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Master Degree in Biomedical Engineering

STUDY OF UV LAMP FOR THE SANITIZATION OF AUTOMATIC DRUG COMPOUNDING SYSTEMS

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1 Abstract

This thesis originated from the need to improve the sanitization phase of automatic robotic systems which deal with the preparation of injectable solutions containing drugs. Considering the context in which these systems operate, the fulfilment of the requirements necessary to ensure the essential level of sterility represents a topic of great interest.

At present, one of the technologies mostly utilized in the microbiological sterilization is the ultraviolet radiation. This study, in fact, wants to investigate new solutions able to enhance the sterilization process and wants to analyse the comparison between two UV lamp types: the UV low-pressure mercury vapor lamps and the UV LEDs. Specifically, this research has as its final goal to assess the feasibility of moving from the traditional technology of the UV mercury-vapor lamp to the innovative technology of LEDs.

In particular, the present study of UV irradiation is applied to automated robots used in hospital pharmacies for the preparation of hazardous and non-hazardous injectable drugs, such as APOTECAchemo and APOTECAunit, respectively.

In these systems the UV irradiation is employed to guarantee the microbial sanitization and consequently, this study has also the secondary aim of verifying if the level of disinfection assured by the UV low-pressure lamps which are currently used is adequate for an homogeneous germicidal action inside the internal chamber of the systems. Therefore, the comparison between the two types of UV source (i.e. traditional lamp and LED) aims also to understand if this germicidal action shall or may be improved in some way.

The first phase of this analysis consisted in collecting some information about the ultraviolet radiation and the two types of UV source through classic research methods. Then, was carried out a market survey to find which solutions of the LED technology are nowadays available in commerce and subsequently were selected two different

typologies of UV LED. After that, were made some tests on both systems, APOTECAchemo and APOTECAunit, for the evaluation of the irradiance produced by UV lamps through the use of a photo-radiometer. After this first session of tests, in order to make a comparison between the irradiation produced by the UV lamps and the LEDs, a test chamber was designed in order to simulate the optimal experimental conditions. With this chamber were tested the intensity of the irradiation produced by the two types of LEDs and by the traditional UV lamp. Finally, was evaluated also the germicidal power of the three UV sources by analyzing how microorganisms react at UV exposure. This latter test was conducted in collaboration with the University of Urbino.

The results obtained from this study confirms that the new UV LED technology could represents a valid alternative to the traditional technology. In details, with the adequate numbers of LEDs (an array of LEDs), the irradiance produced by these two technologies could be comparable: from our tests, in fact, it appears that the irradiance produced by one lamp is equal to 1.720 $^{W}/_{m^2}$, the irradiace produced by the first type of LEDs is equal to 1.362 $^{W}/_{m^2}$ while the irradiace produced by the second type of LEDs is equal to 0.416 $^{W}/_{m^2}$. The difference of irradiance produced by the two typologies of LEDs is due to the different number of LEDs utilized for the test (7 LEDs for the first type and 4 for the second one). So basically, by increasing the number of LEDs can be achieved the same level of irradiance of a traditional lamp. This, however, also implies the increase in costs; the cost of 1 lamp is infact € 15.26, the cost of the 7 LEDs is about € 279.23 and the cost of the 4 LEDs is about € 87.52. The usage of LEDs would be able to cover the gaps in the current technology of mercury-vapor lamp because: they have a smaller dimension (they can be distributed in the space so as to cover in a more homogenous way the zone of interest); they operate in a different wavelength (that can guarantee a more effective germicidal activity); they have a longer lifetime (which reduces the need to change lamps) and they are more environmentally-friendly (they do not contain mercury). But, on the other hand, for

equal irradiance emitted by the different UV sources, the high cost linked to the evolving technology significantly increases prices of LED solutions. Moreover, the transition to LED technology would require a mechanical and electrical adaptation of both APOTECA systems. This would require a further increase in costs and a new design resulting in a time-consuming process. In conclusion, keeping in mind that however the lamps currently used still guarantee good results in terms of irradiance emitted and germicidal power, the transition to the innovative UV LED technology will probably take some more time.

However, as soon as the costs of the LEDs will be reduced, most certainly there will be the transition to LED technology.

2 Introduction

2.1 APOTECA systems

In the last few years fully automated robots are increasingly employed in hospital pharmacies for the preparation of hazardous and non-hazardous drugs. Compounding of drugs must be carried out with strictly aseptic procedures and for this reason it is important to guarantee an appropriate level of sterility in the areas where the blending of drugs take place [1].

Robots of this type are APOTECAchemo and APOTECAunit (Figure 2.1). APOTECAchemo is an automatic robotic system that can prepare, in controlled atmosphere, injectable solutions containing chemotherapeutic drugs following instructions and procedures planned by the operator. Instead, APOTECAunit is an automatic system for the preparation in controlled atmosphere of injectable solutions containing non-hazardous drugs, such as antiemetics and antibiotics. Both systems consist of an equipment (APOTECAchemo/APOTECAunit appliances) and an external dedicated server on which the APOTECAmanager software application is installed. APOTECAmanager software is used for the management of files, the planning of preparations and the creation and management of documents related to the activities carried out.

Figure 2.1 *APOTECAchemo (left) and APOTECAunit (right).*

The server sends the information related to the arrangement of the preparations through the APOTECAmanager to the APOTECA equipment which, based on the information received, carries out all the operations necessary to complete the preparations.

The APOTECA equipment sends the APOTECAmanager all the data related to the preparations arranged, including the accuracy percentages achieved in the measuring operations. The preparations arranged are unequivocally identified by a barcode that records the main information: active principle, dosage, patient's name/name of the production batch, destination department, preparation date and time.

The APOTECAchemo equipment (Figure 2.2) consists of a Main Cabinet which can be divided into 6 areas (loading, warehouse, preparation, electric panel, main filter and waste materials) and an Air Treatment System (ATS), consisting of a set of devices (piping, Venturi, fan, HEPA filters class H14, plenum and contraplenum, cover panels, etc.).

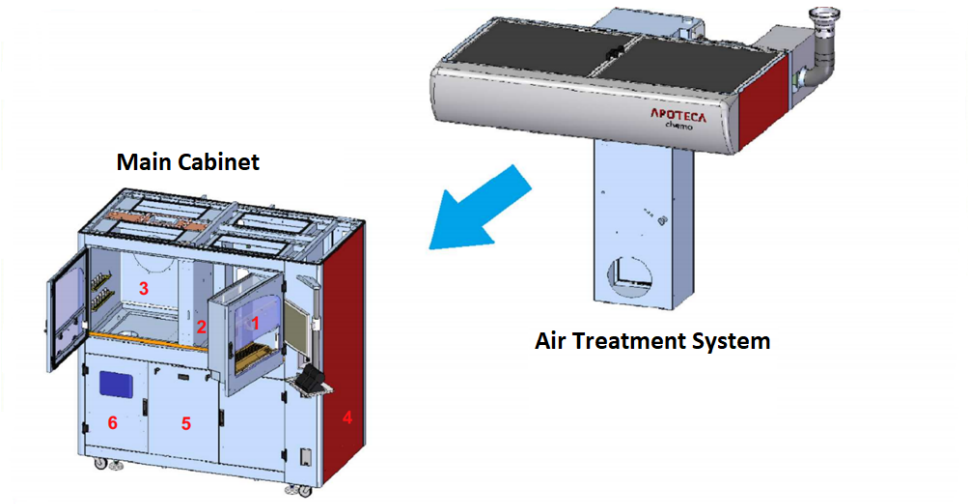

Figure 2.2 *APOTECAchemo equipment. 1. Loading area, 2. Warehouse area, 3. Preparation Area, 4. Electric panel, 5. Main filter area, 6. Waste materials area.*

Similarly, the APOTECAunit equipment (Figure 2.3) consists of a Main Cabinet divided into 6 areas (loading, unloading, warehouse, preparation, electric panel and waste materials) and an Air Treatment System (ATS), consisting of a set of devices (4

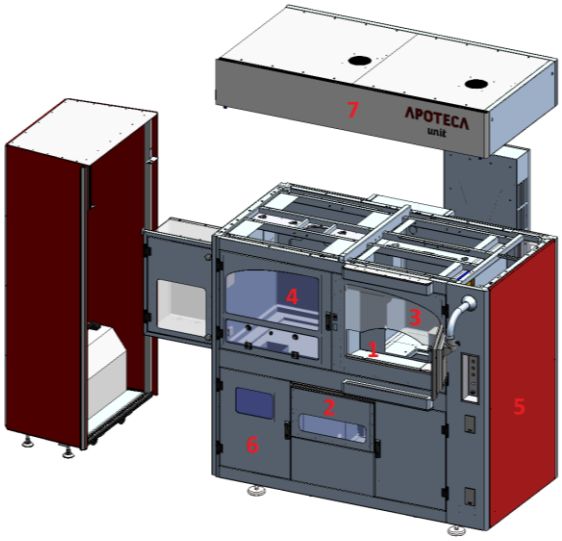

Figure 2.3 *APOTECAunit equipment. 1. Loading area, 2. Unloading area, 3. Warehouse Area, 4. Preparation area, 5. Electric panel, 6. Waste materials area, 7. Air Treatment System.*

fan filter units, 1 exhaustive fan, 4 HEPA H14 class filters).

The preparation arrangement operations are subjected to constant control by means of a precision weighing device, presence sensors and automatic vision systems with image recognition.

Loading and unloading of the components used to arrange preparations are carried out in the absence of contamination and under health conditions, in complete safety for patients and healthcare operators who use the APOTECA equipment. Work areas are microbiologically controlled and separated from the laboratory environment.

One of the main differences between APOTECAchemo and APOTECAunit is related to the pressure gradient in relation to the external environment. In particular, for what concerns APOTECAchemo, the loading/unloading area, the warehouse area and the preparation area are characterized by an air recirculation and change system through HEPA filters. These areas are characterized also by the constant maintenance of negative pressure gradient in relation to the external environment, by a laminar air flow that covers each point of internal volumes of preparation and by continuous easy-to-clean surfaces. In the APOTECAunit instead, the loading/unloading area, the warehouse area and the preparation area are characterized by 100% air recirculation

and air purging through HEPA filters, positive pressure gradient, laminar air flow in every point of the internal preparation compartment and by continuous, easy-to-clean, internal surface [2] [3].

With all of this, the APOTECA equipment allows to improve the patient's safety in relation to the preparation and processes with drugs. The patient is assured their dosage is accurate because of the following:

- computer-aided preparation prescription process;
- planning and scheduling management for preparations;
- automation of arrangement operations and control of measuring accuracy;
- identification of preparations through barcode.

APOTECA systems also permits to reduce risks and improve safety for the personnel that use the equipment. It allows the following:

- considerably reduce the presence of personnel in the proximity to the preparation area;
- avoid excessive handling of drugs, minimizing the operator's exposure to such substances;
- avoid environmental contamination;
- avoid dangers related to the management of needles;
- manage waste disposal safety and with no exposure for the operator to the materials present inside the waste container;
- reduce manual efforts.

Another no less important aim of APOTECA systems is that of increasing the effectiveness of the work process by:

- automating the preparation work process;
- reducing workflow downtime;
- planning the preparation arrangement effectively;
- ensuring traceability of all the work process operations.

2.2 Cleaning procedure

In order to ensure that the preparation activities are carried out by the APOTECA system under healthy conditions without contamination risks, it is necessary to adopt a proper cleaning procedure.

The work areas inside the equipment are microbiologically controlled and separated from the environment of the laboratory thus the preservation of these conditions is strictly conditioned by the cleaning level maintained in the equipment premises and obviously by the condition of the components inserted in the system during the preparation phases.

The cleaning procedure consists of a first step of chemical decontamination and a second one of sanitization/disinfection. Chemical decontamination is intended as the process of physical removal or inactivation of residues of cytotoxic substances from surfaces by the use of chemical agents. In particular, for the daily cleaning of the equipment it is recommended to use sterile gauzes soaked with 70% ethyl alcohol or Fenplus emulsion concentrate. The areas that must be cleaned daily are monitor, loading area and preparation area.

The sanitization/disinfection procedure is performed by using ultraviolet (UV) mercury-vapor lamps to sanitize workspaces and tools. UV irradiation usually destroys or distorts nucleic acids thereby inhibiting DNA replication. For this reason, UV irradiation is commonly used as sanitising means to inactivate microorganisms in compounding robots. Therefore, for the daily disinfection, to ensure bacteriostaticity, on the system are installed two UV panels equipped with UV-ray lamps (Figure 2.4a). In addition to the UV panels, the systems are equipped with three fixed UV lamps mounted in the warehouse area for the APOTECAunit equipment and two fixed UV lamps mounted in the warehouse area for the APOTECAchemo equipment (Figure 2.4b). The extra lamp installed in the APOTECAunit equipment is located in the middle of the carousel.

The first UV panel, with two integrated lamps, is applied to the preparation area

door in the slot dedicated to the service opening, while the second UV panel, with two integrated lamps, is applied to the loading area door in the access slot for the loading of materials [2] [3].

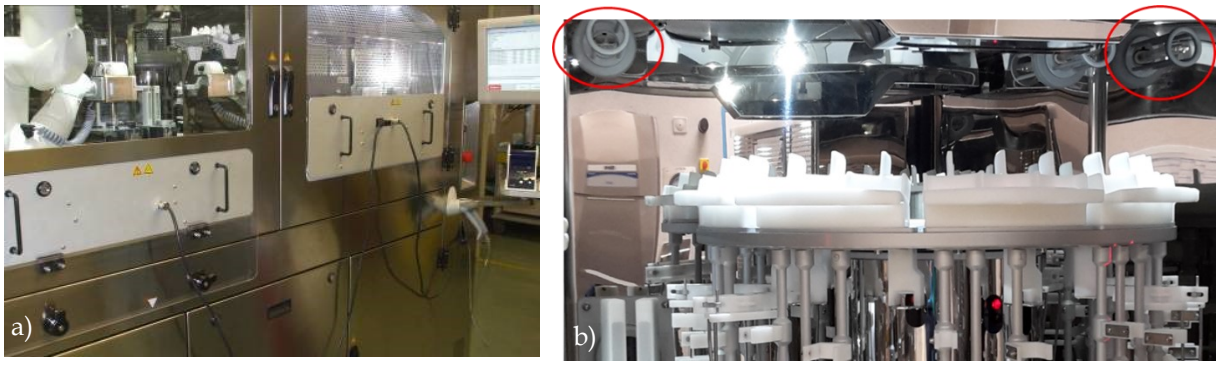

Figure 2.4 *a) UV panels and b) UV fixed lamps.*

The proper execution of this disinfection procedure ensures an aseptic process for all working steps. This condition is required for the intravenous preparations manufacturing in order to prevent any microbial contamination.

In fact, according to Good Manufacturing Practice [1] guidelines for clean-areas classification, aseptic compounding has to be performed in a Class-A environment; which means the environmental class with the stricter conditions. Specifically, the GMP classification requires compliance with the limits for the microbial load and the particle monitoring (Figure 2.5). Qualification of the Class-A inner environment of the robot was performed through microbial air and surface quality assessment utilizing settle and contact plates, swabs and particulate matter monitoring as described in [4].

Grade	Air sample cfu/m3	Settle plates (diameter 90mm) cfu/4 hours*	Contact plates (diameter 55 mm) cfu/plate
A**	<1	<1	<1

Grade	Recommended maximum limits for particles ≧ 0.5 µm/m^3		Recommended maximum limits for particles ≧ 5 µm/m^3	
	in operation	at rest	in operation	at rest
A	3 520	3 520	20*	20*

Figure 2.5 *Microbial load requirements for grade A (top) and particle monitoring requirements for grade A (bottom).*

In APOTECA systems there was found no evidence of microorganism growth on the surfaces "at rest" and during compounding. Therefore, APOTECA systems meet the requirements for advanced aseptic processing in the hospital pharmacies and the pharmaceutical industry in general respecting the recommended maximum limits set for grade A for both microbial load and particle monitoring [4].

These requirements are achieved also thanks to the UV lamps system which are able to maintain the values of microbial load and particle monitoring within the limits necessary for class A.

Obviously, inside the equipment not all points are reached in the same way by the ultraviolet radiation. Some points, in particular the most hidden points, are reached by a quantity of radiation that is lower than that received by the most exposed points. These points that receive less ultraviolet radiation may be subject to conditions that favour the growth of microorganisms.

For that reason it appears necessary to find some solutions that can guarantee a more homogeneous level of irradiation for all the points inside the equipment in such a way that can be guaranteed the same level of sterilization for all the points.

This work has precisely this goal: to find an alternative solution to the UV lamps that are currently utilized in order to guarantee an homogeneous level of sanitization. This alternative can be represented by the ultraviolet light-emitting diodes (UV LEDs). For this purpose in this essay, the LED technology will be analysed in every aspect and will be compared to the actual technology of the UV lamps.

2.3 Ultraviolet Radiation

In order to understand how UV lamps works are firstly needed some notions about ultraviolet radiation. Ultraviolet is a form of electromagnetic radiation with wavelengths that go from 10 up to 400 nm and that are shorter than that of visible light, but longer than X-rays (Figure 2.6). In particular, the UV spectrum can be subdivided

into following bands:

- UV-A (long-wave; 400 to 315 nm);
- UV-B (medium-wave; 315 to 280 nm);
- UV-C (short-wave; 280 to 100 nm);
- Vacuum ultraviolet (VUV, wavelengths shorter than 100 nm).

