{\bullet}$ radicals, is not significant although H_2O_2 presents more efficient values.

This allows us to determine that the operation of the Wadu02® air purifier, based on the emission of ozone in low concentrations (< 20 ppb) and the evaporation of standardized amounts of d-limonene or H_2O_2 from the cartridge (as a result of the execution of the advanced oxidation process) is safe, harmless and effective in reducing suspended particles, VOCs and formaldehyde.

2.5 Application of OH● radicals as a broad spectrum biocide

Free radicals and ions cause irreversible alterations in macromolecules (proteins, membranes and DNA) as a consequence of the movement of electrons, resulting in a morbid effect. Reactive Oxygen and Nitrogen Species (RONS) are the most unstable and reactive, meaning these are the first ones to react with others. Within this group, the $OH^{\bullet}$ radicals are the species with a more ephemeral half-life due to its high reactivity, and therefore the most dangerous [49].

The efficacy of the concentration of $OH^{\bullet}$ radicals in the elimination of pathogenic microorganisms was studied. Under conditions of spray density of 21µL/cm^2 and at processing times of 4 seconds, a dramatic decrease in surviving cells has been reported for *S. Marcescens* at concentrations just above 0.15mg/L and almost entirely in concentrations of 0.41mg/L. In *B. subtilis*, the levels were practically undetectable at concentrations of 0.5 mg/L, whereas in *bacillus* spores the reduction was significant at levels of 0.3mg/L and practically entirely at maximum concentrations of 0.8mg/L [50].

The biocidal function of $OH^{\bullet}$ radicals is based on the advanced oxidation process, a cellular stress mechanism initiated by the "respiratory explosion" (similar to the mitocondrial) and heightened through a cascade of reactions by the release of reactive oxygen species [51-52], such as the hydrogen peroxide, which can pass through biological membranes, and the hypochlorite ion, which modifies and degrades all biological molecules.

The main effects of these reactive forms occur on membranes, lipids and sulfhydryl bonds of DNA's proteins and nucleotides [53], producing:

- Lipid peroxidation, the resulting peroxides of which initiate a catalytic chain reaction leading to further loss of unsaturated fatty acids and extensive membrane damage.
- Production of cross links between proteins, through the formation of disulfide bonds.
- Mutations in the genetic material of the pathogenic microorganism.

During the cellular oxidation process, unsaturated chains are easily attacked by $OH^{\bullet}$ radicals. The peroxidation of the fatty acids in the membranes generates peroxyl radicals ($ROO^{\bullet}$), decreasing their functionality. These radicals have a lower reactivity than the $OH^{\bullet}$ radicals and, therefore, their half-life is somewhat longer.

The presence of cell damage caused by oxidative stress provokes an antioxidant response in the cell: they try to pass electrons from one species to another until radicals are inactive and stability is restored. On the other hand, these interactions can generate cascades spreading the damage [54].

2.5.1 Lipid oxidation.

Biological membranes are made up of unsaturated fatty acid chains and are easily oxidized. $OH^{\bullet}$ radicals attack the double bonds of these structures and leave an unpaired electron in the chain that will bind to an oxygen molecule to re-stabilize, giving rise to a peroxyl radical. The presence of peroxide radicals modifies the membrane's functionality irreversibly, since it changes its spatial distribution causing instability [55]

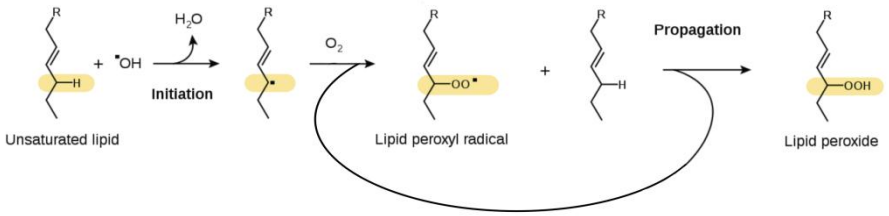

FIGURE 2: ●OH radicals attack the bonds of these structures and generate a chain with an unpaired electron, which will react with an oxygen molecule (O_2) to re-stabilize. As a product peroxyl radicals appear in the membrane, which act as positive feedback further increasing damage.

2.5.2 Protein oxidation: direct (produced by RONS) or indirect (produced by lipid peroxidation)

Free radicals cause changes in the molecular structure of amino acids by modifying their charge, which can end up breaking the polypeptide chain, fragmenting the protein.

