Inactivation of Enveloped Viruses (Coronavirus, H5N1 Virus) and Disinfection of the Air with Legionella-X 100 Via Ultraviolet Germicidal Irradiation (UVGI)

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Abstract— In November 2002 the Severe acute respiratory syndrome induced by coronavirus (SARS-CoV) was first identified in China. It caused a global outbreak with 8,098 probable cases including 774 deaths [1]. World Health Organization (WHO) review comprehensive protocol for cleaning and disinfection of hospitals and other settings after the occupation of people with Severe acute respiratory syndrome [2]. In view of the above, the Legionella-X 100 Air Sterilizer using Photochemical Reaction [3] was developed to combat the Enveloped Viruses such as Coronavirus and Influenza Viruses. Legionella-X 100 Air Sterilizer will emit ultraviolet light which is absorbed by proteins, RNA and DNA in a given microorganism. Absorption of UV by proteins in membranes at high **fluences (UV doses)** ultimately leads to the disruption of the cell membrane destroying the protein coat and hence death of the cell.
Legionella-X 100 that consists of a low-pressure dual band lamp using a wavelength from 185 nm to 253.7 nm.

This article covers the fundamentals of inactivation of viruses via Ultraviolet Germicidal Irradiation (UVGI) (32) using wavelength of 185nm to 253.7 nm

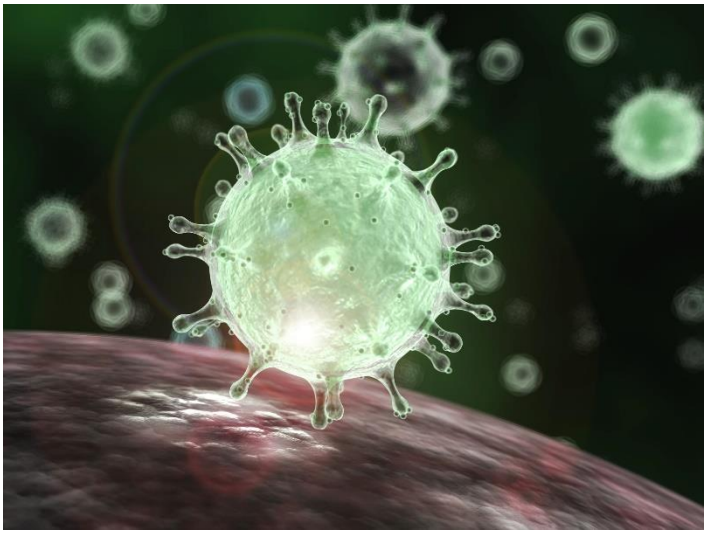
Keywords- Enveloped Virus, Coronavirus, H5N1 Virus, Legionella-X 100, Air Sterilizer, UV Lamp, MERS, SARS

INTRODUCTION- Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses short-wavelength ultraviolet (UV-C) light to kill or inactivate microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions.

Viruses are small, independent particles, built of crystals and macromolecules, unlike bacteria, they multiply only within the host cell. They transform protein of the host cell into proteins of

their own. UV destroys viruses by high energy electrons passing through or diffusing through the protein coat into the nucleic acid core, resulting in damage of the viral RNA. (33)

Coronaviruses (CoVs) are enveloped positive-sense RNA viruses, associated with the subfamily Coronavirinae and are characterized by club-like spikes that protrude from their surface (see picture 1).



Picture 1

It has an exceptional large RNA genome, and an uncommon replication strategy [4]. Coronaviruses (CoVs) are the largest group of viruses belonging to the Nidovirales order, which includes Coronaviridae, Arteriviridae, and Roniviridae families [4] The Coronavirinae comprise one of two subfamilies in the Coronaviridae family, with the other being the Torovirinae.

They are a comprehensive classification of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known coronaviruses are circulating in animals that have not yet infected humans [5].

The Coronavirinae are further subdivided into four groups, the alpha, beta, gamma and delta coronaviruses. The viruses were initially sorted into these groups based on serology but are now divided by phylogenetic clustering.

Alpha, Beta, Gamma and Delta are the four main sub-grouping of Coronaviruses. There are four main sub-groupings of coronaviruses, known as alpha, beta, gamma, and delta [6].