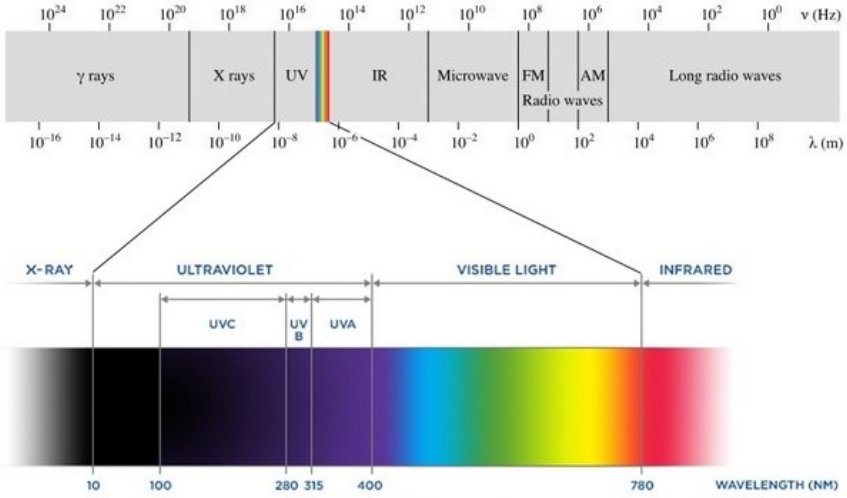

Figure 2.6 *Ultraviolet spectrum.*

All UV ranges and bands are invisible to the human eye. The band of our interest is the UV-C band because it contains the most effective wavelengths for germicidal control. This germicidal UV band, specifically for wavelengths between 200 nm and 300 nm, is particularly effective against microorganisms because the radiation at these wavelengths is strongly absorbed by nucleic acids. The absorbed energy can result in defects including pyrimidine dimers. Pyrimidine dimers are molecular lesions formed from thymine or cytosine bases in DNA via photochemical reactions (organic reactions induced by the action of light). These dimers can prevent replication or can prevent the expression of necessary proteins, resulting in the death or inactivation of the organism. This process is similar to the effect of longer wavelengths (UV-B) producing sunburn in humans.

Microorganisms have less protection against UV-C and therefore cannot survive at a prolonged exposure to it.

3 Materials and Methods

In Figure 3.1 are reported all the working steps of this thesis: starting with the examination of the different UV sources and their comparison up to the different tests made for the comparison of their features.

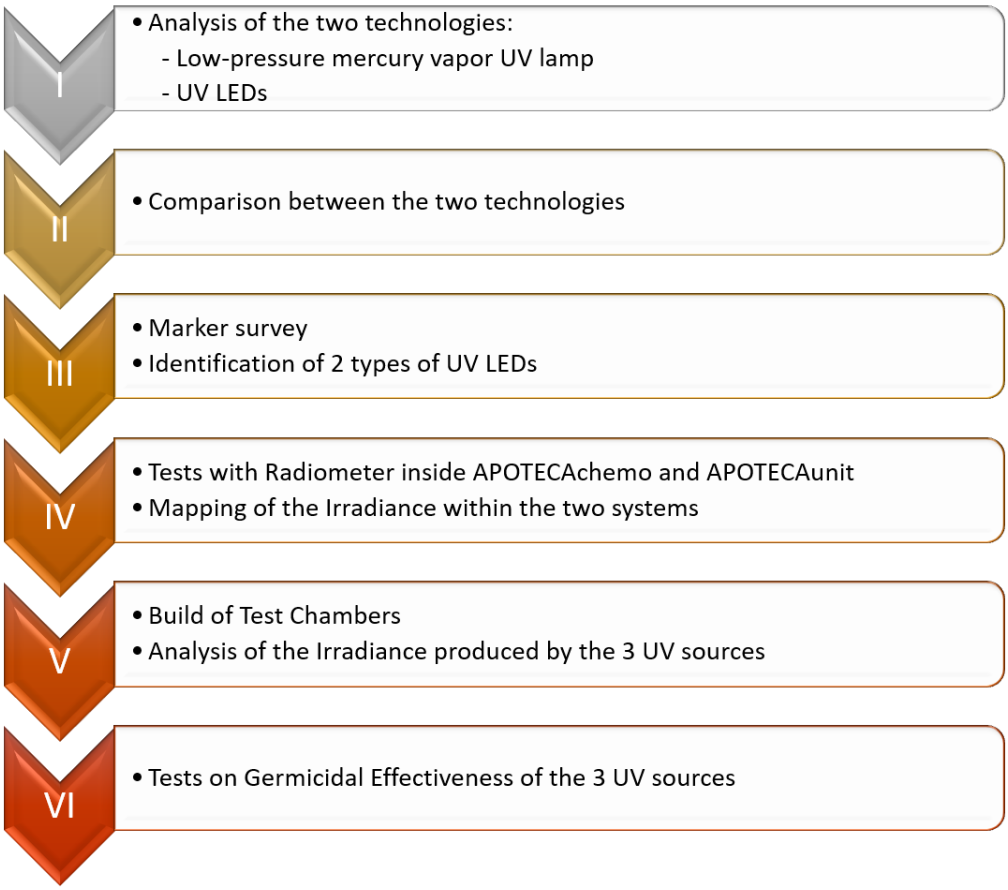

Figure 3.1 *Work steps.*

3.1 Ultraviolet low-pressure lamps

The two most common types of UV lamps are high intensity discharge (HID) lamps (also called high pressure or medium pressure mercury vapor lamps) and low-pressure mercury vapor lamps. UV lamps are often called mercury or "amalgam" lamps because they contain solid amalgam "spots" that controls the mercury vapor

pressure. In addition to mercury, UV lamps also contain a starter gas, typically argon.

In particular, low-pressure UV lamps contain mercury gas at pressures of about 10 torr or less, and when this gas is stimulated by an electrical charge, it emits UV light in a narrow band of wavelengths centred around 254 nm (253,7 nm to be exact).

Low pressure lamps consist of an envelope made of quartz glass or other UV-transmitted glass, a pair of electrodes, and a mercury amalgam (an amalgam is an alloy of mercury with another element, such as indium or gallium). Ballasts are required to provide the necessary starting voltage across the electrodes and to provide the proper lamp current. The electric current that passes between the electrodes heats up the mercury vapor which stimulates electronic transitions and causes emission of ultraviolet light. In low pressure lamps about 60% of the electrical input power is converted to light of which about 85% occurs near 254 nm (Figure 3.2). The overall efficiencies of low-pressure lamps tend to be about 30–31% [5].

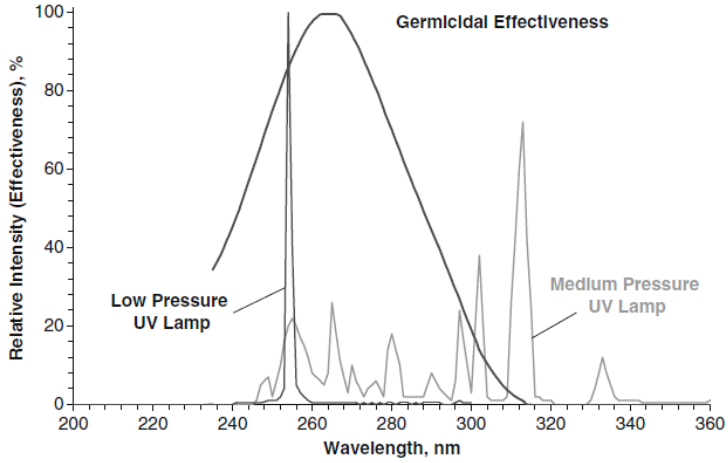

Figure 3.2 *Spectrum of low-pressure UV mercury-vapor lamps compared with germicidal effectiveness. In light grey can be observed also the spectrum of medium-pressure UV lamp.*

For what concerns the daily sterilization procedure, APOTECA systems mount ultraviolet low-pressure lamps that run at temperatures around 45°C and operate with an output at 254 nm (Figure 3.3).

Figure 3.3 *Low-pressure UV lamp, model: HNS S 9 W G23, manufacturer: OSRAM.*

3.2 Ultraviolet light-emitting diodes

Ultraviolet light emitting diodes are small devices that emit UV-C light to destroy dangerous pathogens in water, surface, and air. Due to their small size, UVC LEDs can be easily integrated into a wide range of products - such as water purification systems, medical devices, air purifiers, and more - to provide effective disinfection [6].

Ultraviolet light emitting diodes are compact light sources that come in a variety of shapes, including tiny bulb shapes, hemispherical, and flat chips.

More specifically, the LED is a p-n junction semiconductor lamp that emits radiation when biased in a forward direction. Two semiconductor materials are used to create the junction: one having an excess of electrons (negative or n-type material), and one having a shortage of electrons (positive or p-type material).

Although LEDs are relatively low power (i.e. about 100 mW), they can be installed in larger arrays to produce power levels suitable for disinfection. LEDs can be modelled as point sources for analytical purposes. One of the main advantages of UV LEDs is that they can produce UV at the optimum wavelength (about 265 nm) for germicidal effectiveness (Figure 3.4) [5].

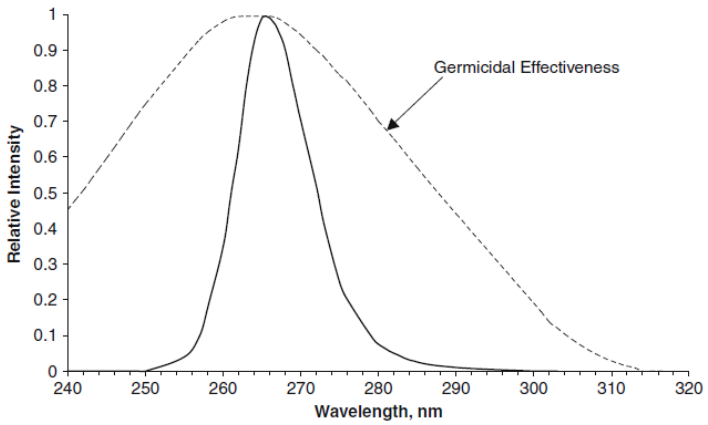

Figure 3.4 *Spectrum of UV LED compared with germicidal effectiveness.*

3.3 Comparison between UV low-pressure lamps and UV LEDs

Even though their application is the same, namely that both solutions are used for sterilization purposes, the low-pressure UV mercury-vapor lamps and UV LEDs are two technologies very different from each other.

The first solution, the UV mercury-vapor lamps, represents the traditional solution because it is the oldest technology and therefore it is the most diffused choice when talking about UV sterilization. However, with the passing of the years, this technology will almost certainly be replaced by LED technology, as is happening for the lamps in the visible spectrum. It is enough to think to how the white-light lamps in our houses (those we use daily for lighting) have been replaced in recent years by the LED technology. Very likely the traditional UV lamps will have the same fate.

UV-C LEDs perform the same functions of conventional mercury-vapor lamps but have many benefits in comparison. First of all, UV LEDs are smaller and do not require an electronic ballast for starting, therefore they may be located where there is not enough space to guarantee the regular installation of UV lamps. The dimensions of one single LED are about few millimetres (3-5 mm) while for a traditional UV lamp

are required several centimetres (15-20 cm). For this reason, the use of one LED allows to reach even the most hidden points in such a way to guarantee the right level of illumination/irradiation also in areas with complicated geometries.

UV-C LEDs are more environmentally friendly with respect the UV mercury-vapor lamps. The latter, in fact, use heavy metals (mercury) that are difficult to handle and cost a great deal to dispose them safely. Even if in the most recently developed lamps the mercury is present in small quantities, they however require special attention at disposal because mercury is considered a hazardous waste. In this context it was concluded the Minamata Convention [7]. This convention treats a series of guidelines about the reduction in the usage of mercury with the purpose of protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds. The Minamata Convention provides controls over a myriad of products containing mercury, the manufacture, import and export of which will be altogether prohibited by 2020. These products include certain types of batteries, compact fluorescent lamps, relays, soaps and cosmetics, thermometers, and blood pressure devices. This convention contains, in support of its objective, provisions that relate to the entire life cycle of mercury, including controls and reductions across a range of products, processes and industries where mercury is used, released or emitted. Also due to this convention, mercury will be used less and less and the mercury-vapor lamps with it. Therefore, the transition to LED technology will be natural, if not mandatory.

Another advantage of the LED technology is that it is characterized by an instantaneous switch-ON and an instantaneous switch-OFF. It means that LEDs can start working immediately and they have no need for a warm-up time. On the contrary, the traditional UV mercury-vapor lamps require several minutes to start working at full capacity and therefore can take up to 10 minutes of warming-up. This can represent a critical constraint especially in that operations of sanitization that cannot take a lot of time and must be quickly carried out. Therefore in such situations can be preferable to opt for a technology with an instantaneous on/off.

This possibility to turn ON and turn OFF instantaneously the light emitting diode allows to work not only with a continuous illumination but also with a pulsed illumination at different frequencies. This option of unlimited cycling allows to reduce consumptions and at the same time does not impact the life of the LEDs. Moreover, the lifetime of a LED is considerably longer than the lifetime of a traditional UV lamp. In particular, UV-C LEDs have a lifetime 5 times higher compared to traditional mercury-vapor lamps: a low-pressure mercury vapor UV lamp has a mean lifetime of 10,000 hours while a UV LED can reach also 50,000 hours of irradiation. This longer lifetime of the product makes it possible to save on costs necessary for the replacing of the lamps. In practical terms, with the same working time, the cost of LEDs could be compared to the costs of 5 lamps. In the long term, in addition to the lower energy consumption ensured by the LED technology, such reasoning could then guarantee a remarkable saving in terms of costs [8].

Another of the great benefits of the LED technology is the possibility to select the wavelength. LEDs in fact can be configured to choose a specific wavelength that is best suited for maximum absorption of light for the chosen microorganism. As we will see later, for sterilization purposes, each microorganism reacts differently at a specific wavelength. Therefore, the possibility to act at specific wavelengths allows to work at optimal germicidal efficiency.

Despite all these advantages in favour of the LED technology (Figure 3.5), there are still a couple of features on which the traditional lamp behaves better than LED. In particular, a LED has a lower power output with respect to a mercury-vapor lamp: the power output of a UV LED is some milliwatts (10-150 mW) while the power output of the traditional UV lamp is in the order of some watts (2-10 W). Obviously should be considered also the reduced dimensions and the lower energy consumption of a LED but, in terms of power output the comparison of one lamp with one LED is clearly in favour of the low-pressure UV lamp. To reach a power output which can be compared with the one obtained from a traditional lamp should be utilized more LEDs at the same time (an array of LEDs) but utilizing this solution the overall benefit of the energy

saving is reduced. This trade-off between power output and energy consumption can therefore represents one of the main limits of this technology.

Finally, due to the fact that the UV-C LED technology can be considered as a technology that is still under development, the cost of this solution is higher than that of UV traditional lamp. The single price of a LED can be indeed higher than the price of a single UV lamp. However, it is not easy to make such kind of consideration because, as said previously, the lifetime of a LED is much longer than that of a traditional lamp. All these parameters should be taken into account in order to decide, according to the final aim, which ones are the most important.

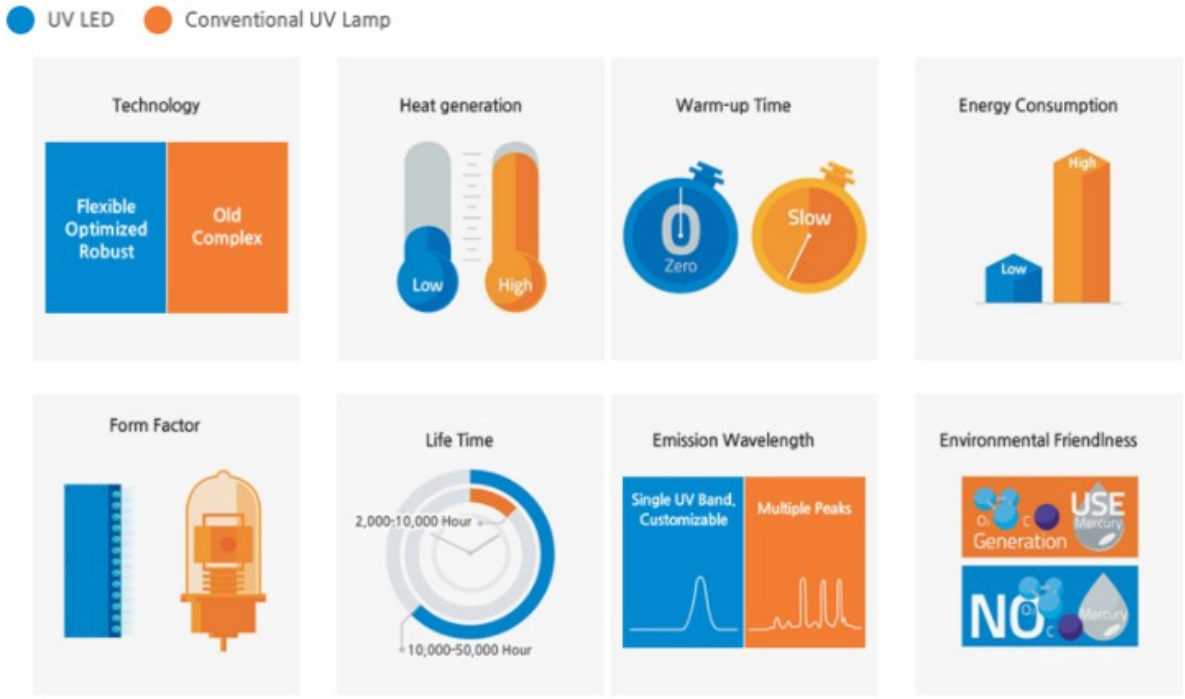

Figure 3.5 *Benefits of UV LED technology [16].*

3.4 Ultraviolet germicidal effect

The principal aspect of major interest, in terms of sterilization, about the comparison between the traditional UV-C mercury-vapor lamp and the innovative UV-C LED is the germicidal effectiveness. Studies have demonstrated that the UV-C efficiency against pathogens (i.e. viruses, bacteria, fungi) of the UV-C LEDs is comparable to the

one of the traditional mercury-vapor lamps. In some instances, thanks to the different wavelength of emission, UV-C LEDs (that emit at wavelengths up to 280nm) seem to be more effective in disinfection procedures with respect to the traditional lamps (that emit in the wavelength of 254nm) (Figure 3.6).