Peroxyl radicals give rise to substances with aldehyde groups, highly reactive species that establish covalent bridges between amino acids producing intra and interprotein cross-linking. Finally, proteins lose conformation and form aggregates, leading to the decrease or inhibition of the correct functioning of the protein

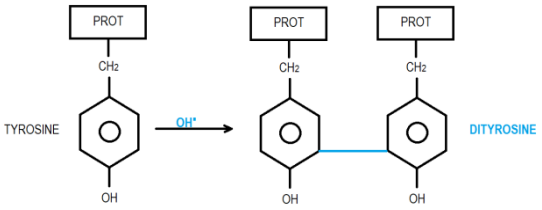

FIGURE 3. The $OH^{\bullet}$ radicals form irreversible covalent bridges between two tyrosines. The product (dityrosine) is not recognized by the kinases of the signaling pathways, meaning the information that the tyrosine had to transmit is lost. In addition, this structure is not degradable and therefore non-functional proteins with intra and intermolecular bridges accumulate.

2.5.3 Morphological changes of microorganisms.

The study by Bai et al. 2012 [50] verified the morphological changes of *B. subtilis* and *Bacillus* spores with treatment with $OH^{\bullet}$ radicals under microscopic observation in *B. Subtilis, S. Marcescens* and *Bacillus* spores.

It was clearly observed that *B. subtilis* in the form of intact cane and evenly distributed cytoplasm after treatment with $OH^{\bullet}$ radicals, greatly lost the integrity of the membrane. In the other hand, the *Bacillus* spore cells feature a tough, multi-layered outer coating that makes it impossible to quickly kill *Bacillus* spores with conventional chemical disinfectants such as chlorine, alkali and alkali-alcohol-amine. However, after treatment with $OH^{\bullet}$ radicals, the *bacillus* spores also ruptured and the round-shaped cells disappeared. Consequently, *Bacillus* spores require a higher concentration of $OH^{\bullet}$ radicals scavenging, spray density - dispersion and time. Concentration, dispersion spray density and processing time are the three important parameters for the destructive effect of $OH^{\bullet}$ radicals on microorganisms.

The demonstration that the effect of $OH^{\bullet}$ radicals on a microorganism will be with greater biocidal efficacy according to its more superficial structure (collected in previous studies) determines the need to recognize the morphological characteristics of pathogenic microorganisms from their outer layer to the interior of the specific cell [56,57].

Some microorganisms have been able to reverse this oxidative process through superoxide dismutase (SOD), a family of three metalloenzymes (FeSOD, MnSOD and CuZnSOD) with a high capacity to interact with oxidants, neutralizing them and reducing oxidative damage.

MnSOD is synthesized by *Escherichia coli* [58] after exposure to oxygen and is induced by the presence of superoxide radicals. Both SOD and catalase activity have been detected in the cytosol of microbial cells and in the periplasmic space (located between the plasma membrane and the cell wall) of the bacteria. Likewise, a protective role has been demonstrated against ROS generated in the catalase respiratory burst in *Staphylococcus aureus.* [59-61]