Two strains of human coronavirus, 229E and OC43 [7] are known to cause close to 25% of colds that exhibit symptoms similar to those caused by the rhinoviruses (e.g. runny nose, sneezing, and

cough). However, recent zoonotic strains of coronavirus characterized by species-jumping from animals to humans have gained notoriety and become of particular concern over the past decade.

The SARS-CoV (Severe Acute Respiratory Syndrome coronavirus) outbreak of 2002-2003 originated in bats and spread indirectly to humans via intermediate animals (e.g. civet cats) [8] From the earliest reported cases in southern China, the virus eventually spread to 28 countries over the course of eight months; thousands are believed to have been infected and 774 deaths were reported .

SARS-CoV is thought to be transmitted most readily by respiratory droplets (droplet spread) produced when an infected person coughs or sneezes. The **virus** also can spread when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eye(s)

More recently, the MERS-CoV (Middle East Respiratory Syndrome Coronavirus) outbreak originating in Saudi Arabia in April of 2012 has made headlines due to its high mortality rate of 45% and rapid spread to 9 countries (6); clusters of cases have continued to be reported in the Middle East through the end of 2013 [9].

The transverse species from bats to become endemic in humans, coronaviruses 229E and OC43 are proliferate from human-to-human person by way of contaminated aerosols. However, the potentiality for transmission from tainted fomites remains of concern as proven by the continued activity of strain 229E more than three hours after drying onto porous and non-porous materials, including aluminum and sterile sponges; strain OC43 remained infectious up to one hour after drying on the same surfaces [10]. The SARS-CoV virus is infectious and its incursion rate is approximated to range from 10%-60%. Predominantly, some victims are considered great spreaders with the capability to spread the disease to large number patients (usually more than 4), with some reports documenting transmission of the virus to more than 100 contacts. Despite of the fact that steroids and ribavirin have been used empirically for therapy, no efficacy data from controlled studies exist to prove that either drug affects outcome favorably.

The zoonotic SARS coronavirus strain desiderated both respiratory and intestinal replication routes for human hosts. A contemplative study was carried out on 138 patients infected with SARS-CoV found that almost 40% of patients developed diarrhea [14] and that SARS-CoV genomic material was detectable in the stool of patients for more than 10 weeks after onset of the initial illness.

Environmental transmission of coronaviruses via fomites and liquids can be curtailed given the proper application of disinfection protocols [10].

In view of the above, Magna International has developed Legionella-X 100 air sterilizer using ultraviolet bactericidal irradiation technology and a complete range of Legionella-X high-level disinfectants capable of killing most pathogens, including all types of viruses, vegetative bacteria, mycobacteria, and bacterial spores, were developed to meet each unique application of inactivating Enveloped Viruses, bacteria using synergistic chemical composition of Quaternary

Ammonium Compound, surfactants, alcohol, essential oils and a synergistic chemical composition of Chlorhexidine Gluconate, surfactant, essential oil and water [15,16].

How does SARS transmit?

Most coronaviruses spread the same way other cold-causing viruses do, through infected people coughing and sneezing, by touching an infected person's hands or face, or by touching things such as doorknobs that infected people have touched

SARS is transmitted principally by close human-to-human contact. In the context of SARS, close contact means having cared for or lived with someone with SARS or having direct contact with respiratory secretions or body fluids of a patient with SARS.(Examples of close contact include kissing or hugging, sharing eating or drinking utensils, talking to someone within 3 feet, and touching someone directly [12].

Close contact does not include activities like walking by a person or sitting across a waiting room or office for a brief time.) The virus that causes SARS is transmitted by the spread of respiratory droplets produced when an infected person coughs or sneezes [12]

When a person coughs or sneezes, small amounts of fluid are propelled for about 3 feet through the air and land on the mouth, nose or eyes of persons who are nearby. The virus also can spread when a person touches a surface or object contaminated with these infectious droplets and then touches his or her mouth, nose, or eyes. It is possible that the SARS virus might spread more broadly through the air (airborne spread) or by other ways that are not now known [12]

Human coronaviruses were first identified in the mid-1960s. The seven coronaviruses that can infect people are: Common human coronaviruses 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS) SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS) 2019 Novel Coronavirus (2019-nCoV) [19].