This result can be explained by the fact that some microorganisms seem to be more affected by UV light at higher wavelengths. However, each microorganism is more sensible at a specific wavelength. For this reason the wavelength at which the maximum germicidal efficacy can be observed varies depending on the type of microorganism.

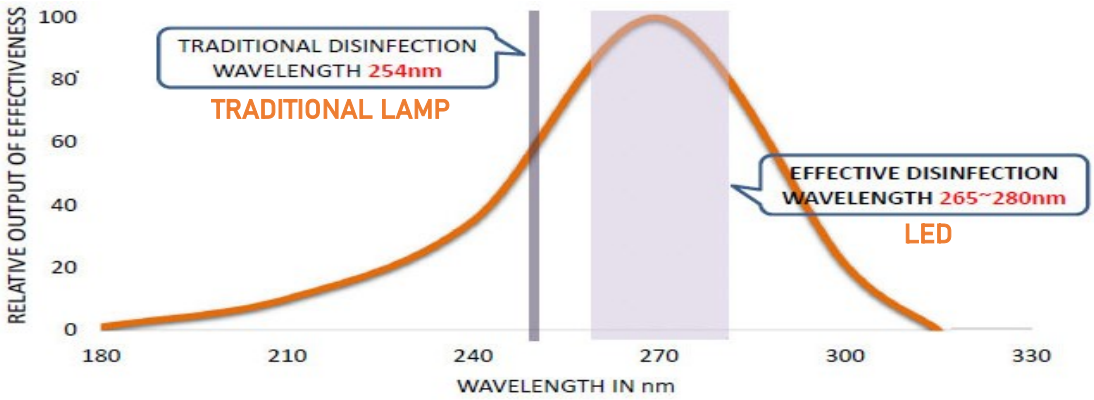

Figure 3.6 *Absorption spectra of microorganisms (orange line) in relation to the wavelength of emission of the traditional UV lamp and the UV LED.*

As can be seen in Figure 3.6, the emission of radiation at a wavelength of 254 nm from the UV-lamps intersects the absorption spectrum of the microorganism below its peak absorption. This means that low-pressure mercury lamps are not optimized for efficient DNA inactivation. This can be explained by the fact that, although mercury-based lamp systems are useful for disinfection, their outputs are fixed at specific wavelengths by the reaction mechanisms of mercury and therefore they are not able to provide the maximum efficacy in terms of germicidal power.

On the other hand, the continuous spectral response of UV-C LEDs, with wavelengths that go from 265nm to 280 nm, is largely within the desired UV-C range. This range, that corresponds to the maximum absorption of radiation by

microorganisms, allows for a more effective germicidal power [9].

This means that, on equal terms, microorganisms exposed to a radiation at higher wavelengths require a smaller amount of radiation than the same microorganisms exposed to a radiation at lower wavelengths.

The definitions mentioned above of "on equal terms", that means to obtain the same level of reduction of the same microorganism, and "amount of radiation" are concepts that require a further examination and for this reason they will be clarified in the subsequent paragraphs.

3.5 Exposure Dose

The concept of "amount of radiation" is linked to the exposure dose. The exposure dose represents the radiant exposure ($\frac{J}{m^2}$, unweighted) incident on biologically relevant surface [10].

The UV dose is given by the product of UV irradiance and exposure time on a given microorganism or surface, typically reported in millijoules per square centimetre ($\frac{mJ}{cm^2}$) or joules per square meter ($\frac{J}{m^2}$). In formulas:

$$Dose = I * \Delta t \quad \left[\frac{J}{m^2}\right]$$

The irradiance is the amount of energy received by microorganisms or a surface and it is specifically defined as the power of incident electromagnetic radiation on a surface ($\frac{W}{m^2}$). The exposure time is measured in seconds (s).

Germicidal efficacy depends on the exposure dose (UV dose). In particular, increasing the UV dose, so increasing the irradiance or the exposure time, the germicidal efficacy of a UV source increase.

Microbial populations decay exponentially under UV exposure. The slope of the logarithmic decay curve is defined by the rate constant, which is designated as *k*. The

UV rate constant k has units of $\frac{m^2}{J}$, and is also known as the UV susceptibility.

The primary model used to evaluate the survival of microorganisms subject to UV exposure is the classical exponential decay model. Figure 3.8 shows a first-order decay rate model.

In other situations, can be observed that a tiny fraction of the microbial population exhibits a higher level of resistance to UV irradiation. Most microbial populations, in fact, behave as if two separate populations were present, one relatively susceptible and one relatively resistant, resulting in a two-stage decay (Figure 3.7) [5].

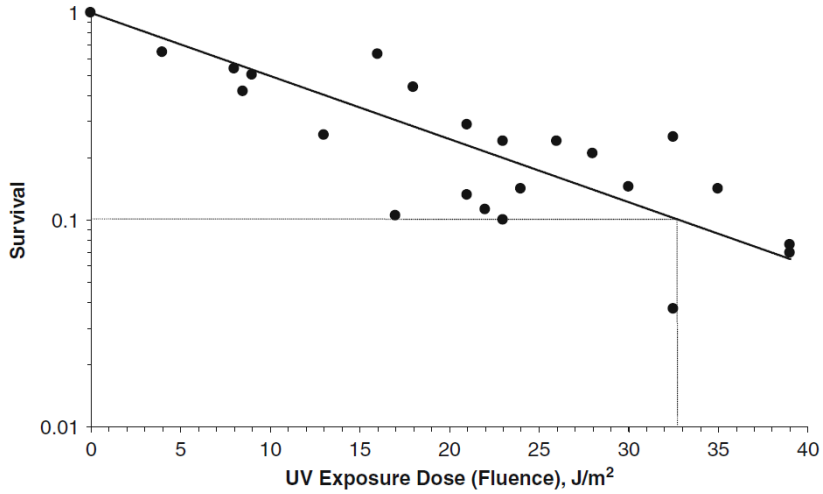

Figure 3.7 *Example of first-order decay. Line is curve fit of the indicated exponential decay equation.*

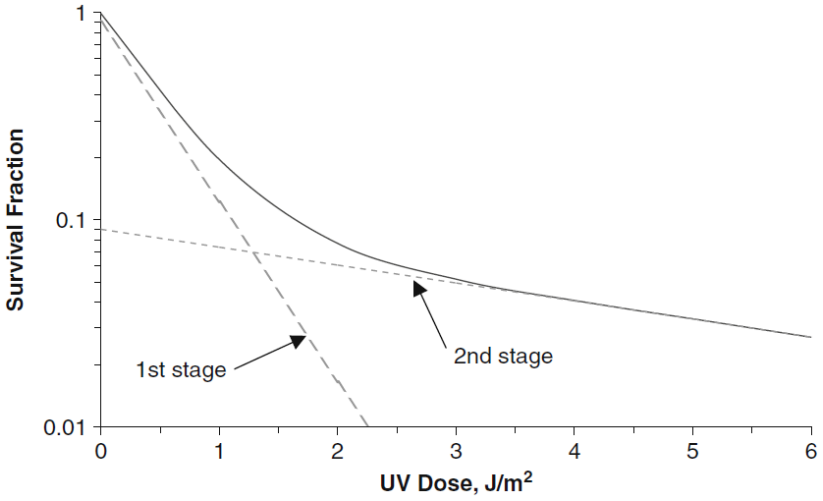

Figure 3.8 *Example of two stage decay curve.*

Microbial susceptibility to ultraviolet light varies widely between species of microbes. Bacteria, viruses, and fungal spores respond to UV exposure at rates defined in terms of UV rate constants. For constant and uniform irradiance, the germicidal effect of UV-C energy on a single microorganism population can be expressed as follows [10]:

$$\frac{N_t}{N_0} = e^{(-k * E_{ff} * \Delta t)} = e^{(-k * Dose)}$$

where

N_0 = initial number of microorganisms;

N_t = number of microorganisms after any time Δt;

$\frac{N_t}{N_0}$ = fraction of microorganisms surviving;

k = microorganism-dependent rate constant, $\frac{cm^2}{\mu W \cdot s}$;

E_{ff} = effective (germicidal) irradiance received by microorganism, $\frac{\mu W}{cm^2}$.

This equation describes an exponential decay in the number of living organisms as a constant level of UV-C exposure continues.

3.6 Log Reduction

When designing disinfection systems, one of the starting requirements is selecting the targeted reduction level of a specific microbe or, more specifically, reduction of colony forming units (CFU) of the targeted microbe.

For practical purposes in fact, it would be too time consuming, complex and expensive to use a microscope and count every individual microbial cell of a sample. Instead, by diluting a sample and spreading this across a petri plate, microbiologists can count groups of microbes, called colonies. Each colony is assumed to have grown from a single CFU.

Similarly, when calculating and reporting the changes in CFUs after disinfection,

rather than state the magnitude of change in individual CFUs, microbiologists express the performance as a percentage reduction in terms of a reduction factor and, for convenience, typically in factors of 10 using a logarithmic (log) reduction scale – a log reduction factor (LRV).

Log reduction is a mathematical term that is used to express the relative number of living microbes that are eliminated by disinfection [11].

$$Log\ reduction\ =\ \log_{10}(\frac{N_0}{N})$$

Where:

N_0 = colony forming units of the microorganisms before exposure to UV light;

N = colony forming units of the microorganisms after exposure to UV light.

In practice, a 1-log reduction corresponds to inactivating 90 percent of a target microbe with the microbe count being reduced by a factor of 10. Thus, a 2-log reduction will see a 99 percent reduction, or microbe reduction by a factor of 100, and so on (Table 3-1).

Table 3-1 Correlation between Log Reduction, Reduction Factor and percentage reduction.

LOG REDUCTION	REDUCTION FACTOR	PERCENT REDUCED
1	10	90%
2	100	99%
3	1,000	99.9%
4	10,000	99.99%
5	100,000	99.999%
6	1,000,000	99.9999%

Effective disinfection systems achieve the desired log reduction factor by ensuring that the process delivers a microbe specific UV-C dose.

As said above, every microorganism, according to its biological characteristics, has a unique spectral sensitivity "fingerprint". Depending on the UV-C wavelength range being used, each microbe will require a different amount of energy to be inactivated. Therefore, by selecting proper UV-C wavelengths and greater doses of energy, the amount of disinfection (i.e. LRV of the microbe) can be improved.

For these reasons, the most accurate method for an effective germicidal inactivation would involve first knowing the specific microorganism to be inactivated in order to choose the precise wavelength range of the microbe's action spectrum.

However, regardless of the precise wavelength, can be seen that higher wavelength (i.e. the ones in the LEDs range) require less dose to achieve the same inactivation level and are therefore more efficient (Table 3-2 and Table 3-3). Microorganisms selected for this analysis have been chosen by relying on previous studies and because they represent the most frequently detected source of microbiological contamination in healthcare settings [12].

Table 3-2 Dose required to achieve a specific Log-reduction for UV low-pressure mercury vapor lamp.

		UV DOSE ($^{mJ}/_{cm^2}$)			
Microorganism	**Wavelength**	**1**	**2**	**3**	**4**
Pseudomonas aeruginosa	254 nm	3.8	6.5	10	17
Staphylococcus aureus	254 nm	3.9	5.4	6.5	10.4
Bacillus subtilis spores	254 nm	24	35	47	79

Table 3-3 Dose required to achieve a specific Log-reduction for UV-C LED.

		UV DOSE ($^{mJ}/_{cm^2}$)			
Microorganism	**Wavelength**	**1**	**2**	**3**	**4**
Pseudomonas aeruginosa	280 nm	-	-	-	8.5
Staphylococcus aureus	256 nm	-	-	-	12.1
Bacillus subtilis spores	282 nm	3	11	18	26

3.7 Market Survey

Once obtained enough information about the ultraviolet radiation and about the principal characteristics of the UV lamps/UV LEDs linked to the sterilization process, the attention was focused on the new LED technology. In particular, was carried out a market survey to find which solutions are nowadays available in commerce in such a way to be able to make an exact comparison with the actual low-pressure mercury vapor technology.

The main goal of this survey was to collect several information of the LED technology such as costs, dimensions, power, wavelength of emission. In this way was possible to select two types of LEDs that best suit the purpose of this study and therefore to proceed with a direct comparison with the traditional UV lamp.

In order to have the exact terms of comparison, in Table 3-4 are summarized the specific characteristics of the UV lamp currently utilized in APOTECAchemo and in APOTECAunit (Figure 3.3).

Table 3-4 Characteristics of the low-pressure mercury vapor UV lamp currently installed in APOTECAchemo and APOTEC unit.

Manufacturer	Model	Wavelength	Size	Radiated Power	Power Dissipation	Price
OSRAM	HNS S 9 W G23	254nm	165.5mm length 28.00mm diameter	2.5W	9.00W	€15,26

The principal solutions found with the market research for UV-C LED technology are listed in Table 3-5. This market survey was carried out on-line both on search engine and on official search channel internal to the company looking in particular for LEDs with emission wavelength in the UV-C range (specifically in the range 260-280nm). Another parameter to which attention has been paid is the power output trying to avoid LEDs with too low emission power.

Table 3-5 Characteristics of UV-C LEDs found with the market survey (the items that do not have the price were not were not immediately available or their purchase was bound by a minimum number of pieces).

Manufacturer	Model	Wavelength	Size	Radiated Power	Power Dissipation	Price
Crystal IS	KL265-50V-SM-WD	260nm~270nm	3.50mm*3.50mm	70mW	4W~4.5W	€21,88
Crystal IS	KL265-50U-SM-WD	260nm~270nm	3.50mm*3.50mm	60mW	4W~4.5W	€18,76
Inolux	IN-C39BTKU1	270~285nm	3.9*3.9*2.6mm	8~16mW	1.35W	€25.49
Inolux	IN-C39ATKU1	270~285nm	3.9*3.9*3.2mm	8~16mW	1.35W	€36.34
Lextar	PU35CM1 V0	270nm~283nm	3.50mm*3.50mm	15mW	0.57W	€3,98
LITE-ON	LTPL-G35UVC275GH	270nm~280nm	3.45mm*3.45mm	72mW	5.2W	€39,00
LITE-ON	LTPL-G35UVC275GZ	270nm~280nm	3.45mm*3.45mm	47mW	3.5W	€16,71
Lite-On	LTPL-G35UV275GR-E	270~285nm	3.45mm*3.45mm	17.5mW	1.6W	€6,28
Lite-On	LTPL-G35UV275GC-E	270~285nm	3.45mm*3.45mm	10mW	1.2W	€3,81
Luminus	LST1-01G07-UV01-01	275~286nm	----------	45mW	2.26W	€39.89
Luminus Devices	XST-3535-UV-A60-CE280-01	280~285nm	3.65mm*3.65mm	40~45mW	2.63W	€30.14
Luminus Devices	XFM-5050-UV3-A130-FA275-00	275nm~285nm	5.0 mm*5.0 mm	135mW	7.9W	---
NICHIA	NCSU334B	275nm~285nm	6.80mm*6.80mm	70mW	3.49W	---
Stanley Electric	LJU1106EAE-275	270nm~280nm	3.50mm*3.50mm	70mW	4.6W	---
Stanley Electric	ZEUBE265-2CA	259nm~269nm	3.50mm*3.50mm	50mW	4.6W	---

The two types of LEDs selected for the comparison with the UV low-pressure mercury vapor lamp are:

- Crystal IS, KL265-50V-SM-WD (Figure 3.10);
- Luminus, LST1-01G07-UV01-01 (Figure 3.9).

Figure 3.10 *Crystal IS, KL265-50V-SM-WD.*

Figure 3.9 *Luminus, LST1-01G07-UV01-01.*

These two types of LEDs have been chosen taking into consoderation different characteristics. The first parameters taken into account was the radiated power: in particular were chosen a LED with a relatively high power output (70mW, Crystal IS) and a LED with a mid-range power (45mW, Luminus). LEDs with too low power output have been discarded to avoid the need of an excessive number of pieces for comparison. Other parameters considered are the power efficiency and the price. In detail, the efficiency in terms of radiated power compared with the power dissipation have been analysed; LEDs have been preferred, since having the same power output, they require a smaller amount of input power. With a similar reasoning, were preferred LEDs that for equal radiated power have a lower price.