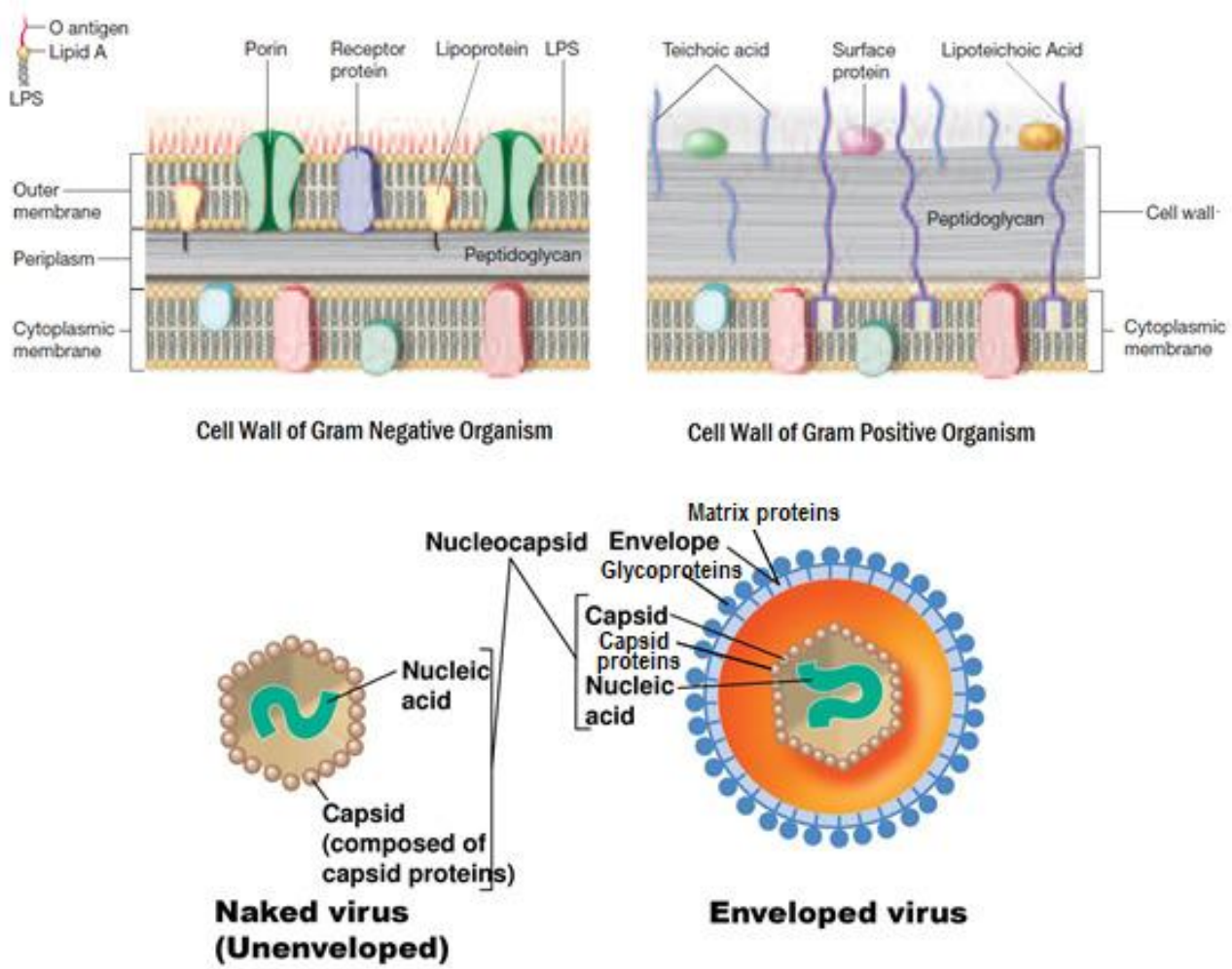

FIGURE 4: Cellular envelopes according to the type and morphology of viruses and bacteria. Adapted from Pearson education, Inc ©2015 & laboratoryinfo.com

In recent years, different laboratories, external certifiers and university research centers have developed various studies to check the biocidal efficacy of the Wadu02®, in the presence of pathogenic microorganisms in different spaces.

The results are reflected in Table 1, in which we can show that in the case of gram + and gram - bacteria the reduction reaches an average of 99.9% in the first hour of exposure to the advanced oxidation process, in both air and surfaces.

In the case of viruses, the results are observed depending on the conditions of relative humidity and the morphology of the virus. The efficacy results of Wadu02® in non-enveloped viruses indicate that in humid conditions the efficacy is less than in dry environments, averages of 99% are reached; while in enveloped viruses humidity favors the advanced oxidation process and virus elimination than in dry environments.

2.5.4 Report on stability and disinfection of 2019-nCoV

The 2019-nCoV is a new strain of coronavirus that was first detected in Wuhan City (China) in December 2019. The number of infected patients grown rapidly in recent weeks, becoming a serious public health concern. The transmission of the virus occurs mainly via respiratory droplets produced by an infected person that can land in the mouth or nose of people nearby or possibly be inhaled into the lungs. Coronaviruses are a large family of viruses common in many different species of animals, including camels, cattle, cats and bats. Rarely, animal coronaviruses can infect people, and spread among us such as with MERS, SARS and now with 2019-nCoV [62].

The new coronavirus has been classified as a *Betacoronavirus*, like MERS and SARS, both of which have their origins in bats. Coronaviruses are in the subfamily *Coronavirinae* in the family *Coronaviridae*, in the order *Nidovirales*. They are divided in 4 subgenders *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus* and *Gammacoronavirus*[63].