The H5N1 highly pathogenic influenza viruses' subtype have infected more than 600 people since 1997, resulting in the deaths of approximately 60% of those infected. [20] The influenza A viruses circulating in avian species rarely infect humans. However, since 1997, highly pathogenic avian influenza viruses of the H5N1 subtype have infected more than 600 people. Infection of humans with these viruses typically leads to severe respiratory disease that often progresses to multiorgan failure; approximately 60% of confirmed cases of highly pathogenic H5N1 influenza infection have resulted in death. The first fatal infections of humans with highly pathogenic avian H5N1 influenza viruses were reported in Hong Kong in 1997 [25].

Since their emergence in the late 1990s, highly pathogenic avian H5N1 influenza viruses have undergone multiple reassortment events with avian influenza A viruses of different subtypes, including H6N1, H9N2 and H5N1 [26,27,28,29,30,31]. Hence, the currently circulating highly pathogenic H5N1 viruses represent a diverse group of viruses. Moreover, the viral surface

glycoprotein HA (the major viral antigen), has evolved through point mutations, leading to several genetically and antigenically distinct clades and subclades.

The major clades circulating during the past years include clades circulating in Egypt, Israel, the Gaza strip and the West Bank, circulating in China, Bangladesh India and circulating in Indonesia. [8,9,10]. Although genetically and antigenically diverse, highly pathogenic avian H5N1 viruses share the ability to cause high mortality in poultry and infect humans. Recently, the HA gene of highly pathogenic avian H5N1 influenza viruses of clade 2.3.4.4 has reassorted with the neuraminidase (NA) and other viral genes originating from different avian influenza viruses, giving rise to novel viruses of the H5N2, H5N6 and H5N8 subtypes.

Many studies have assessed the virulence and pathogenicity of highly pathogenic avian H5N1 influenza viruses in different cell types and animal models including chickens, ducks, mice, guinea pigs, ferrets, pigs and nonhuman primates (reviewed in [11,12]). Mice are typically used to assess the virulence and immunogenicity of influenza viruses because they are inexpensive and multiple immunological reagents are available.

However, mice are not a natural host of influenza viruses and typically do not transmit viruses. Ferrets infected with influenza viruses show signs of respiratory infection like those observed in humans, and influenza viruses can transmit among ferrets via respiratory droplets.

For decades it was assumed that infectious diseases were spread primarily by the airborne route or through direct patient contact, and the surrounding environment played little or no role in disease transmission. Up until 1987 the Centers for Disease Control and the American Hospital Association focused on patient diagnosis due to the belief that nosocomial infections were not related to microbial contamination of surfaces (21). Over the years of studies have changed the perspective on viral transmission to include a more complex multifactorial model of disease spread. There is now growing evidence that contaminated fomites or surfaces play a key role in the spread of viral infections (22, 23, 24).

Disinfection define as a process that eliminates innumerable or all pathogenic microorganisms, excluding bacterial spores, on inanimate objects. In health-care settings, objects usually are disinfected by liquid chemicals.

The length of survival of Coronaviruses ranges from 24 to 72 hours on fomites and in stool samples; Up to 72–96 hours on dry inanimate surfaces [17]. Hence chemical disinfectants need to be employed to disinfect all fomites to prevent infection.

The Mechanism of the Inactivation of the Viruses through Legionella-X 100 Air Sterilizer via Ultraviolet Germicidal Irradiation is herein described.

Virus is made up have core genetic material, either RNA or DNA surrounded by a protective coat called capsid which is made up of protein. The nucleic acid may be single or double-stranded. The entire infectious particle, called virion, consists of the nuclei acid and outer protein. The simplest viruses contain enough RNA or DNA to encode proteins. (34,35)

Fundamentals of UV light

Ultraviolet or UV light is light that has a higher frequency than visible light. As violet is the color of the highest frequency of visible light; ultraviolet light is the term we apply to light that has frequencies higher than visible light.