As it can be seen from Table 3-4 and Table 3-5, and as expected, the mean wavelength of emission is higher for the LED technology with respect to the traditional UV mercury lamps. This represents certainly an advantage because all the UV-C LEDs have the wavelength that falls exactly in the range of maximum absorption for microorganism DNA. This helps in reducing the quantity of dose to be delivered to reach a particular Log-reduction (i.e. 4-Log reduction).

Another advantage that can be seen from these tables is represented by the dimensions. In fact, the dimensions of one single LED are significantly lower than that of a traditional mercury vapor lamp (Figure 3.11). This helps in positioning the LEDs in places where a traditional lamp cannot be placed. In this way, with the application of LEDs at critical points, it is possible to illuminate even the most hidden areas.

Finally, another important parameter for the comparison between the two technologies is represented by the power. In particular, the LED technology is characterized by a smaller power dissipation, but at the same time is able to provide a much lower power output.

Figure 3.11 *Comparison of dimensions between UV lamp and UV LED.*

This aspect, together with the cost, is possibly the key-parameter for the comparison of the two technologies. For the purpose of this thesis, in fact, the characteristic that matters most is the germicidal effectiveness and this one is strictly linked to the power output (in turn linked to irradiance).

In practical terms, considering that the recommended exposure time suggested by the company is 4h, the dose delivered by the lamps is:

$$irradiance \cdot time = dose$$

$$0.25\frac{W}{m^2} \cdot 4h \cdot 3600\frac{sec}{h} = 3600\frac{J}{m^2}$$

$3600\frac{J}{m^2}$ is the dose for 2.5 W mercury vapor lamp, considering an irradiance of $0.25\frac{W}{m^2}$.

$$0.007\frac{W}{m^2} \cdot 4h \cdot 3600\frac{sec}{h} = 100.8\frac{J}{m^2}$$

$100.8\frac{J}{m^2}$ is the dose for 70mW LED lamp, considering an irradiance of $0.007\frac{W}{m^2}$.

$$0.0045\frac{W}{m^2} \cdot 4h \cdot 3600\frac{sec}{h} = 64.8\frac{J}{m^2}$$

$64.8\frac{J}{m^2}$ is the dose for 45mW LED lamp, considering an irradiance of $0.0045\frac{W}{m^2}$. Irradiance has been calculated based on previous studies [13].

In order to make a more detailed comparison, have been analysed the characteristics of the 3 UV sources (2.5W UV mercury-vapor lamp, 70mW UV LED and 45mW UV LED) by relying on the dose necessary to reach the 4-Log reduction after an ideal irradiation of 4 hours. This analysis has been repeated for the three different microorganisms listed above (see section 3.6).

A) For 4 Log-reduction of Bacillus subtilis spores at a wavelength of 254nm it is needed a dose of $790\frac{J}{m^2}$ while for 4 Log-reduction of Bacillus subtilis spores at a wavelength of 282nm it is needed a dose of $260\frac{J}{m^2}$.

   1) Considering that the dose delivered by the 2.5W UV mercury-vapor lamp is $3600\frac{J}{m^2}$, this last is sufficient to reach the 4-Log reduction or rather, the dose obtained in this way is 4.5 times higher than that required for 4 Log-reduction. So it is enough one single lamp to reach a 4-Log reduction, with a length of 165.5mm, a price of €15.26 and a power dissipation equal to 9W.
   2) Considering that the dose delivered by the 70mW UV LED is $100.8\frac{J}{m^2}$, to reach a 4 Log-reduction of Bacillus subtilis spores with a wavelength of 282nm are necessary 3 LEDs with a total dose delivered of $302.4\frac{J}{m^2}$. In this case, the total power dissipation is equal to 13.5W, the total length (considering 3 LEDs in line) is equal to 10.5mm and a total price of € 65.64.
   3) Considering that the dose delivered by the 45mW UV LED is $64.8\frac{J}{m^2}$, to reach a 4 Log-reduction of Bacillus subtilis spores with a wavelength of 282nm are necessary 5 LEDs with a total dose delivered of $324\frac{J}{m^2}$. In this case, the total power dissipation is equal to 11.3W, the total length (considering 5 LEDs in line) is equal to 10cm and a total price of € 199.45.

B) For 4 Log-reduction of Staphylococcus aureus at a wavelength of 254nm it is

needed a dose of 104 $\frac{J}{m^2}$ while for 4 Log-reduction of Staphylococcus aureus at a wavelength of 256nm it is needed a dose of 121 $\frac{J}{m^2}$. In this case the wavelength of emission is quite similar for both UV mercury-vapor lamp and UV LED and in fact the dose necessary for a 4 Log-reduction is almost the same.

1) Considering that the dose delivered by the 2.5W UV mercury-vapor lamp is 3600 $\frac{J}{m^2}$, this last is sufficient to reach the 4-Log reduction or rather, the dose obtained in this way is 34 times higher than that required for 4 Log-reduction. So it is enough one single lamp to reach a 4-Log reduction, with a length of 165.5mm, a price of €15.26 and a power dissipation equal to 9W.
2) Considering that the dose delivered by the 70mW UV LED is 100.8 $\frac{J}{m^2}$, to reach a 4 Log-reduction of Staphylococcus aureus with a wavelength of 256nm are necessary 2 LEDs with a total dose delivered of 201.6 $\frac{J}{m^2}$. In this case, the total power dissipation is equal to 9W, the total length (considering 2 LEDs in line) is equal to 7mm and a total price of € 43.76.
3) Considering that the dose delivered by the 45mW UV LED is 64.8 $\frac{J}{m^2}$, to reach a 4 Log-reduction of Staphylococcus aureus with a wavelength of 256nm are necessary 2 LEDs with a total dose delivered of 129.6 $\frac{J}{m^2}$. In this case, the total power dissipation is equal to 4.52W, the total length (considering 2 LEDs in line) is equal to 4cm and a total price of € 79.78.

C) For 4 Log-reduction of Pseudomonas aeruginosa at a wavelength of 254nm it is needed a dose of 170 $\frac{J}{m^2}$ while for 4 Log-reduction of Pseudomonas aeruginosa at a wavelength of 280nm we need a dose of 85 $\frac{J}{m^2}$.

1) Considering that the dose delivered by the 2.5W UV mercury-vapor lamp is 3600 $\frac{J}{m^2}$, this last is sufficient to reach the 4-Log reduction or rather, the dose obtained in this way is 21 times higher than that required for 4 Log-reduction. So it is enough one single lamp to reach a 4-Log reduction, with a length of 165.5mm, a price of €15.26 and a power dissipation equal to 9W.

2) Considering that the dose delivered by the 70mW UV LED is $100.8\frac{J}{m^2}$, to reach a 4 Log-reduction of Pseudomonas aeruginosa with a wavelength of 280nm one single LED is enough. In this case, the total power dissipation is equal to 4.5W, the total length is equal to 3.5mm and a total price of € 21.88.
3) Considering that the dose delivered by the 45mW UV LED is $64.8\frac{J}{m^2}$, to reach a 4 Log-reduction of Pseudomonas aeruginosa with a wavelength of 280nm are necessary 2 LEDs with a total dose delivered of $129.6\frac{J}{m^2}$. In this case, the total power dissipation is equal to 4.52W, the total length (considering 2 LEDs in line) is equal to 4cm and a total price of € 79.78.

It is necessary to specify that all these considerations are ideal. In practice it should be taken into account the real radiation incident on the surface while here it has been made the assumption that the radiation incident on the surface is equal to the total power output. In reality, it should be considered factors such as reflection, scattering, distance that in some way can alter the effective irradiance measured on the surface with respect to the power output emitted from the lamp.

On the basis of these analyses and, to continue with the subsequent tests, it was decided to proceed with the purchase of:

- n° 5 of 70mW LEDs (Crystal IS, KL265-50V-SM-WD) at a total cost of € 109.40;
- n° 8 of 45mW LEDs (Luminus, LST1-01G07-UV01-01) at a total cost of € 319,12.

3.8 Irradiance distribution tests on APOTECA Systems

The second step of this work includes some tests on the systems APOTECAchemo and APOTECAunit for the evaluation of the irradiance produced by UV lamps. This test arises from the need to assess how ultraviolet radiation is distributed within the systems in such a way as to be able to understand which areas within the systems are less exposed to UV radiation and consequently understand whether these areas can

possibly be covered with the use of LEDs.

These tests were carried out through the use of the photo-radiometer HD2102.2 (Figure 3.12) together with the probe LP 471 UVC (Figure 3.13).

This irradiance evaluation test was carried out in the APOTECAchemo and repeated also in the APOTECAunit.

In details, once that the UV panels have been positioned, the LP 471 UVC probe for irradiation measurement was connected to the photo-radiometer HD2102.2. After that, the photo-radiometer was turned on for 15 minutes by setting the measurement interval at 30 seconds and the probe was positioned inside the system.

Figure 3.12 *Photo-radiometer HD2102.2.*

Figure 3.13 *Probe LP 471 UVC.*

This first test inside the system is needed to verify the turning-on timing of UV lamps. In particular, it should be assessed how long the lamps take to enter into full operation. In this way it will be possible to know exactly how much time is needed to the irradiance to settle on the maximum values. By doing this, is it possible to know how much time it is necessary to wait before making the actual measurement.

From this first measurement, have been seen that the lamps take about 6 minutes to enter into full operation. So, all the other measurements were taken for 7 minutes by setting the interval of measurements at 1 second. Have been taken into account only

the values recorded between the sixth and seventh minute. Finally, it has been made the average of these sixty values in such a way to obtain a mean value to evaluate the irradiation.

To perform this test the probe was placed in different points within the two systems while the UV lamps were turned on. In detail, have been analysed 65 points in the APOTECAchemo and 73 points in the APOTECAunit divided as in Table 3-6.

In Figure 3.14 and Figure3.15 are showed two example of probe position for the test.

Table 3-6 Positions of the acquisitions in APOTECAunit and APOTECAchemo.

APOTECAunit		**APOTECAchemo**	
Acquisition Zone	**Number of Acquisitions**	**Acquisition Zone**	**Number of Acquisitions**
Loading Area	**11**	Loading Area	**11**
Warehouse Area	**25**	Warehouse Area	**17**
Preparation Area	**37**	Preparation Area	**37**

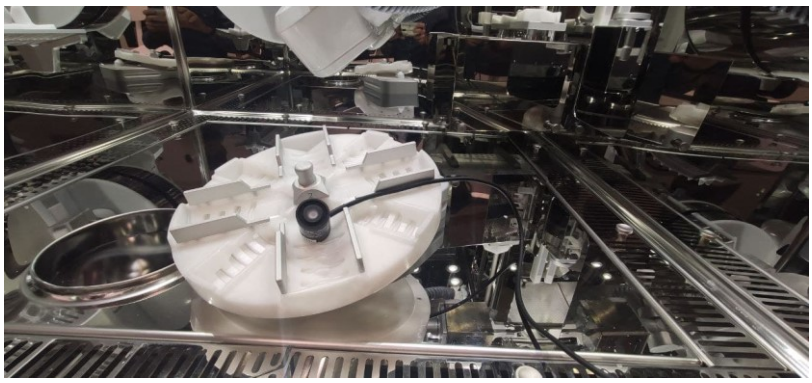

Figure 3.14 *Probe placed on the shaker (Preparation Area).*

Figure 3.15 *Probe placed on the carousel (Warehouse Area).*

The detailed procedure containing also all the description of the positions in which the irradiance has been measured is described in the protocol in Appendix 7.1.

3.9 Test Chamber

Tests made on the APOTECAchemo and on the APOTECAunit allowed to understand which level of effective UV irradiation can be guaranteed by the low-pressure mercury vapor lamps. Therefore, in order to make a comparison with the LED technology, are needed the same information of irradiance produced by LEDs.

Given that the substitution of the UV conventional lamps with the LEDs directly into the systems is not a feasible solution because it would be time consuming and too expensive, to have a practical solution that allows to obtain comparable information about the irradiance produced by the UV lamp and the LEDs, it has been decided to

build up a chamber test. In this test chamber have been mounted alternately both the UV lamps and the LEDs.

Using this chamber, was then evaluated, under the same conditions, the irradiance produced by the three UV sources; in this way it was possible to collect uniform information and therefore make a comparison.

The test chamber has the form of a hollow cylinder with a diameter of 30cm and 50cm high and is opaque black in colour in such a way to simulate as closely as possible an ideal irradiation condition (Figure 3.16). In this chamber test, the lamps or the LEDs are positioned at the top of the cylinder while in the lower base of the cylinder is situated the probe of the radiometer for the recording of irradiation. The chamber was designed to be easily replicated, if necessary, and to be easy to use especially considering that in a second step it will be used by untrained personnel.

The diameter of 30cm guarantees space for the positioning of the probe and for the positioning of the Petri dishes (used later with the tests for germicidal effectiveness).

The height of 50cm, on the other hand, ensures a quite uniform distribution of the radiation on the lower base so as to cover all the different positions.

The UV lamp and UV LEDs have been mounted on different superior supports in such a way to allow the exclusive use of one or the other solution (Figure 3.17). For this reason, it has been decided to build up three different test chambers: one for each UV radiation source.

The UV lamp was simply encased in a 3D printed box and connected to the power supply (Figure 3.17b). While, with regard to the two typologies of LEDs, the electrical connection was more complicated. It was necessary to use a driver to keep the current constant and different resistors were applied to avoid voltage fluctuations (Figre 3.17a and Figure 3.17c). To facilitate heat dissipation the LEDs were welded in a high temperature furnace and a specific heatsink was applied at the top of the support. All three chambers are switched-on through the use of a socket, without the need for any switch.

In the lower base of the test chamber, a 3D printed guide has been installed to

facilitate positioning of the UV-C probe and Petri dishes (Figure 3.16c).

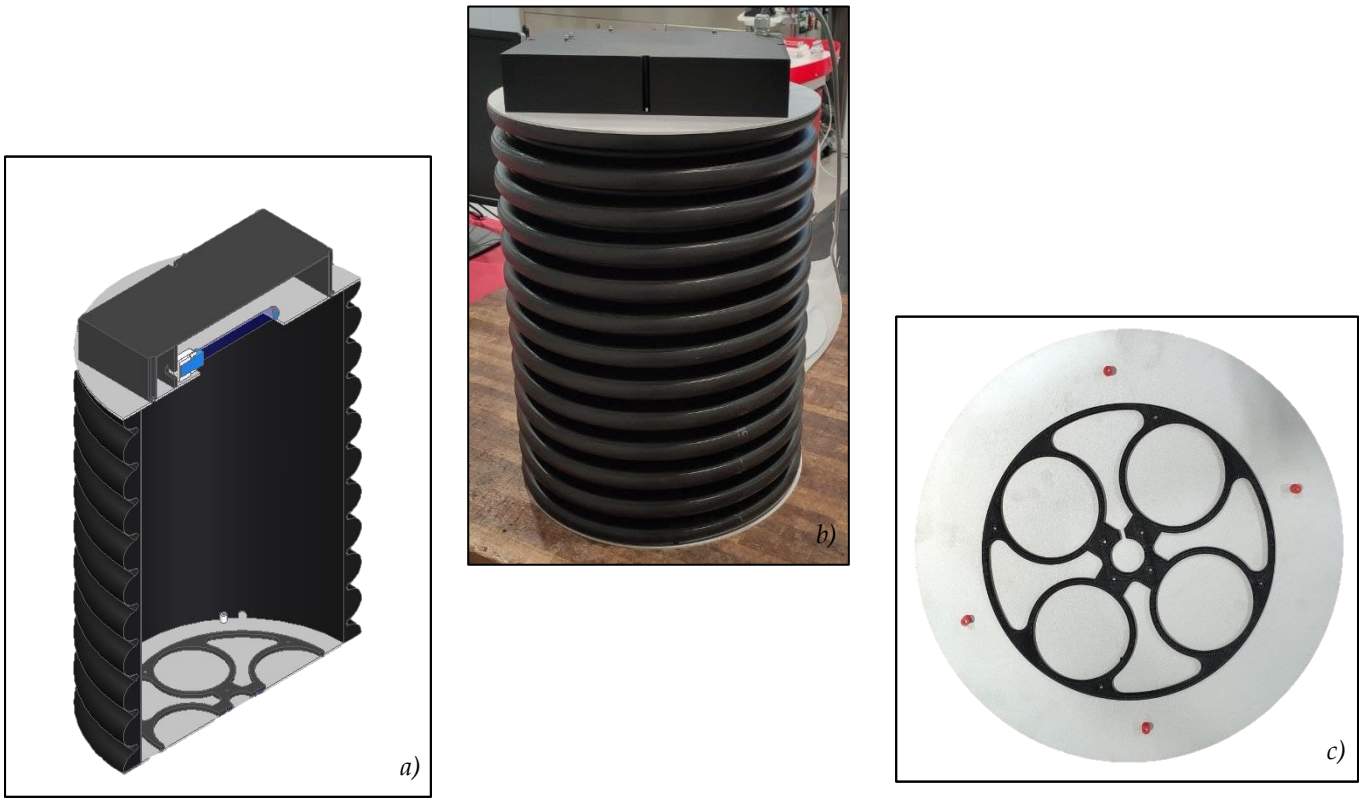

Figure 3.16 *Test chamber: a) Designing section, b) Real test chamber, c) Inferior support.*