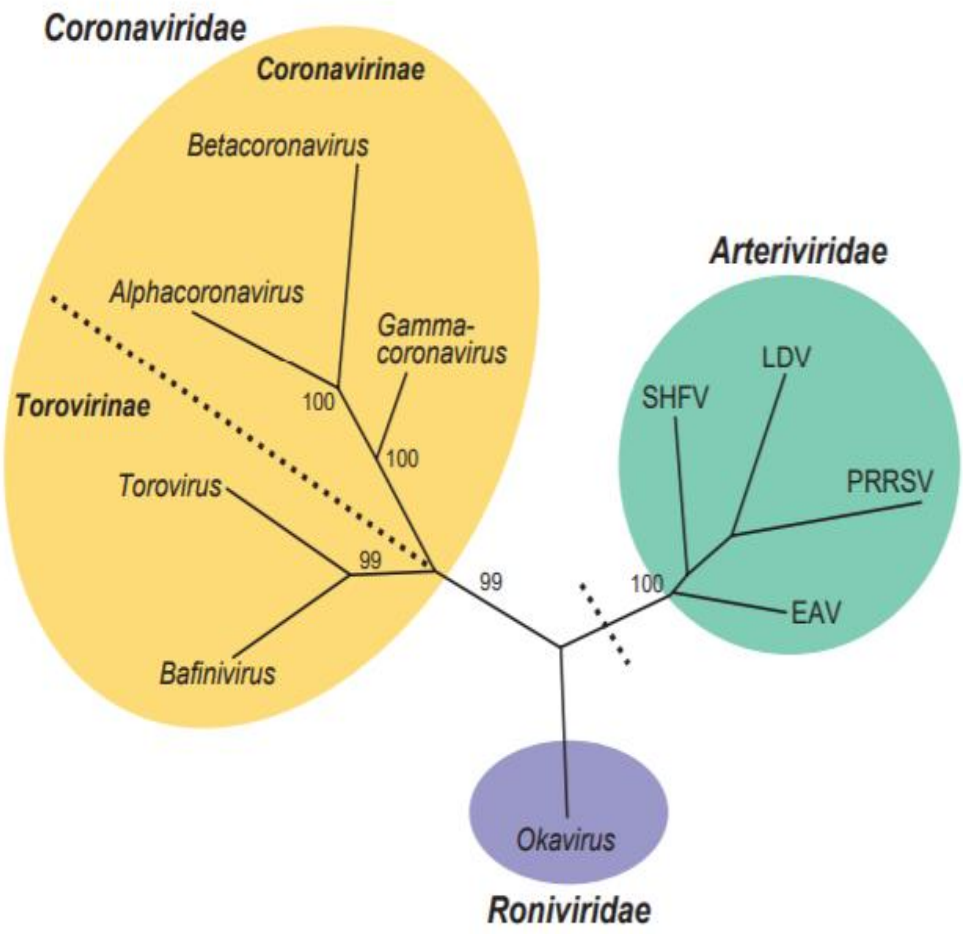

FIGURE 5: *Nidovirus* phylogeny. The Nidoviral order consists of three families: *Coronavirinae, Roniviridae and Arteriviridae* from International Committee on Taxonomy of Viruses, 2012 ©.

Based on the genetic material, these viruses are included in group IV of the Baltimore classification, as the viral particle contains only a single positive-sense stranded RNA. Therefore, the genetic material itself acts as an RNA messenger since they both are positive-sense. When translated, the RNA polymerase and the different structural proteins that form the capsid are synthesized [64].

Coronavirus' diameter is around 60-200 nm. They present a nucleocapsid with helical symmetry and a lipid sheath, that derives from the membrane of the previously infected host cell and contains glycoproteins and surface antigens. From the lipid sheath the characteristic projections of this genus arise forming a solar corona around it that is visible under a microscope and gives the family its name. Despite what might be expected, having an envelope implies that the virus is sensitive to different factors and external agents such as heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation [63].

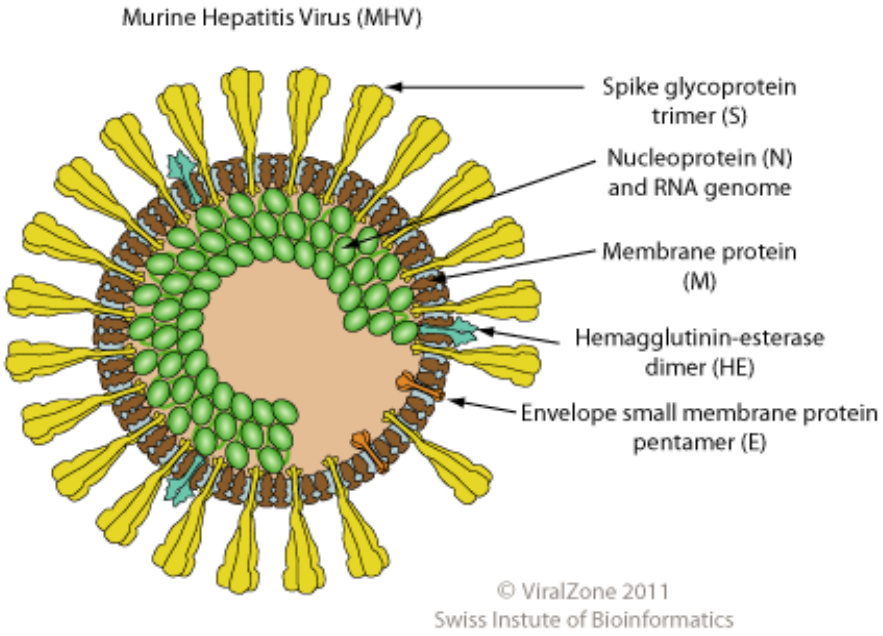

FIGURE 6: Structural proteins of *coronavirus* (from ViralZone©)