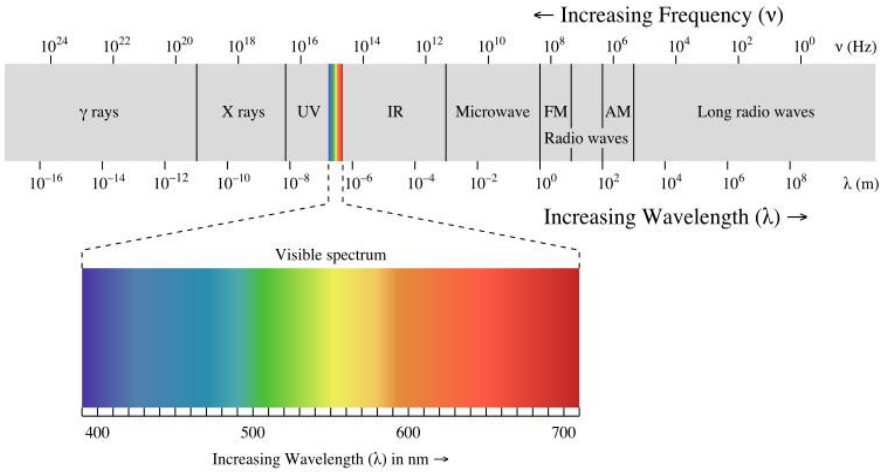


Table 1

Visible light is normally measured in wavelengths with the unit of nanometers (nm). As the wavelength (nm) increases the frequency of light decreases. Visible light is usually defined as having wavelengths in the range of 400 – 700 nm. Ultraviolet light is usually defined as the range between 100 – 380 nm.

It is important to note that every commercial UV light will produce a range of UV light, and not only one single wavelength. A 185 nm wavelength UV light is "tuned" to produce UV light at 185 nm, but may create UV light from 100 – 240 nm, or even higher

Ultraviolet germicidal irradiation (UVGI) is a disinfection method that uses short-wavelength **ultraviolet** (**UV**-C) **light** to **kill** or inactivate **microorganisms** by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions

Ultraviolet germicidal irradiation (**UVGI**) is a disinfection method that uses short-wavelength ultraviolet (UV-C) light to kill or inactivate microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions.[1] UVGI is used in a variety of applications, such as food, air, and water purification.

The application of UVGI to disinfection has been an accepted practice since the mid-20th century. It has been used primarily in medical sanitation and sterile work facilities. Increasingly it has been employed to sterilize drinking and wastewater, as the holding facilities are enclosed and can be circulated to ensure a higher exposure to the UV. In recent years UVGI has found renewed application in air purifiers.

UV-C light is weak at the Earth's surface as the ozone layer of the atmosphere blocks it.[2] UVGI devices can produce strong enough UV-C light in circulating air or water systems to make them inhospitable environments to microorganisms such as bacteria, viruses, molds and other pathogens. UVGI can be coupled with a filtration system to sanitize air and water.

Photochemical wavelength ranges

The usual wavelength range n photochemistry is 100 – 1000 nm (100,000 – 10,000 cm^{-1}). Light photons with wavelengths longer than 1000 nm have a proton energy too small to cause chemical change when absorbed, and photons with wavelengths shorter than 100 nm have so much energy that ionization and molecular disruptions characteristic of radiation chemistry prevail. The total photochemical wavelength range is divided up into bands with specific names as given in Table 2..

Table 2. Spectral ranges of interest in Photochemistry

Range Name	Wavelength Range / nm	Wavenumber Range / cm^{-1}	Energy Range (kJ $Einstein^{-1}$)
Near Infrared	700 – 1000	10,000 – 14,286	120 - 171
Visible	400 – 700	14,286 – 25,000	171 – 299
Ultraviolet UVA UVB UVC	 315 – 400 280 – 315 200 - 280	 25,000 – 31,746 31,746 – 35, 714 35,714 – 50,000	 299 – 380 380 – 427 427 - 598
Vacuum Ultraviolet (VUV)	100 – 200	50,000 – 100,000	598 - 1196