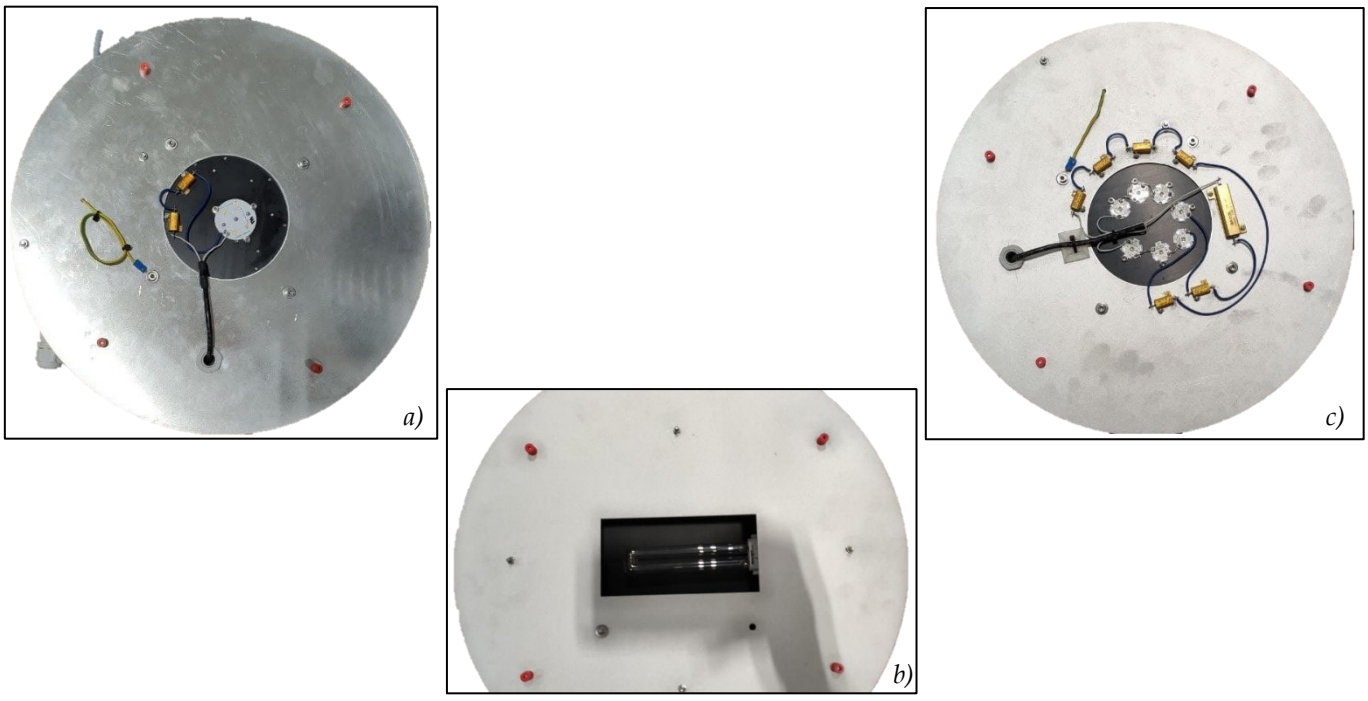

Figure 3.17 *Superior supports: a) 70mW UV LEDs, b) 2.5W UV lamp, c) 45mW UV LED.*

3.10 Germicidal Effectiveness Tests

The last set of tests involve the need to understand the germicidal effectiveness of the three UV sources.

In collaboration with the University of Urbino it has been decided to study microorganisms viability under the UV exposure. The final goal of these tests is indeed the interpretation of the relationship that exists between the dose received by a specific microorganism and its effective Log-reduction.

These tests were carried out at University of Urbino through the use of test chambers previously described (Section 3.9). In particular, the microorganisms were placed on the inferior support together with the UV-C probe and were irradiated by the three different UV sources.

The microorganisms chosen for these tests are the ones reported in Table 3-2; these microorganisms have been chosen because they are among the most common in hospital setting as described also in Bruscolini et al. [14]. In particular, these microorganisms are characterized by a different level of resistance to UV radiation and in this way allow to have a wide range of control on the relationship dose-killing rate. In order to obtain more information, it was decided to analyse also different concentrations for each microorganism. In fact, four concentrations of each microorganism have been analysed: 10^8 CFU/ml, 10^6 CFU/ml, 10^4 CFU/ml and 10^3 CFU/ml (Figure 3.18).

Each microorganism, for all the four concentrations, has been irradiated with 4 different amount of dose. Each dose is related to a certain level of log-reduction. In detail, as can be seen in Appendix 7.2, dose 1 corresponds to a reduction of 2 log, dose 2 to a reduction of 3 log, dose 3 to a reduction of 4 log and dose 4 to a reduction of 6 log. All the dose values necessary to obtain a certain log-reduction were taken from the literature [12].

The dose needed to obtain a certain log-reduction was then calibrated considering the dose actually measured in the position where the plates containing the

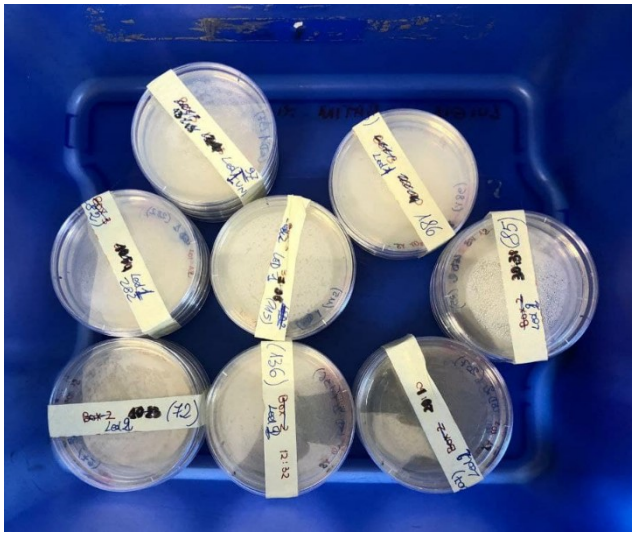

Figure 3.18 *Petri dishes utilized for tests of one microorganisms (plates for the three UV sources and with 4 concentrations).*

microorganisms were placed. This procedure was carried out because it has been noted a decrease in irradiation between the central position of the chamber (where the probe is positioned) and the most lateral portions (positions occupied by the Petri dishes). In this way, on the Petri dishes, has been ensured the necessary dose to reach the required log-reduction.

For each test has been used the radiometer in order to be sure that the dose delivered was the one previously determined (Figure 3.19).

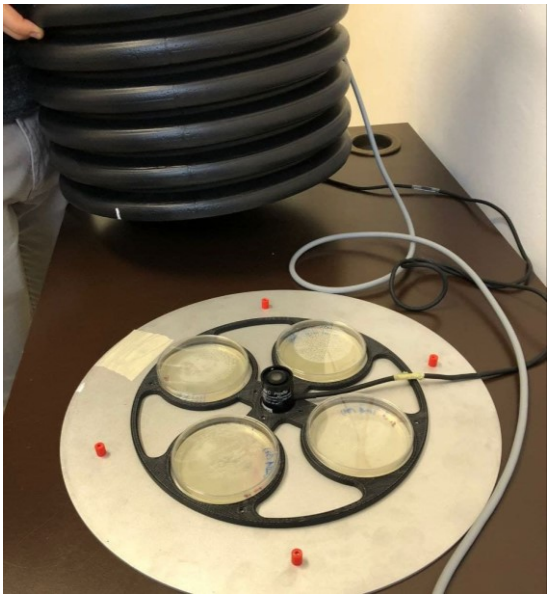

Figure 3.19 *Germicidal effectiveness test.*
Irradiation of four dishes with four different concentrations of microorganism.

In this way, once that the desired dose was reached, the UV source is switched off in order to be sure that the dose delivered is exactly the one calculated before.

The detailed protocol utilized to perform the tests is reported in Appendix 7.2.

4 Results and Discussion

In this chapter will be presented and discussed the results of the three different types of tests described above: Irradiance distribution tests, Chamber tests for the evaluation of the irradiance produced by the three UV sources and Germicidal Effectiveness tests.

4.1 Irradiance Distribution Test on APOTECA systems

As said before in section 3.8, in order to have an exact value of the irradiance present into the systems, it has been decided to measure the irradiance produced by the low-pressure mercury vapor UV lamps with the radiometer (equipped with the probe for the UV-C). In this way, it was possible to obtain the real value of irradiance in almost all points inside the systems. The values of irradiance measured inside the APOTECAchemo are summarized intoFigure 4.1, 4.2, 4.3, and 4.4 while irradiance measured inside the APOTECAunit is summarized into Figure 4.5, 4.6, 4.7, and 4.8.