As can be seen in Figure 6, the coronavirus capsid consists of the following structural proteins:

The Spike glycoprotein (S) protrudes from the outer envelope of the virus forming the "corona" visible under a microscope. Its function is to stick to the proteins found on the cell's surface and infect them. In some cases, the S protein causes the infected cell to fuse with other adjacent cells, thus favoring the spread of the virus. The Envelope protein (E) is responsible for the formation of new viral particles and their release from the infected cell, being necessary for virus diffusion. The Membrane protein (M) is attached to the inner part of the virus membrane and causes this membrane to bend, determining the spherical shape of the virions. M also interacts with the nucleocapsid formed by the RNA of the virus and the N protein. And finally, the Nucleocapsid protein (N) is phosphorylated and binds to the viral genome during assembly.

They are viruses distributed worldwide due to their genetic diversity, their short incubation periods and the high mutation rate they present. The combination of these factors allows the pathogen to infect not just animals but also humans.

It is well known that ozone, at concentrations above 100ppm and high humidity rates, is an effective disinfection treatment especially for RNA-viruses with or without envelope [65-67]. However, high ozone concentrations may be harmful to coexist in habitable urban environments. Reactive oxygen species (ROS) including $OH^{\bullet}$ radicals, hydrogen peroxide (H_2O_2) and ozone (O_3) have been reported to enhance disinfection efficiencies of several microorganisms [68,69].

III. RESULTS

According to the results obtained with Wadu02® in viruses with similar structures to Covid-19 (RSV), we can expect that the efficacy of the device will have an expected elimination result of an average of 99 to 92% depending on the relative humidity conditions.

IV. CONCLUSIONS

The results show that the use of $OH^{\bullet}$ radicals in the advanced oxidation process produced by the Wadu02® purifier is a new, safe and effective method to quickly eliminate pathogenic microorganisms in large air spaces and surfaces.

The application of $OH^{\bullet}$ radicals in different studies has shown that their use in advanced oxidation processes, standardized as a safety measure carried out by Wadu02®, is safe, innocuous and effective in the control of pathogenic microorganisms and the elimination of suspended particles, formaldehyde and VOCs.

The evidence on the efficacy of $OH^{\bullet}$ radicals as a biocide shows that their use is endorsed for being a strong oxidant, capable of eliminating microorganisms in low concentrations (0.8 mg/L) equivalent to 10 thousandths of the dose of conventional

chemical disinfectants. Its spray density - dispersion is 22ml/cm^2 representing one thousandth of other disinfectants, its constant high reaction rate 10^9L/mol • sec in the processing of $OH^•$ radicals is shorter than 4 seconds, which is one thousandth of chemical disinfectants. Finally, the damage that has been observed to pathogens under a microscope is irreversible.

Basing our homeostatic state on the correct functioning of our internal antioxidant system and the experimental demonstration of the use of $OH^•$ radicals effectively in the disinfection of air and surfaces, we can issue a safety statement on the use of Wadu02® technology to achieve safe, effective and harmless advanced oxidation processes in humans in the purification and decontamination processes of air and surfaces.

TABLE 1
WADU02© BIOCIDE EFFICACY TEST RESULT WITH LIMONENE

Pathogen	Means of dispersion	Exposure	effectiveness %	Documented testing
Bacillus subtilis (Gram +)	Surface	1 h	99,4	Bacillus, Esch, Staph - KNU
	Air	20 min	99,6	Bacillus, Esch, Staph - KNU
Staphylococcus aureus (Gram +)	Surface	1 h	52,3	Bacillus, Esch, Staph - KNU
	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL
	Air	1 h	99,9	Bacillus, Esch, Staph - KNU
Resistant Staphylococcus aureus (MRSA) (Resistant Gram +)	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
	Air	4 h	99,9	MRSA - KCL
Pseudomonas aeruginosa (Gram -)	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL
Enterobacter species: Salmonella (Gram -)	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
Enterobacter species: Klebsiella (Gram -)	Surface	4 h	99,9	Salm, Kleb, MRSA - KCL
	Air	4 h	99,9	Klebsiella - KCL
Enterobacter species: Escherichia coli (Gram -)	Surface	1 h	99,9	Bacillus, Esch, Staph - KNU
	Surface	4 h	99,9	Esch, Pseudo, Staphy- KCL
	Air	20 min	99,9	Bacillus, Esch, Staph - KNU
Influenza virus (Enveloped)	Wet	30 min	86	Influenza A - UB
	Dry	30 min	38	Influenza A - UB
VRS -Respiratory Syncytial Virus (Enveloped)	Wet	2 h	99	VRS - UB
	Dry	2 h	92	VRS - UB
Rotavirus (Naked)	Wet	2 h	37	RoV - UB
	Dry	2 h	99	RoV - UB

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Wellisair: Eficiencia contra bacteria Klebsiella pneumoniae

Informe de prueba de bacterias (nº 027130): 24 de marzo de 2016, Korea Conformity Laboratories (KCL).