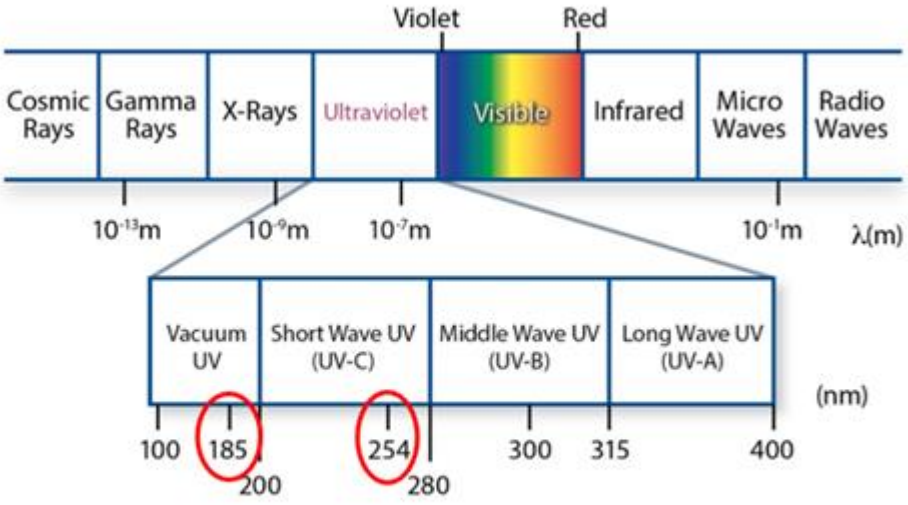
Most studies in photochemistry involve the Ultraviolet ranges. The division into three sub-ranges is connected with human skin's sensitivity to ultraviolet light. The UVA range causes changes in the skin that lead sun tanning. The UVB range can cause sun burning and is known eventually to induce skin cancer. The UVC range is extremely dangerous, since it is absorbed by proteins, RNA and DNA and can lead to cell mutation, cancer and/or cell death. The UVC range is sometimes called the germicidal range, since it is very effective in inactivating bacteria and viruses. The vacuum ultraviolet range is absorbed by almost all substances (including water and air). Thus, it can only be transmitted in a vacuum. The absorption of a VUV photon causes one or more bond breaks.

Ultraviolet wavelength and Ozone production

The Ultraviolet Germicidal Irradiation (UV-C) wavelength is an invaluable tool for an HVACR system. By leveraging germicidal energy to keep refrigeration coils free of microbial growth, facility managers also enjoy the benefit of reducing the spread of airborne infectious agents. However, some facility managers may hesitate to leverage these benefits for their application due to a concern about ozone. While the Ultraviolet spectrum contains four separate wavelengths—UV-A, B, C and Vacuum UV each operates at different energy levels and only one is capable of producing ozone (Vacuum UV).

As you'll note in the graphic below, Vacuum UV operates in the 100-200nm range, where it is capable of producing ozone. UV-C, conversely, reaches its optimal germicidal strength near 253.7nm. Because ozone may only be produced below 200nm, at 253.7nm (rounded to 254nm), the germicidal wavelength does not generate ozone.

In addition to the stronger 254nm wavelength that *does not produce ozone,* UV-C lamps offer another layer of ozone protection.
Most germicidal lamps are produced with doped quartz glass, which blocks the transmission of the 185nm ozone-producing wavelength.



253.7nm UV-C LAMPS = GERMICIDAL EFFICIENCY WITHOUT OZONE PRODUCTION

Table 3

The doped quartz glass allows the 253.7nm radiation to pass through, but it blocks the 185nm wavelength from escaping. Therefore, germicidal lamps with doped glass CANNOT produce ozone.

WHAT IS OZONE?
Ozone is present in low concentrations throughout the earth's atmosphere. Some researchers say that this chemical is "good up high, but bad down low." Without the ozone layer protecting our Earth's stratosphere, for example, the Sun's ultraviolet radiation would make life on Earth uninhabitable. At street level, however, a high concentration of ozone is toxic to plants and animals. In humans, ozone can irritate nasal passages, cause nausea and extended exposure can lead to lung inflammation.
Ozone, also called Vacuum Ultraviolet (UV-V), is a gas molecule that contains three (3) oxygen atoms – and as such, it has a destabilizing effect on oxygen in the air (leading to its irritation and danger to humans). A UV lamp "tuned" to 185nm can create ozone from oxygen (O2) by disrupting the O2 molecule and splitting it into two oxygen atoms. These two oxygen

atoms attempt to attach to other oxygen molecule (O2). It is the attachment of this third oxygen atom that creates ozone (O3).
Ironically, UV light in the 240-315nm wavelength will break this third oxygen atom attachment above and convert it back to oxygen. The peak ozone destruction occurs at the 254nm wavelength. So, a UV-C lamp at the 253.7nm wavelength will actually destroy ozone!
ASHRAE has said that certain air cleaners produce ozone and thus, its position is to recommend discontinuing utilizing "devices that use the reactivity of ozone for the purpose of cleaning the air." [36]

Observance of Performance Sustainability

Keeping buildings operating at their most efficient level and sustaining that performance over the life of a building is one of today's key challenges for specifying engineers, HVACR contractors and facility managers. Today, with germicidal technology, virtually *all* HVACR systems are potential candidates because of the many proven operational benefits it offers, including destruction of surface and airborne microorganisms and greatly improved indoor air quality by Legionella-X 100 via ultraviolet germicidal irradiation.