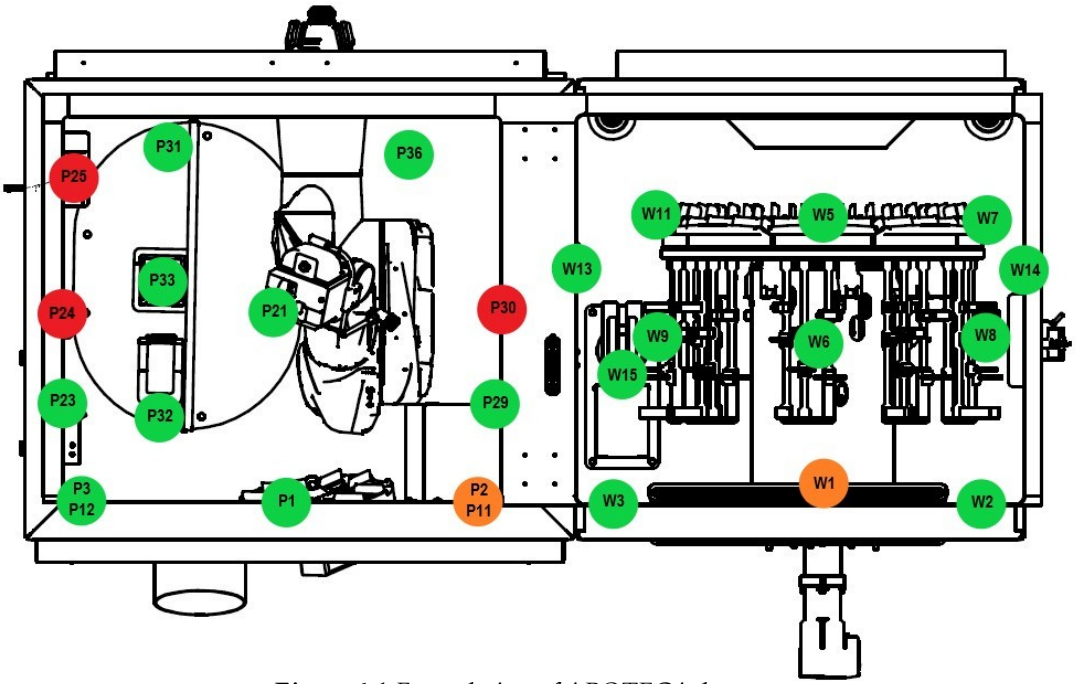

Figure 4.1 *Frontal view of APOTECAchemo system.*

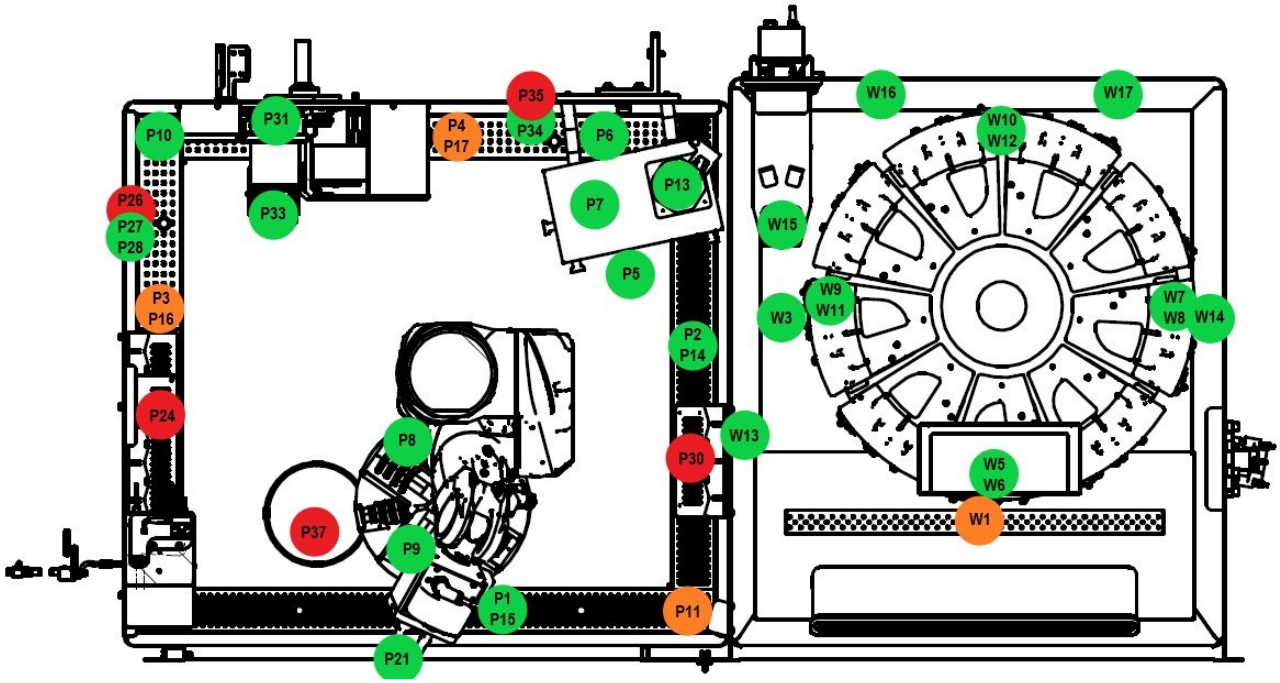

Figure 4.2 *Superior view of APOTECAchemo system.*

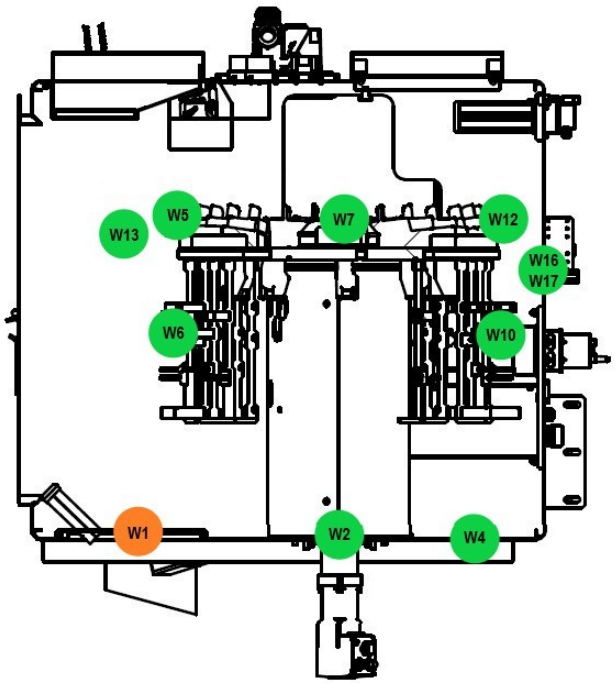

Figure 4.3 *Lateral view of the warehouse area of APOTECAchemo system.*

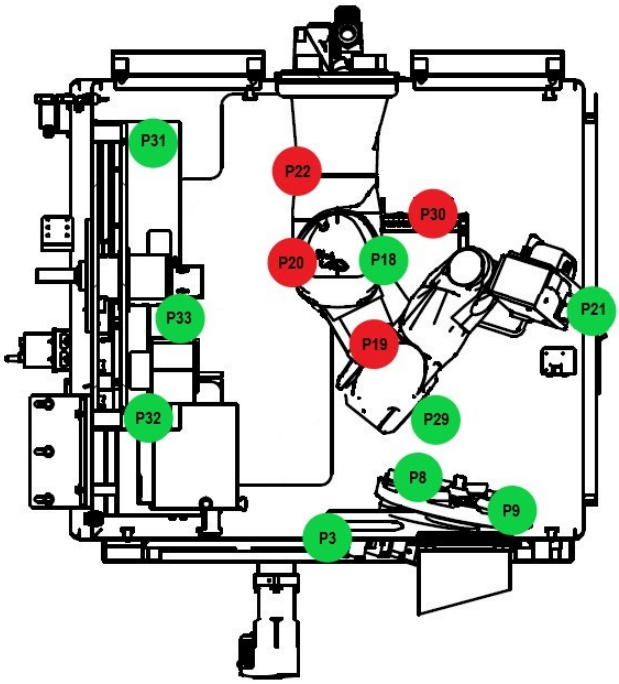

Figure 4.4 *Lateral view of the preparation area of APOTECAchemo system.*

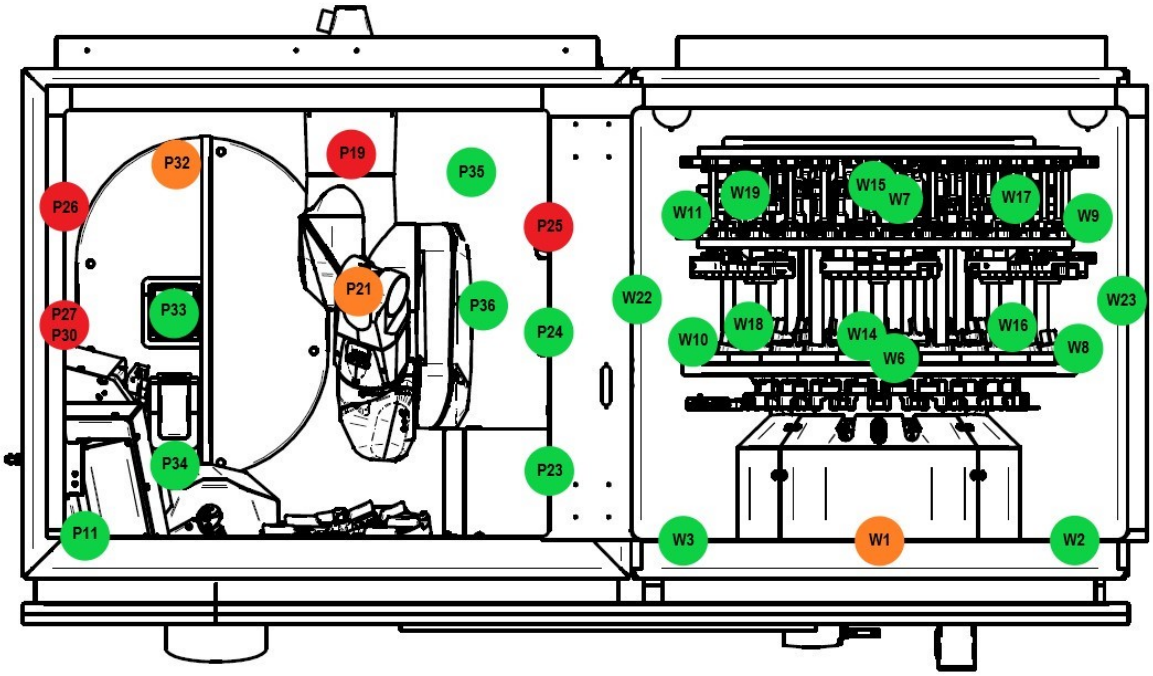

Figure 4.5 *Frontal view of APOTECAunit system.*

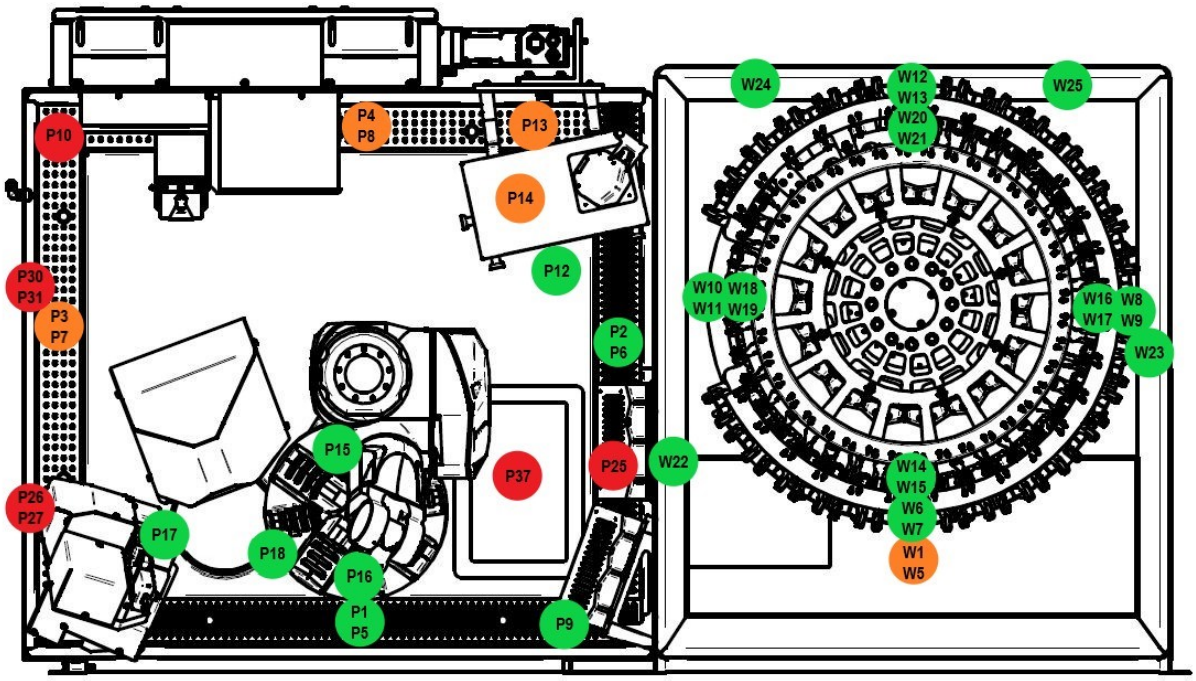

Figure 4.6 *Superior view of APOTECAunit system.*

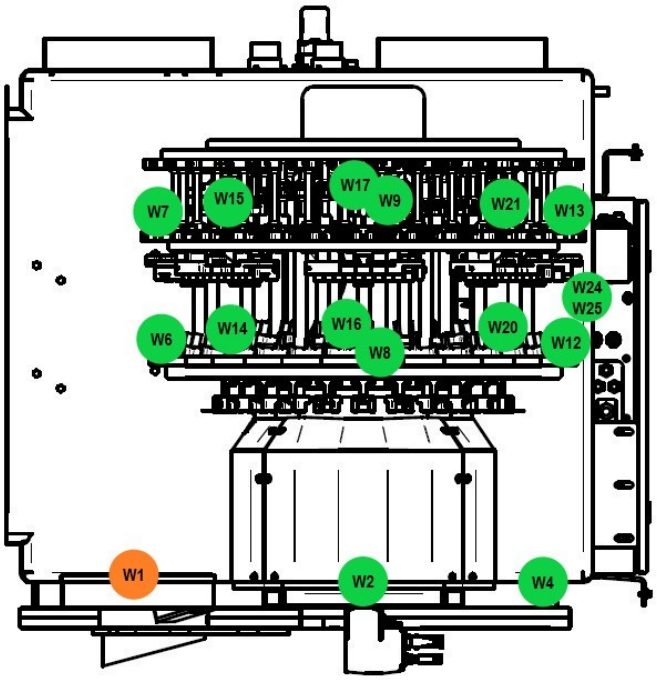

Figure 4.7 *Lateral view of the warehouse area of APOTECAunit system.*

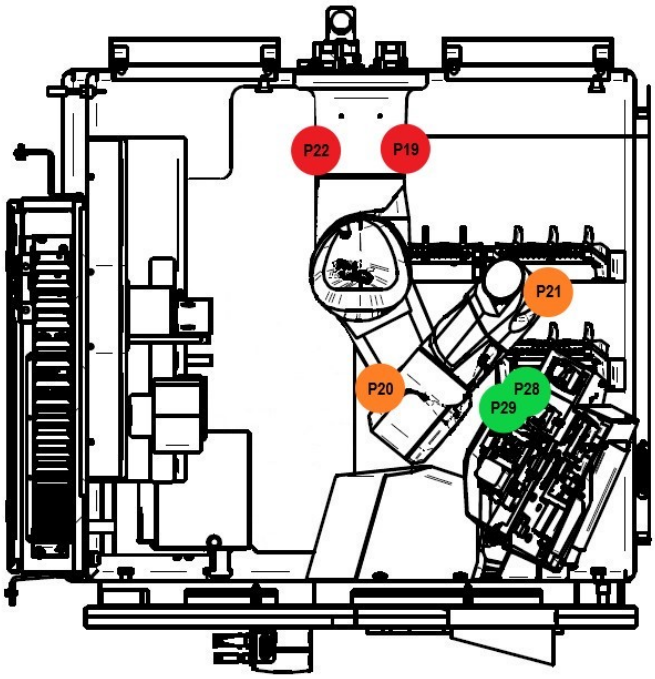

Figure 4.8 *Lateral view of the preparation area of APOTECAunit system.*

The values of irradiance obtained with these tests were then analysed in order to understand which of these values can guarantee an adequate level of sterilization inside the equipments.

With this purpose, it has been decided to determine a "threshold level". Values that are above this threshold have been considered sufficient to guarantee sterility and therefore have been indicated in the previous figures with a green dot, while values that fall below the threshold represent critical points in which the sterilization is not always obtained and for this reason they have been indicated with a red dot. The points indicated with an orange dot have shown irradiance values very close to the threshold and for this reason, even if slightly higher they still require attention because in these points the sterility could not always be guaranteed.

It should be noted, however, that none of the points identified above, not even the red critical points, has any influence on the goodness of the preparations. As can be seen in [15],in fact, the mediafill tests carried out on final products have always indicated the total absence of contamination with microorganisms.

The threshold has been calculated based on the worst-case scenario. In particular,

has been estimated the value of the threshold by relying on the dose necessary to obtain a 4-Log reduction of Bacillus subtilis spores (which represents the most resistant microorganism to UV radiation) considering an exposure time of 4 hours (that is the time required for the sterilization protocol of the systems).

In calculations:

$$irradiance \cdot time = dose$$

$$irradiance = \frac{dose}{time} = \frac{790\ \frac{J}{m^2}}{4h \cdot 3600 \frac{sec}{h}} = 0.054 \frac{W}{m^2}$$

Therefore, the minimum level of irradiance needed to ensure a 4-Log reduction after 4 hours of irradiation is equal to $0.054 \frac{W}{m^2}$. All the values of irradiance measured inside the systems are reported in Table 7-1 and in Table 7-2 (see Appendix 7.3).

Then, by knowing the minimum level of irradiance needed, it has been identified which are the points inside the systems where the UV irradiation is not sufficient to guarantee the minimum level of sterilization. Therefore, these points are the most exposed to a growth of the microbial load.

The results obtained after the analysis of the positions of the critical points are quite similar for both systems (APOTECAchemo and APOTECAunit). The disposition of these points is mainly due to the arrangement of the UV lamps inside the systems.

In fact, it has been found that the majority of these critical points are situated into the preparation area. This area is irradiated by only 2 lamps that are contained in the UV panel, as said earlier. Moreover, this panel is mounted into the door and so in the anterior and inferior part of the preparation area. This disposition of the lamps explains why the critical points are located in the higher and posterior part of this area. Obviously, these positions are the more distant ones with respect to the UV lamps and therefore are the least irradiated.

Other points that do not reach the minimum level of irradiation are the most hidden ones. For example, points under the grids or in the posterior part of the robot. Other critical points are the ones in the waste hatch or in the bucket for the final products.

Also these points, despite they are not far from the lamps, are less irradiated because they are in shadow areas or however behind some obstacles that stops the direct exposure to UV irradiation.

The irradiance distribution found through this study could definitely be improved with the use of LEDs. These, in fact, thanks to their small size, could be positioned in such a way as to irradiate directly the critical points and thus ensure a more uniform distribution of UV radiation within the APOTECA systems.

However, the transition to this alternative solution would require a high number of LEDs. Considering their lower power output and the need to cover large areas within the equipment, several tens of LEDs would be necessary. Then, considering their price per unit, the total cost of adopting this solution would be very high with respect to the solution currently utilized.

The transition to LED technology would then require a radical change of the equipments both mechanically and electrically. This is because, it would be necessary to shift from the current number of sources (6 UV lamp for APOTECAchemo and 7 for APOTECunit) to a considerably larger number (tens of points for LED sources). For this reason it would be necessary a new mechanical and electrical design that would require time to analyze all the characteristics/needs and at the same time would increase even more the costs for a possible modification of the components of the equipment.

As a result, the best solution to ensure a better distribution of UV radiation within the equipment could be to implement the system with one or two additional UV low-pressure mercury vapor lamps in order to cover critical areas. This solution could ensure a more homogeneous sterilization level and at the same time not increase much the costs of the modifications.

4.2 Chamber Tests for Irradiance Evaluation

After having carried out tests to assess how the irradiance is distributed within the APOTECA systems, have been performed some tests to compare the three sources of UV radiation (i.e. UV lamp and the two types of LEDs). For this purpose, as described in section 3.9, was utilized the test chamber to ensure that the irradiance produced by the three sources was analysed under the same conditions. This made it possible to obtain results which could be easily compared.

In particular, the irradiance produced by each UV source has been measured in five different positions: the central position representing the actual housing of the UV-C probe and the four positions where the Petri dishes will then be positioned for the germicidal effectiveness tests (Figure 4.9).

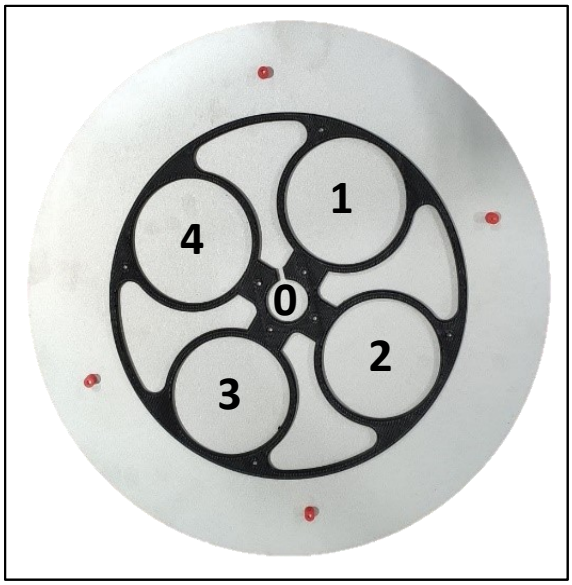

Figure 4.9 *Description of the five position for irradiance recording.*

The results obtained from these tests, for each UV source, are reported in Table 4-1 for UV lamp, in Table 4-2 for 45mW LEDs and in Table 4-3 for 70mW LEDs.

Table 4-1 Values of irradiance for UV lamp.

LOW-PRESSURE MERCURY VAPOR UV LAMP

POSITION	IRRADIANCE [W/m²]
0	1.720
1	1.540
2	1.420
3	1.540
4	1.600

Table 4-2 Values of irradiance for 45mW UV LEDs.

LED, LUMINUS

POSITION	IRRADIANCE [W/m²]
0	1.363
1	0.835
2	0.764
3	0.724
4	0.791

Table 4-3 Values of irradiance for 70mW UV LEDs.

LED, CRYSTAL IS

POSITION	IRRADIANCE [W/m²]
0	0.416
1	0.354
2	0.312
3	0.304
4	0.322

To perform these tests, have been used respectively:

- n°1 UV lamp;
- n°7 45mW UV LEDs (Figure 3.17c);
- n°4 70mw UV LEDs (Figure 3.17a).

It was decided to keep aside 1 LED of each type in order to overcome any possible malfunctioning.

As can be seen from the tables, the highest value of irradiance registered in the central position was that of the UV lamp (1.720 W/m^2), followed by the 45mw LEDs (1.363 W/m^2) and finally by the 70mw LEDs (0.416 W/m^2). These absolute values, particularly with regard to the two types of LEDs, are of relative importance as they are dependent on the number of LEDs used for testing.

In practice, it would be interesting to evaluate the value of irradiance in relation to the number of LEDs used; in fact, by analysing this ratio it can be seen that:

$$1.363 \frac{W}{m^2} \Big/ _7 = 0.195 \frac{W}{m^2}$$

is the average value of irradiance per LED for the 45mW LEDs and

$$0.416 \frac{W}{m^2} \Big/ _4 = 0.104 \frac{W}{m^2}$$

is the average value of irradiance per LED for the 70mW LEDs.

This ratio between measured irradiance and number of LEDs employed is quite similar for both types of LEDs, slightly in favor of 45mW LEDs. But, if in this reasoning we include also the price per unit of each LED, then you get:

$$0.195 \frac{W}{m^2} \Big/ _{€\ 39.89} = 0.0048$$

for 45mW LEDs (with a price per unit of € 39.89) and

$$0.104 \frac{W}{m^2} \Big/ _{€\ 21.88} = 0.0047$$

for 70mW LEDs (with a price per unit of € 21.88).

By doing this type of reasoning and so taking into account the irradiance produced, the number of LEDs used and the respective price, it can be seen that the two types of LEDs are practically equivalent.

Another interesting information for the comparison between the two types of LEDs is represented by the distribution of the irradiance on the lower base of the test chamber and so in the different five positions previously described. As can be seen from Table 4-2 and Table 4-3 in fact, the maximum reduction between the central position and the lateral position in percentage is:

$$1.362\frac{W}{m^2} : 100 = 0.724\frac{W}{m^2} : X$$

$$X = \frac{72.4}{1.362} = 53.2$$

Therefore, for 45mW LEDs, the reduction is about 46.8%.

$$0.416\frac{W}{m^2} : 100 = 0.304\frac{W}{m^2} : X$$

$$= \frac{30.4}{0.416} = 73.1$$

Therefore, for 70mW LEDs, the reduction is about 26.9%.

With regard to this comparison, between the two types of LEDs there is a clear difference. The distribution of the irradiance produced by 70mw LEDs is in fact much more homogeneous than the 45mW LEDs. The former show a maximum reduction of 26.9% compared to 46.8% of seconds. This value is is caused by a particular feature of the two LEDs: the beam angle. The first type of LED (Crystal IS) has a beam angle of 130° while the second one (Luminus) shows a beam angle of 60°.