Alcance: medir la eficacia de Wellisair para la desinfección contra microbios en el aire de Klebsiella pneumoniae (bacteria causante de infecciones del tracto urinario, neumonía, sepsis, infecciones de tejidos blandos e infecciones quirúrgicas por heridas).

Procedimiento:

- Método de prueba: medición de la tasa de reducción de Klebsiella pneumoniae después de inyectar una concentración constante de bacterias dentro de una cámara de ensayos de 8m^3 durante 4 horas.
- Equipo de medición: sistema de toma de aire MAS-100NT.
- Condiciones de prueba: 23ºC 50,1% R.H.

Resultados:

Antes de tratamiento con Wellisair

Después de tratamiento con Wellisair

Descomposición de la concentración en el aire de Klebsiella pneumoniae			
Tiempo	Antes de tratamiento con Wellisair	Después de tratamiento con Wellisair	
	Microbios (CFU/m^3)	**Microbios (CFU/m^3**	**Reducción (%)**
4h	1,2x10^4	<10	99,9%

Conclusiones: el desinfectante de aire Wellisair fue capaz de reducir el 99,9% de la concentración inicial en el aire de los microbios Klebsiella pneumoniae después de 4 horas de tratamiento.

Wellisair: Eficiencia contra bacterias Salmonella & Klebsiella & SARM

Informe de prueba de bacterias (nº 027129): 24 de marzo de 2016, Korea Conformity Laboratories (KCL).

Alcance: medir la eficacia del Wellisair para la desinfección contra **Salmonella Typhimurium** (bacteria causante de diarrea, dolor abdominal, vómitos y náuseas, que puede ser mortal), contra **Klebsiella pneumoniae** (bacteria que causa infecciones del tracto urinario, neumonía, sepsis, infecciones de tejidos blandos e infecciones en heridas quirúrgicas) y contra el **SARM** (bacteria contraída en hospitales a través de la inserción de un tubo de ventilación en el paciente que produce una neumonía nosocomial, enfermedad que puede ser mortal).

Procedimiento:

- Método de prueba: medición de la tasa de reducción de las diferentes bacterias con una distancia entre el medio inoculado con la cepa y la muestra de 5cm después de 4 horas
- Condiciones de prueba: 37ºC 33,1% R.H.

Resultados:

Salmonella Typhimurium		Klebsiella pneumoniae		SARM	
Antes de Wellisair	Después de Wellisair	Antes de Wellisair	Después de Wellisair	Antes de Wellisair	Después de Wellisair
					

Elementos de prueba		Resultados del ensayo		
		Inicial (CFU/mL)	Después de 4 h (CFU/mL)	Tasa de reducción (%)
Salmonella Typhimurium	Sin tratamiento	$1,6x10^4$	$1,6x10^4$	-
	Tratamiento con Wellisair	$1,6x10^4$	<10	99,9%
Klebsiella pneumoniae	Sin tratamiento	$2,0x10^4$	$2,0x10^4$	-
	Tratamiento con Wellisair	$2,0x10^4$	<10	99,9%
SARM	Sin tratamiento	$1,2x10^4$	$1,2x10^4$	-
	Tratamiento con Wellisair	$1,2x10^4$	<10	99,9%

Conclusiones: el desinfectante de aire Wellisair fue capaz de reducir el 99,9% de la concentración inicial de microbios de Salmonella, Klebsiella y SARM después de 4 horas de tratamiento.

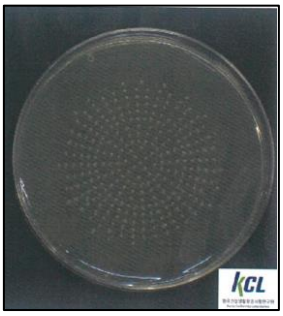
Wellisair: Eficiencia contra bacteria Staphylococcus Aureus Resistente a la Meticilina (SARM)

Informe de la prueba de bacterias (nº 027131): 24 de marzo de 2016, Korea Conformity Laboratories (KCL).

Alcance: medir la eficacia de Wellisair para la desinfección en el aire contra el Staphylococcus aureus resistente a la meticilina (SARM), bacteria contraída en hospitales a través de la inserción de un tubo de ventilación en el paciente que produce una neumonía nosocomial, una enfermedad que puede ser mortal.

Procedimiento:

- Método de prueba: medición de la tasa de reducción de SARM después de inyectar una concentración constante de bacterias dentro de una cámara de ensayos de 8m^3 durante 4 horas.
- Equipo de medición: sistema de toma de aire MAS-100NT.
- Condiciones de prueba: 23ºC 50,2% R.H.