Ultraviolet Germicidal Irradiation (UVGI) Mechanism

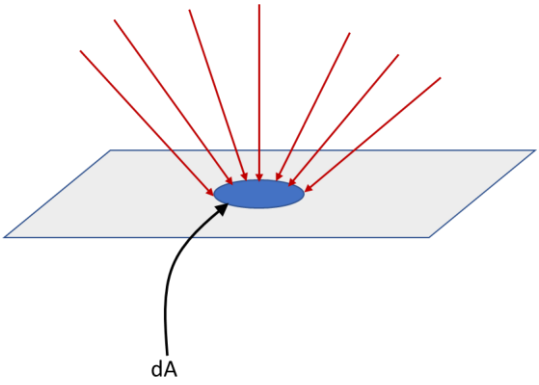
Terms and concepts associated with the receipt of light

When light is emitted from a source, it radiates outward at the speed of light ($c = 2.99792458 \times 10^8\ ms^{-1}$). When the light impinges on an object, it may be reflected, transmitted or absorbed. There are several terms that related to the receipt of light.

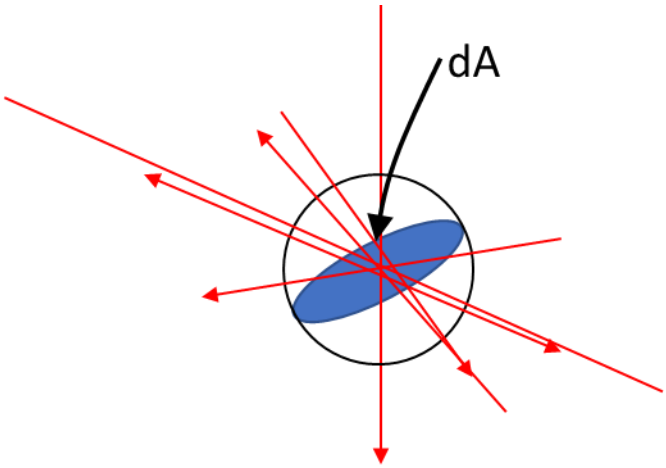
Irradiance
Irradiance (symbol; E; units $W\ m^{-2}$) is defined as the total radiant power incident from all upward directions on an infinitesimal element of surface of area dS containing the point under consideration divided by dS (see Illustration 1a).

Fluence Rate
Fluence Rate (symbol E'; units Wm^{-2}) is defined as the total radiant power incident from all directions onto an infinitesimally small sphere of cross-sectional area dA, divided by dA (see Illustration 1b)

(a)



(b)



Illystration 1. Illustration of the concepts of irradiance and fluence rate: (a) irradiance onto a surface; (b) fluence rate through an infinitesimally small sphere of cross-sectional area dA.

The appropriate term for UV disinfection is “fluence rate” because a microorganism can receive UV power from any direction, especially when there is more than one UV lamp in the vicinity.

Fluence (UV Dose)

Fluence (symbol H’, units J m^{-2}) (also called UV Dose) is defined as the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere pf cross-sectional area dA, divided by dA.

The term UV Dose is often used in UV disinfection literature. It represents the UV exposure of a given organism in the germicidal range. The units are mW.scm^{-2} or mJ cm^{-2}.
The term “fluence” is preferred over that of “UV Dose”, since the term “Dose” is used to imply total absorbed energy (e.g., the “UV Dose” required to induce sunburn on the skin). Fluence represents the radiant energy “incident” on a microorganism, and in most cases only a small fraction (about 1%) of radiant energy is absorbed.