In summary, cosindering all the features examined, the second type of LEDs (70mW, Crystal IS) are the most functional. This is because, even if they show a lower absolute irradiance value (linked to the number of LEDs employed), in relation to the price they are are not inferior to the second type but at the same time they have the advantage of ensuring a more homogeneous irradiance distribution.

Finally, comparing the UV LED technology with the UV low-pressure mercury vapor lamp it is possible to see that the comparison is undoubtedly in favor of the UV

lamp.

This in fact shows the highest value of irradiance in relation to the number of lamps ($1.720\frac{W}{m^2}$ per lamp) and at the same time shows also the lowest price per unit (€ 15.26). Furthermore, it has also the lowest level of reduction in terms of irradiance distribution. It has a maximum reduction equal to 17.4%.

$$1.720\frac{W}{m^2} : 100 = 1.420\frac{W}{m^2} : X$$
$$= \frac{142}{1.720} = 82.6$$

Therefore to conclude, from the point of view of convenience and functionality, there is no comparison between the two technologies. The "standard" technology of UV lamps is still the best from this point of view.

4.3 Germicidal Effectiveness Tests

The last set of tests is the one carried out, as described in section 3.10, at University of Urbino to analyze the germicidal efficacy of the three UV sources.

In this section are given the results obtained from irradiation on two microorganisms: Staphyllococcus aureus and Pseudomonas aeruginosa. The following data are preliminary results since the tests performed are only a part of the protocol previously described.

These tests showed that all three UV sources have demonstrated a high germicidal power. In Figure 4.10 can be seen indeed how, compared to the control plate, the plates containing the microorganism irradiated by the three UV sources show a significant decrease in bacterial load.

As can be seen also from Table 4-4 and Table 4-5, S. aureus is more resistant to UV radiation than P. aeruginosa. In fact, to obtain a 6-log reduction of S. aureus it is necessary to arrive at Dose 3 for UV lamp and LED 2 while for LED 1 it is sufficient Dose 1. A 6-log reduction of P. aeruginosa is instead reached with Dose 1 for all three

UV sources.

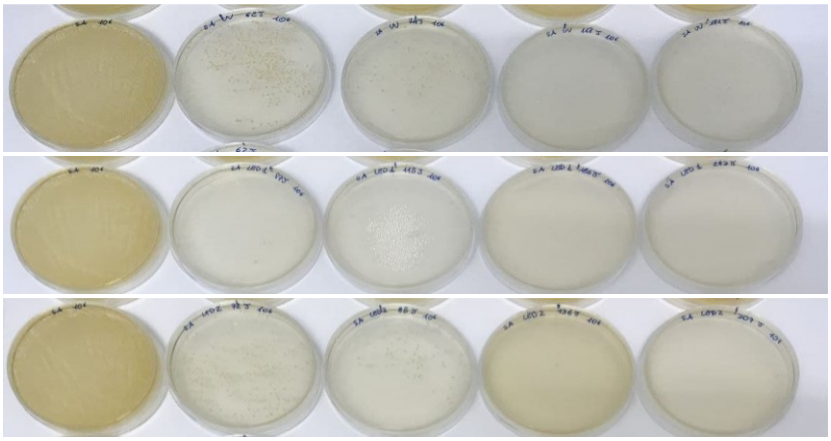

Figure 4.10 *Concentrations of S. aureus after exposure to the three UV sources: top UV lamp, middle LED1 and bottom LED2.*
From left to right: control, dose 1, dose 2, dose 3 and dose 4.

Table 4-4 Concentrations of S. aureus after exposure to the three UV sources. Columns represent the different dose levels. nc stands for "not countable".

S. aureus	**UV lamp**				
		55 J/m^2	65 J/m^2	105 J/m^2	160 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	256 CFU	76 CFU	0 CFU	2 CFU
	10^4 inoculum	10 CFU	0 CFU	0 CFU	0 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	LED 1 (45 mW)				
		55 J/m^2	65 J/m^2	105 J/m^2	160 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	2 CFU	5 CFU	0 CFU	0 CFU
	10^4 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	LED 2 (70 mW)				
		55 J/m^2	65 J/m^2	105 J/m^2	160 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	226 CFU	71 CFU	3 CFU	0 CFU
	10^4 inoculum	2 CFU	0 CFU	1 CFU	0 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU

Table 4-5 Concentrations of P. aeruginosa after exposure to the three UV sources. Columns represent the different dose levels. nc stands for "not countable".

P. aeruginosa	**UV lamp**				
		65 J/m^2	100 J/m^2	179 J/m^2	250 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	0 CFU	4 CFU	1 CFU	6 CFU
	10^4 inoculum	0 CFU	0 CFU	0 CFU	1 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	LED 1 (45 mW)				
		65 J/m^2	100 J/m^2	170 J/m^2	250 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	10^4 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	LED 2 (70 mW)				
		65 J/m^2	100 J/m^2	170 J/m^2	250 J/m^2
	10^8 inoculum	nc	nc	nc	nc
	10^6 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	10^4 inoculum	0 CFU	0 CFU	0 CFU	0 CFU
	10^3 inoculum	0 CFU	0 CFU	0 CFU	0 CFU

The values in the two tables above summarise the number of CFU measured for each microorganism after each exposure at different dose levels. Each colum represents a different level of dose needed to reach a specific log-reduction (as describer in section 3.10) and each row represents four different concentrations of the specific microorganism.

Analyzing these data it can be seen that, concerning P. aeruginosa, no colony has been detected already at the lowest dose level (65 J/m^2) for all the UV sources. This means that to obtaion a reduction of the 99.9999% it is sufficient Dose 1. As regards S. aureus instead, to obtain the same level of reduction are necessary Dose 3 for UV and LED 2 and Dose 1 for LED 1.

In fact, Figure 4.11 and Figure 4.12 show the comparison among the three different UV sources. In particular, from Figure 4.11 can be seen that the three UV sources

behave in the same way, namely that all of them reach a 6-log reduction already at the first dose administered.

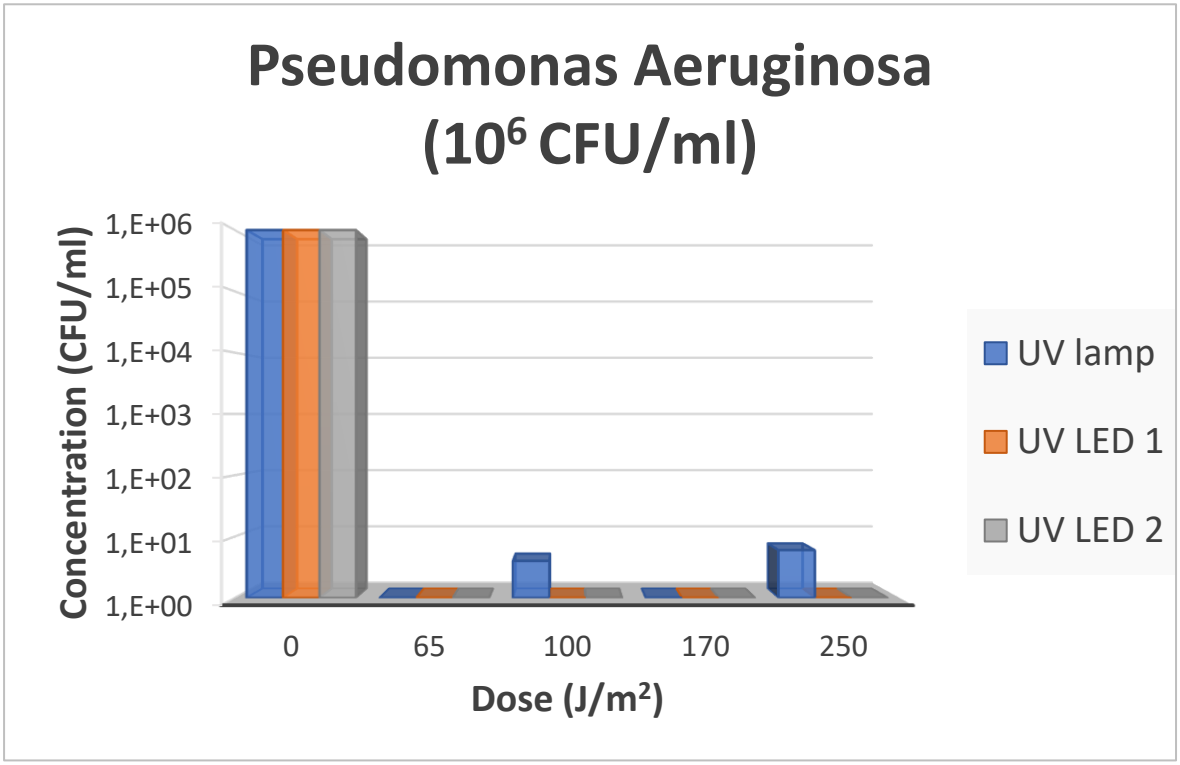

Figure 4.11 *Comparison of germicidal efficacy for P. aeruginosa among the three UV sources.*

It is more interesting to see from Figure 4.12 how, with a slightly stronger microorganism (S. aureus), there is a UV source that behaves better than the others. LED 1, in fact, reaches a 6-log reduction already after the first dose administered while the other two sources reach a 6-log reduction only after Dose 3.

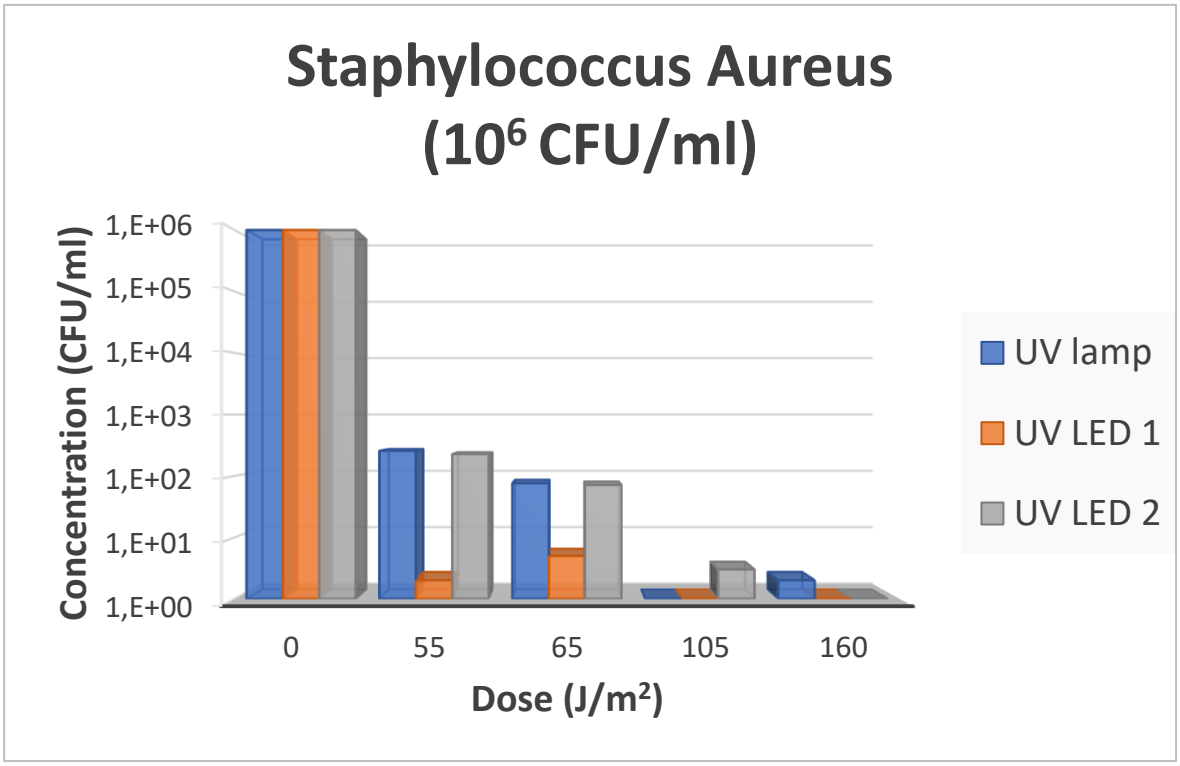

Figure 4.12 *Comparison of germicidal efficacy for P. aeruginosa among the three UV sources.*

4.4 Evaluation of economic sustainability

Finally, to complete the comparison, this section define an economic assessment between the types of UV sources examined until now.

By considering that the life-time of APOTECA system is about 10 years and that currently the UV lamps are replaced annually, in the average life of an APOTECA system will be used on average 70 lamps (considering the APOTECAunit system that mounts the highest number of lamps: 7). Therefore, considering the price per unit of a UV lamp (€ 15.26), can be seen that the long-term cost is of about € 1070.

In section 3.3, it was said that LEDs have a life-time 5 times higher than that of UV lamps, so considering an average life of 10 years for APOTECA systems, these should be replaced only once.

Dividing the long-term cost by 2 (2 set of LEDs, considering only one replacement in 10 years), one gets:

$$\frac{€\ 1070}{2} = €\ 535$$

Considering then the price of one 70mW LED (€ 21.88):

$$\frac{€\ 535}{€\ 21.88} = 24.45$$

This means that in order not to increase the long-term cost should be utilized 24/25 70mW LEDs to ensure the sterilization of the entire equipment.

Considering instead the price of one 45mW LED (€ 39.89):

$$\frac{€\ 535}{€\ 39.89} = 13.41$$

This means that in order not to increase the long-term cost should be utilized 13/14 45mW LEDs to ensure the sterilization of the entire equipment.

However, from the analyses that have been made previously taking into account the low power output of LEDs, it has been seen that this number of LEDs is not enough to ensure a homogeneous sterilization in either of the two situations.

To this should then be added that the transition to LED technology would require a new mechanical design to include the new positioning of dozens of LEDs that should be installed. And should be also added a new electric design to ensure the electrical connection to each source.

All these considerations make the transition to LED technology overpriced with respect to the standard technology.

5 Conclusions

This study about sanitization with ultraviolet light has been performed because the initial goal was to improve the sanitization phase and, at the same time, to understand if are present innovative solutions that can be employed to improve this phase of sanitization.

Tests made on APOTECA systems have confirmed the initial doubts: namely that the irradiation provided by the UV low-pressure mercury vapor lamps currently utilized within the two systems cannot provide a sufficient level of sterilization in all the points of the equipment. This issue is most probably related to the disposition of the UV lamps inside the systems. It has been seen, in fact, that the critical points are the ones where the lamps are less present with respect to the dimension of the area and therefore not all the points are reached in the same way by irradiation. At the same time, however, these crucial points are located in areas not critical to the preparation of final products and therefore they have no impact on the goodness of the final product.

In this thesis has been found and analysed a valid alternative to solve this problem and that would allow to enhance/improve the level of sterilization which is currently ensured into APOTECA systems. This alternative is the LED technology. In detail, this technology has some characteristics that have been shown to be better than those present in the low-pressure UV lamps currently used. These characteristics are: the small size, a more effective wavelength, a longer life-time and the absence of mercury. At the same time, however, it has been seen that from a purely operational/functional point of view, this new technology is not at the same level as the one currently used. This is because, by comparing performance and costs, this new solution would require a huge investment of resources both in economic terms and in terms of mechanical and electrical design.

Then, from the point of view of germicidal efficacy it has been seen that the higher

wavelength in which the LEDs act confirms the advantage concerning the dose/killing rate relationship. In fact, the LED operating in the highest wavelength range (UV LED 1, 275-286nm) has been shown to be the most effective having a 6-log reduction (a reduction of 99.9999%) already after the administration of the first dose. The other two sources (UV lamp, 254nm and LED 2, 260-270nm), for equal dose, have shown 4-log reduction. Thus, from this point of view, LED technology and in particular the 45mW Luminus LED exhibit the best features ensuring a longer wavelength and therefore a more effective germicidal power.

It should be remembered, however, that, for equal dose, LEDs require a longer exposure time to UV radiation as they have a power output much lower than the standard technology of UV lamps. Hence, making this kind of reasoning, the trade-off between power output and exposure time becomes fundamental.

Then, it should be taken into account also the high cost of LED technology compared to the standard one since, as also described in section 4.4, this would require the installation of a large number of LEDs. Considering therefore the high cost per unit of a single LED and the requirements for the installation, the final cost of a possible transition to LED technology would be too high compared to the technology present now in APOTECA systems.

In conclusion, all things considered, an immediate transition to LED technology is not a viable solution. However, over time, with the advancement of LED technology and the consequent lowering of prices or the increase of power output and starting to develop a new mechanical/electrical design, this solution could certainly become a valid solution.

At present, one solution to solve the problem of the homogeneous irradiation can be to install one or two UV mercury lamps to cover the zone where the irradiation is not sufficient. This solution would ensure a more homogeneous irradiation of equipment surfaces and at the same time would not have a significant impact on costs.

For the future, a new solution that is developing recently is the sterilization with visible light [15]. This alternative could be added to the sterilization phase. In this way,

a minimum sterilisation level could be guaranteed even in operation. This technology is still in its infancy nonetheless it will deserve consideration.