Antes de tratamiento con Wellisair

Después de tratamiento con Wellisair

Resultados:

Descomposición de la concentración en el aire de SARM			
Tiempo	Antes de tratamiento con Wellisair	Después de tratamiento con Wellisair	
	Microbios (CFU/m^3)	**Microbios (CFU/m^3**	**Reducción (%)**
4h	1,0x10^4	<10	99,9%

Conclusiones: El desinfectante de aire Wellisair fue capaz de reducir el 99,9% de la concentración inicial en el aire de los microbios SARM después de 4 horas de tratamiento.

Wellisair: Eficiencia contra virus Coxackievirus B5

Informe de prueba de virus (nº 20180711): 11 de julio de 2018, Laboratorio de virus contaminantes del agua y de los alimentos de la Universidad de Barcelona.

Alcance: medir la eficacia del Wellisair para la desinfección en superficies del Coxsakievirus B5 (CBV5), enterovirus asociado a patologías importantes en humanos como enfermedades de la mano, los pies y la boca e infecciones del sistema nervioso central.

Procedimiento: Wellisair fue ubicado en una caja en el interior de la cámara de seguridad a con doce piezas de vidrio contaminadas con 100µl de suspensión viral por muestra a temperatura ambiente. Para los experimentos con virus secos, las piezas de vidrio se secaron durante 1 hora en la vitrina de seguridad antes del experimento de desinfección. Todas las pruebas se realizaron con tres réplicas para cada tiempo y condiciones de tratamiento, y las partículas virales se cuantificaron mediante unidades formadoras de placas (PFU).

Resultados:

Inactivación del CVB5 en suspensiones húmedas			
	Sin tratamiento	Tratamiento con Wellisair	
Tiempo	Virus (PFU/ml)	Virus (PFU/ml)	Reducción (%)
0 min	$3{,}80 \times 10^{8}$	$3{,}80 \times 10^{8}$	-
30 min	$4{,}10 \times 10^{7}$	$2{,}42 \times 10^{7}$	40,97
1h	$1{,}33 \times 10^{7}$	$2{,}78 \times 10^{6}$	79,09
2h	$2{,}17 \times 10^{6}$	$7{,}67 \times 10^{1}$	99,99
4h	$5{,}27 \times 10^{5}$	No se detecta	>99,999

Inactivación del CVB5 en suspensiones secas (primer test)			
	Sin tratamiento	Tratamiento con Wellisair	
Tiempo	Virus (PFU/ml)	Virus (PFU/ml)	Virus (PFU/ml)
0 min	$9{,}87 \times 10^{5}$	$9{,}87 \times 10^{5}$	-
30 min	$1{,}67 \times 10^{5}$	$1{,}00 \times 10^{4}$	94,01
1h	$1{,}23 \times 10^{5}$	$1{,}07 \times 10^{4}$	91,30
2h	$4{,}47 \times 10^{4}$	$5{,}47 \times 10^{2}$	98,77
4h	$1{,}20 \times 10^{4}$	$6{,}90 \times 10^{1}$	99,42

Inactivación del CVB5 en suspensiones secas (segundo test)			
	Sin tratamiento	Tratamiento con Wellisair	
Tiempo	Virus (PFU/ml)	Virus (PFU/ml)	Virus (PFU/ml)
0 min	$1{,}53 \times 10^{6}$	$1{,}53 \times 10^{6}$	-
2h	$4{,}77 \times 10^{5}$	$2{,}97 \times 10^{3}$	99,39
4h	$2{,}27 \times 10^{5}$	$1{,}97 \times 10^{1}$	99,99

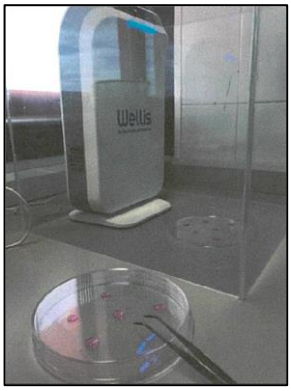
Conclusiones: Wellisair redujo el porcentaje de virus infecciosos CVB5 en superficies húmedas en un 99,99% después de 2 horas de tratamiento y en más del 99,999% después de 4 horas. La reducción del número de virus en superficies secas osciló entre el 99,42 y el 99,99% después de 4 horas.

Wellisair: Eficiencia contra virus VRS (Virus Sincitial Respiratorio Humano) (condiciones húmedas)

Informe de prueba de virus (20191212-3): 12 de septiembre de 2019, Laboratorio de virus contaminantes del agua y de los alimentos de la Universidad de Barcelona.