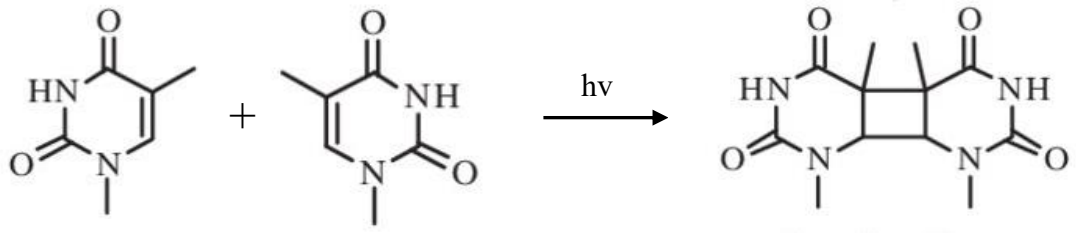
Ultraviolet Disinfection

Ultraviolet light has been used to disinfect both drinking water and secondary effluent from sewage treatment plants over a good part of the 20th century.

Fundamental Mechanism of UV disinfection

Ultraviolet is absorbed by proteins, RNA and DNA in a given microorganism. Absorption of UV by proteins in membranes at high fluence (UV dose) ultimately leads to the disruption of the cell membranes and hence death of cell. However, at much lower fluences (UV Dose), absorption of UV by DNA (or RNA in some virus) can disrupt the ability of the microorganism to replicate. A cell that cannot replicate cannot cause disease.

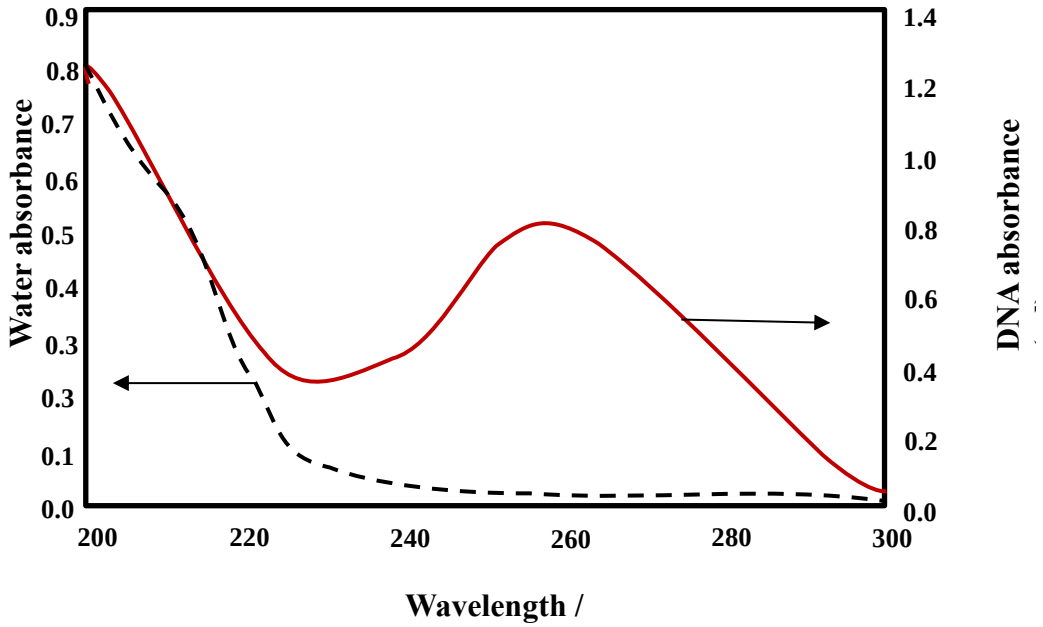
DNA is a nucleic acid polymer in a double-stranded helix linked together by a sequence of four constituent bases (adenine, cytosine, quinine, and thymine), which constitute the genetic code. These form "base pairs" (adenine with thymine and cytosine with guanine) held together by hydrogen bonds. This is the "glue" that holds the two "strands" of DNA together. Of these four bases, thymine undergoes a unique photochemical reaction (See Reaction 1). If two thymine bases are located adjacent to each other, absorption of a UV photon by one of the thymines leads to formation of a chemical bond between the two thymines (called a thymine dimer). The rection spectrum for this photochemical dimerization peaks at 260 nm and follows closely the absorption spectrum of DNA (see Graph 1).[32]