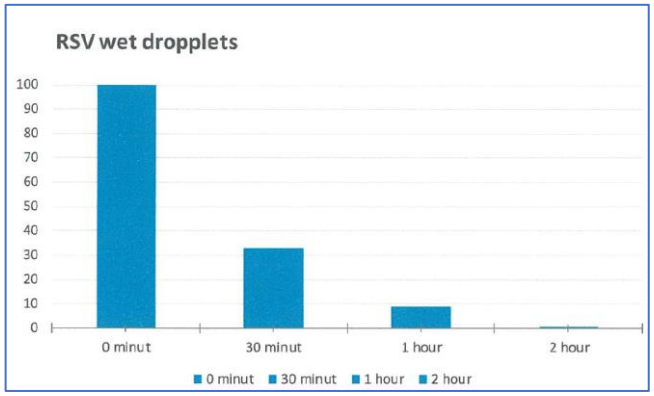
Alcance: medir la eficacia de Wellisair para la desinfección en superficies contra el virus sincitial respiratorio humano (RSV), principal causa viral de las infecciones agudas del tracto respiratorio inferior: bronquiolitis y neumonía.

Procedimiento: Wellisair fue ubicado en una caja de metacrilato junto a cien microlitros en gotas de RSV sobre pequeños trozos de vidrio, humedecidos a temperatura ambiente.
En cada tiempo de prueba, los virus se recuperaron mediante medio de cultivo (MEM) y las partículas virales fueron cuantificadas por $TCID_{50}$ en células Hep2.



Resultados:

Descomposición de la concentración de RSV en condiciones húmedas			
Tiempo	Sin tratamiento	Tratamiento con Wellisair	
	Virus (PFU/ml)	Virus (PFU/ml)	Reducción (%)
0 min	$3,58x10^5$	$3,58x10^5$	-
30 min	$2,60x10^5$	$8,08x10^4$	67%
1h	$2,82x10^5$	$5,00x10^4$	91%
2h	$1,77x10^5$	$6,55x10^3$	99%



Conclusiones: el desinfectante de aire Wellisair fue capaz de reducir el 99% de la concentración inicial de RSV después de 2 horas de tratamiento.
Se podría esperar que la eficiencia de Wellisair, recibiendo dosis equivalentes de aerosoles, sea al menos equivalente.

Wellisair: Eficiencia contra virus VRS (Virus Sincitial Respiratorio Humano) (condiciones secas)

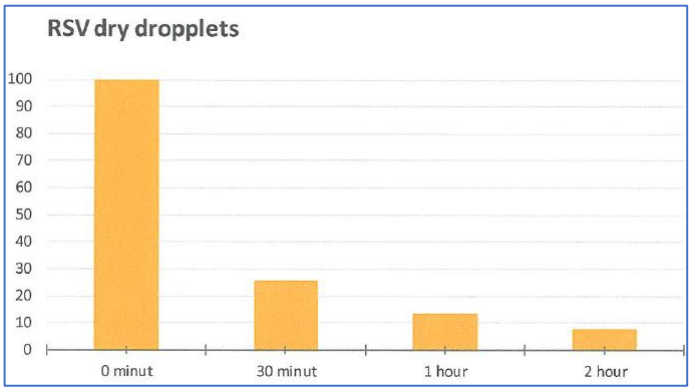
Informe de prueba de virus (nº20191212-4): 12 de septiembre de 2019, Laboratorio de virus contaminantes del agua y de los alimentos de la Universidad de Barcelona.

Alcance: medir la eficacia de Wellisair para la desinfección en superficies contra el virus sincitial respiratorio humano (RSV), principal causa viral de las infecciones agudas del tracto respiratorio inferior: bronquiolitis y neumonía.

Procedimiento: Wellisair fue ubicado en una caja de metacrilato junto a cien microlitros en gotas de RSV sobre pequeños trozos de vidrio, secados a temperatura ambiente.
Para cada tiempo de prueba, los virus se recuperaron mediante medio de cultivo (MEM) y las partículas virales fueron cuantificadas por $TCID_{50}$ en células Hep2.

Resultados:

Descomposición de la concentración de RSV en condiciones secas			
	Sin tratamiento	Tratamiento con Wellisair	
Tiempo	Virus (PFU/ml)	Virus (PFU/ml)	Reducción (%)
0 min	$5{,}30x10^4$	$5{,}30x10^4$	-
30 min	$5{,}00x10^4$	$1{,}29x10^4$	74%
1h	$4{,}30x10^4$	$1{,}23x10^4$	87%
2h	$4{,}71x10^4$	$9{,}05x10^3$	92%



Conclusiones: el desinfectante de aire Wellisair fue capaz de reducir el 92% de la concentración inicial de RSV después de 2 horas de tratamiento.
Se podría esperar que la eficiencia de Wellisair, recibiendo dosis equivalentes de aerosoles, sea al menos equivalente.