thymine thymine thymine dimer

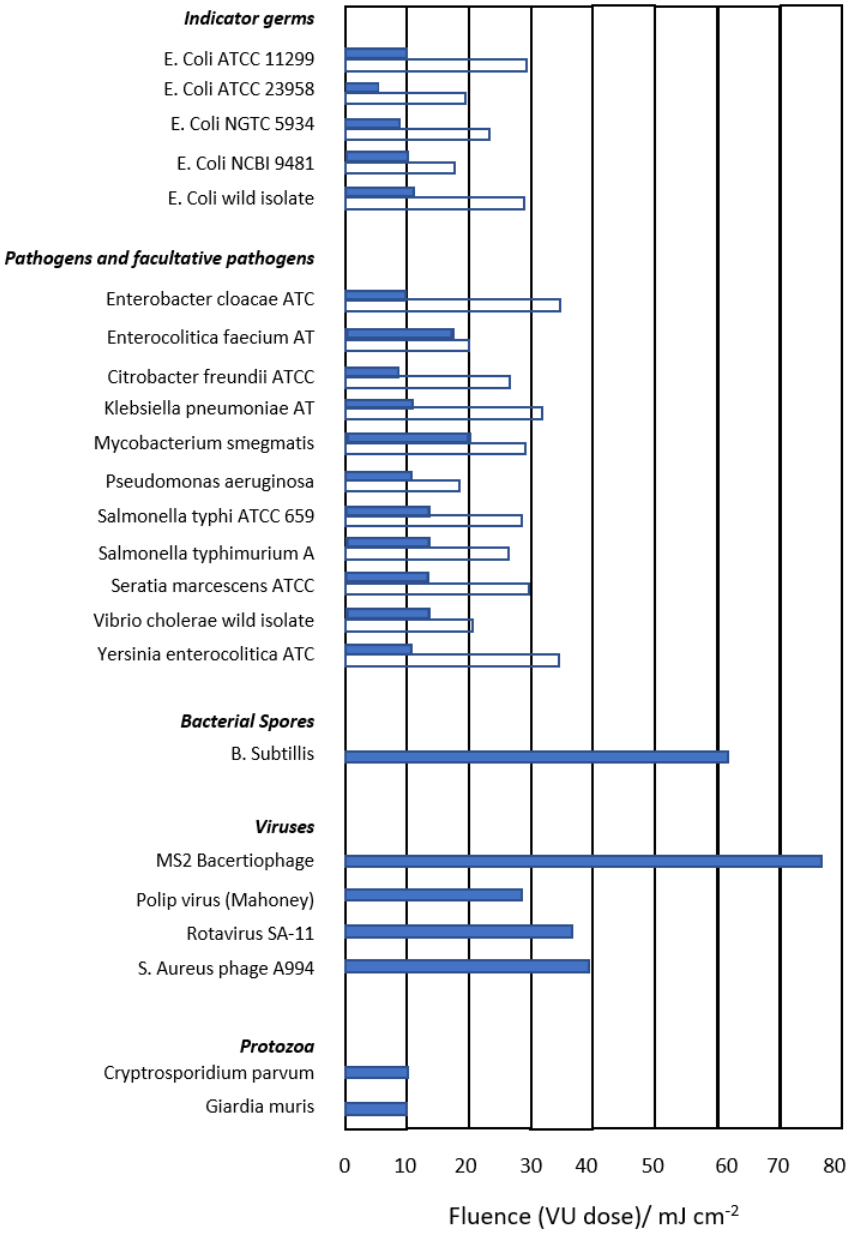
Reaction 1. Photochemical dimerization of two thymine bases

The photochemical dimerization of thymine pairs disrupts the structure of the DNA. So that if enough thymine dimers are formed, the DNA cannot replicate in cell mitosis. This then is the fundamental mechanism of VU disinfection. Some virus contains only RNA; in this case, a similar photochemical dimerization reaction takes place between two uracil bases.
Some microorganisms (particularly bacteria) have a repair mechanism that dissociates the thymine dimers. This process is triggered by the absorption of UV light and is thus called photoreactivation. The repair mechanism can be overcome, but this requires a higher fluence (UV Dose).



Graph 1

Graph 1. Absorption spectrum of DNA (———————) compared to a typical drinking water absorption spectrum **(** ---------- **).**



Graph 2

Graph 2. UV dose required for 4 logs (99.99%) inactivation of bacteria, spores, virus and protozoa. The bars represent “in the presence of photoreactivating light” (open bars) and ‘in the absence of photoreactivating light” (solid bar). [32]

The UVGI effectively inactivate Enveloped Coronavirus and H5N1 virus.

4.0 Conclusion

Based on the above information, Ultraviolet Germicidal Irradiation (UVGI) is effective to inactivate H5N1 and Coronaviruses.

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