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7 Appendix

7.1 UV Radiation Efficiency Test Protocol

Project Code: [redacted]	*Category Code*: [redacted]	*Document Code:* [redacted]	*Progressive document:* [redacted]	*Language:* [redacted]	*Rev:* [redacted]	*Group:*
Document title: **UV Radiation Efficiency Test**						
Description: *Test for the evaluation of the irradiance produced by UV low pressure mercury-vapor lamps in the APOTECAchemo/APOTECAunit through the use of the photo-radiometer HD2102.2.*						
Notes: Documentation saved in [redacted]						

☐ **To be selected if this document is a REPORT**

(pages from x to xx)

If this document is a report, the verifier has to compile the following table at the end of the check.

Register nr	
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Verification date	
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Approver signature	

Rev.	Date	Description	Author	Checked by	Approved by
01	18/02/2016	English translation of the [redacted] version	V.Rosini	D.Paolucci	M.Mengoni

I. Premise

The irradiance evaluation test in the APOTECAchemo/APOTECAunit system arises from the need to assess how ultraviolet radiation is distributed within the machine. The ultraviolet radiation is emitted by the low-pressure mercury vapor lamps that are currently used to ensure microbiological sterilization of the system. The lamps utilized in the APOTECAchemo/APOTECAunit are OSRAM HNS S 9 W G23.

II. Purpose

The purpose of this test is to evaluate the irradiance present within the APOTECAchemo/APOTECAunit system during the sterilization phase with the UV panels installed and with all the UV lamps switched-on.

III. Introduction

The UV Radiation Efficiency Test wants to estimate the irradiance produced by the UV low pressure lamps to verify if the amount of irradiation is sufficient to guarantee an adequate level of microbial sterilization. The test will be divided into multiple measurements in order to have a detailed characterization of the distribution of irradiance within the system.

IV. Test conditions

Before performing the UV Radiation Efficiency Test it will be necessary to verify the switching-on timing of UV lamps. In details, it should be assessed how long the lamps take to start working into full operation. In this way it will be exactly known how long after the irradiance will have settled on the maximum values. Thereby, after waiting this time, it will be possible to start the actual measurement.

The test shall be carried out using UV low pressure mercury vapor lamps (OSRAM HNS S 9 W G23) and the HD2102.2 photo-radiometer with LP 471 UVC probe. It will be necessary to mount the UV panels and then proceed with the switching-on of the UV lamps to perform the measurements. It will also be necessary to place the probe at different points inside the machine and, for measurements involving higher points, it will be necessary to use a support for the probe.

For the visualization of the results and for the eventual use of the radiometer through PC it will be necessary to install the software Deltalog9.

The total number of measurements to be carried out is 67. 11 measurements in the Loading Area, 17 measurements in the Warehouse Area and 37 measurements in the Preparation Area (Table2). In addition, 2 preliminary tests to evaluate the time needed by the lamps to start working into full operation.

V. Compiler instructions

If this document is a report of data for the execution of the verification protocol, it must be indicated in the first page, by putting a tick sign in the related check box.

Moreover, in the first page, at the end of a formal and content analysis, the verifier will have to write:

- Name (a person different from the technician who has performed the tests)
- Verification date

The test result must be registered in the proper fields of this protocol document.

In the document header of each page, the register number of every APOTECA system which the report refers to, needs to be indicated.

For each component, one or more test is reported, with the related acceptance criterion.

Read carefully the text of each test and perform them using the recommended instrumentation.

The result of the test needs to be reported in the Result field. Put a thick sign (X) on the P (pass) or F (fail) column to indicate:

	P	F
Passed test	X	
Failed test		X
Not available test	----	----

It is possible to write eventual observations or comments in the Notes field.

TABLE 1.

Cod.	Test description	Result		Notes
		Pass	Fail	
1	Put the equipment in STOP state and place the UV panels without switching-on the lamps.			
2	Once the UV panels have been positioned, connect the LP 471 UVC probe for the measurement of irradiance to the HD2102.2 photo-radiometer by the use of the dedicated probe input with 8-pole DIN45326 connector located at the top of the instrument.			
3	Turn on the photo-radiometer by pressing the ON-OFF/Auto-OFF button. The switching-on activates, for a few seconds, all segments of the display, starts a self-test that includes the detection of the probe connected to the input and brings the instrument to the standard measuring condition.			
4	Set the interval between two data stores to 30 seconds by going to the seventh menu item (Print and log interval) by pressing the MENU key - CLR Q/T. The first press of the MENU key allows you to access the first menu item; to switch to the next entries, press the ENTER key. To change the displayed item, use the arrow keys. Next, press the MENU button to exit the menu.			
5	Place the probe inside the machine (in order to perform the preliminary measurement and analyse the time required by the lamps to start working into full operation).			
6	Close the front door keeping the radiometer outside the machine (take care of passing the probe wire between the door closure).			

Cod.	Test description	Result		Notes
		Pass	Fail	
	Switch on UV lamps for a period of 15 minutes.			
7	Proceed measuring the irradiance and storing the measured values. Activate the logging function by pressing the LOG button on the photo-radiometer.			
8	After 15 minutes, turn off the UV lamps.			
9	Stop the measurement of irradiance by pressing the LOG button on the photo-radiometer. The stored data constitute a continuous block of data.			
10	Repeat the data acquisition and storage procedure for a period of 10 minutes. (Activate the logging function by pressing the LOG button on the photo-radiometer)			
11	Once the second measurement is completed, connect the photo-radiometer to the PC via the serial cable CP23 (connection cable with Mini-USB connector type B on one side and USB connector type A on the other). The Mini-USB connector output is located on the bottom of the instrument.			
12	Start the Deltalog9 software from your PC and make sure you are connected to the photo-radiometer.			
13	Transfer the data in memory to the PC with the DUMP LOG command (MENU key until ">>>>_Log_dump_or_eras" and then LOG key).			
14	Proceed to the visualization and analysis of the recorded data. The data will be divided into continuous blocks of data each related to a specific measurement.			

Cod.	Test description	Result		Notes
		Pass	Fail	
15	Verify from the data acquired how long the lamps takes to enter into full operation. (approximately it will be 6-7 minutes)			
16	Once these information have been obtained, measurements shall be made for a time equal to the time taken by the lamps to start working into full operation (6-7 minutes) plus one minute (for the actual data acquisition). In this way we will have one minute of recording with lamps in optimal operation.			
17	Before placing the probe in the first measuring point, set the interval between two data stores to 1 second as previously done (seventh menu item, pressing the MENU - CLR Q/T).			
18	Place the probe at the first measuring point. (for the measuring points, see Table 2)			
19	Repeat the measurement procedure. (Activate the logging function by pressing the LOG button on the photo-radiometer)			
20	Switch on UV lamps for a time equal to the time taken by the lamps to start working into full operation (6-7 minutes) plus one minute (for actual data acquisition).			
21	Switch off the lamps and move the probe to the second measuring point.			
22	Repeat the same measurement procedure for all points indicated in Table 2. Measurements points are divided into Loading Area, Warehouse Area and Preparation Area.			

Cod.	Test description	Result		Notes
		Pass	Fail	
23	Once all measurements have been completed, perform the UV lamp panel removal procedure.			
24	Once the removal procedure is complete, connect the photo-radiometer to the PC and transfer the newly collected data as described above. (Connect the photo-radiometer to the PC via the serial cable CP23)			
25	Proceed to the visualization and analysis of the recorded data through the Deltalog9 software. The data will be divided into continuous blocks of data each related to a specific measurement.			

TABLE 2.

	ID	Position – Loading Area	Notes
1	L_1	Loading Area, Base, Right	
2	L_2	Loading Area, Base, Middle	
3	L_3	Loading Area, Base, Left	
4	L_4	Loading Area, Half-height, Left	
5	L_5	Loading Area, Half-height, Right	
6	L_6	Loading Area, Half-height, Middle	
7	L_7	Loading Area, On the top, Middle	
8	L_8	Loading Area, On the top, Left	
9	L_9	Loading Area, On the top, Right	
10	L_{10}	Loading Area, Side, Half-height, Right	
11	L_{11}	Loading Area, Side, Half-height, Left	

	ID	Position – Warehouse Area	Notes
1	W_1	Warehouse Area, Base, Front	

Register nr _____

	ID	Position – Warehouse Area	Notes
2	W_2	Warehouse Area, Base, Right	
3	W_3	Warehouse Area, Base, Left	
4	W_4	Warehouse Area, Base, Back	
5	W_5	Warehouse Area, Carousel, Front, On the top	
6	W_6	Warehouse Area, Carousel, Front, At the bottom	
7	W_7	Warehouse Area, Carousel, Right, On the top	
8	W_8	Warehouse Area, Carousel, Right, At the bottom	
9	W_9	Warehouse Area, Carousel, Left, At the bottom	
10	W_{10}	Warehouse Area, Carousel, Back, At the bottom	
11	W_{11}	Warehouse Area, Carousel, Left, On the top	
12	W_{12}	Warehouse Area, Carousel, Back, On the top	
13	W_{13}	Warehouse Area, Side, Half-height, Left	
14	W_{14}	Warehouse Area, Side, Half-height, Right	
15	W_{15}	Warehouse Area, Above the Reader	

	ID	Position – Warehouse Area	Notes
16	W_{16}	Warehouse Area, Back Panel, Half-height, Middle-Left	
17	W_{17}	Warehouse Area, Back Panel, Half-height, Middle-Right	

	ID	Position – Preparation Area	Notes
1	P_1	Preparation Area, Base, Front	
2	P_2	Preparation Area, Base, Right	
3	P_3	Preparation Area, Base, Left	
4	P_4	Preparation Area, Base, Back	
5	P_5	Preparation Area, Back-Right Corner, Ahead of weighing device	
6	P_6	Preparation Area, Back-Right Corner, Behind the weighing device	
7	P_7	Preparation Area, Above the weighing device	
8	P_8	Preparation Area, Shaker, Back position	
9	P_9	Preparation Area, Shaker, Front position	
10	P_{10}	Preparation Area, Back-Left Corner	

	ID	Position – Preparation Area	Notes
11	P_{11}	Preparation Area, Front-Right Corner	
12	P_{12}	Preparation Area, Front-Left Corner	
13	P_{13}	Preparation Area, Above the weighing device (on white support)	
14	P_{14}	Preparation Area, Under Right Grid	
15	P_{15}	Preparation Area, Under Front Grid	
16	P_{16}	Preparation Area, Under Left Grid	
17	P_{17}	Preparation Area, Under Back Grid	
18	P_{18}	Preparation Area, Robot, Front	
19	P_{19}	Preparation Area, Robot, Front seam	
20	P_{20}	Preparation Area, Robot, Back	
21	P_{21}	Preparation Area, Robot, Clamp	
22	P_{22}	Preparation Area, Robot, Back, On the top	
23	P_{23}	Preparation Area, Left Side, First shelf	
24	P_{24}	Preparation Area, Left Side, Second shelf	

	ID	Position – Preparation Area	Notes
25	P_{25}	Preparation Area, Left Side, On the top	
26	P_{26}	Preparation Area, Left Side, Back, On the top	
27	P_{27}	Preparation Area, Left Side, Back, Half-height	
28	P_{28}	Preparation Area, Left Side, Back, At the bottom	
29	P_{29}	Preparation Area, Right Side, Front, At the bottom	
30	P_{30}	Preparation Area, Right Side, Shelf	
31	P_{31}	Preparation Area, Back Panel, Dosing device, On the top	
32	P_{32}	Preparation Area, Back Panel, Dosing device, At the bottom	
33	P_{33}	Preparation Area, Back Panel, Dosing device, Middle	
34	P_{34}	Preparation Area, Back Panel, Right, At the bottom	
35	P_{35}	Preparation Area, Back Panel, Right, Half-height	
36	P_{36}	Preparation Area, Back Panel, Right, On the top	
37	P_{37}	Preparation Area, Waste hatch	

VI. Conclusions

NOTES

CONCLUSIONS
The request of change n° [redacted] is considered: ☐ VALIDATED

Register nr _____

☐ NOT VALIDATED	

7.2 Germicidal Effectiveness of UV Radiation Test Protocol

Date February 2021

Object Study on germicidal effectiveness of UV-C radiation emitted by traditional UV lamps and UV LEDs

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Introduction

APOTECA robotic systems for the preparation of sterile parenteral drugs must ensure that the production process is carried out in aseptic conditions and that the microbiological contamination is kept below the maximum limits indicated in EU-GMP Annex 1.

The sanitization of the surfaces inside APOTECA through traditional UV lamps (i.e., low pressure mercury vapor UV lamps) has already demonstrated a high effectiveness after a few hours of irradiation (Bruscolini et al 2015, Sabatini et al 2019).

The aim of this study is to evaluate, in ideal conditions, the germicidal effectiveness of UV-C radiation by comparing the effectiveness of UV-C radiation emitted by traditional UV lamps and UV-C radiation emitted by two types of UV-C LEDs.

The final result of this study will be a correlation between the reduction of the microorganisms' concentration and the dose. The dose is represented by the product between the energy received by a given microorganism (irradiance, I) and the exposure time to that energy (t): Dose = I * t $[\frac{J}{m^2}]$.

Materials

The following materials will be required to perform the experimental tests:

- 3x Chamber Test
- 1x Radiometer HD2102.2 with probe LP 471 UVC
- 1x UV-C low pressure mercury vapor lamp OSRAM, HNS S 9 W G23 (UV)
- 4x LEDs UV-C KLARAN, KL265-50V-SM-WD, 70mW (LED1)
- 7x LEDs UV-C LUMINUS, LST1-01G07-UV01-01, 45mW (LED2)
- 4x types of microorganisms:
    - Pseudomonas aeruginosa ATCC 9027
    - Staphylococcus aureus ATCC 6538
    - Bacillus subtilis ATCC 6633 (spore)
    - Candida albicans ATCC 10231
- 600x Petri dish 90mm
- 300x Dish contact plate 55 mm
- 100x Cuvettes polystyrene bio-med semi-micro
- 960x Universal tip 100-1000ul pp blue nonsterile
- 960x Universal tip 2-200ul pp yellow nonsterile
- 500x Test-tube centrifuge pp 15ml
- 500x Test-tube centrifuge pp 50ml
- 500x Snap cap microtube 1.5ml pp
- 5GR Manganese(ii) sulfate monohydrate 98+%
- 500GR Nutrient agar
- 500GR Tryptone soya broth
- 500GR Tryptone soya agar

Materials supplied by Loccioni: Chambers Test, radiometer, UV lamp and UV LEDs.

Materials supplied by UniUrb: microorganisms.

Materials to order (Loccioni) and deliver to UniUrb: Petri dish, Dish contact plate, Cuvettes, Universal tip, Test-tube, Snap cap microtube, Manganese sulfate monohydrate, Nutrient agar, Tryptone soya broth, Tryptone soya agar.

Methods

- The plates will be inoculated with known quantity of each microorganism: 10^8 CFU/ml, 10^6 CFU/ml, 10^4 CFU/ml, 10^3 CFU/ml.
- The germicidal effectiveness will be evaluated by irradiating 4 concentrations of each microorganism with 4 different UV doses.
- During each irradiation 4 plates will be placed inside the chamber test and irradiated at a predefined dose.
- Positive control tests.
- A test cycle includes:
  1. UV irradiation at dose 1 of P.aeruginosa (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  2. UV irradiation at dose 2 of P.aeruginosa (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  3. UV irradiation at dose 3 of P.aeruginosa (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  4. UV irradiation at dose 4 of P.aeruginosa (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  5. UV irradiation at dose 1 of S.aureus (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  6. UV irradiation at dose 2 of S.aureus (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  7. UV irradiation at dose 3 of S.aureus (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  8. UV irradiation at dose 4 of S.aureus (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  9. UV irradiation at dose 1 of B.subtilis spore (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  10. UV irradiation at dose 2 of B.Subtilis spore (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  11. UV irradiation at dose 3 of B.Subtilis spore (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  12. UV irradiation at dose 4 of B.Subtilis spore (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  13. UV irradiation at dose 1 of C.Albicans (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  14. UV irradiation at dose 2 of C.Albicans (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  15. UV irradiation at dose 3 of C.Albicans (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)
  16. UV irradiation at dose 4 of C.Albicans (4 plates at 4 concentrations: 10^8, 10^6, 10^4, 10^3)

Number of tests

- Each test cycle will be replicated 3 times for each UV device used (UV, LED(1), LED(2)).
- Total number of test cycles: 144.

- The duration of each test cycle varies depending on the dose to be delivered.

Inoculum preparation, incubation and sample analysis

- Each microorganism strain was prepared at predefined concentrations and stored according to Sabatini et al. [4].
- The inoculum were spread on the TSA plates in order to achieve the nominal concentrations 108, 106, 104, 103.
- After UV radiation tests TSA plates were incubated at 37° C for 24 hours.
- After incubation CFU were visually counted.
- Results were recored and the correspondent Log-reductions were calculated.

Doses

P. aeruginosa		Chamber 1 (LAMP)	Chamber 2 (LED1) (45 mW)	Chamber 3 (LED2) (70 mW)
Cycle 1	Dose 1 (65 J/m^2)	43s (74 J/m^2)	1min 24s (115 J/m^2)	3min 22s (85 J/m^2)
Cycle 2	Dose 2 (100 J/m^2)	1min 6s (114 J/m^2)	2min 9s (176 J/m^2)	5min 10s (129 J/m^2)
Cycle 3	Dose 3 (170 J/m^2)	1min 52s (193 J/m^2)	3 min 40s (300 J/m^2)	8min 47s (220 J/m^2)
Cycle 4	Dose 4 (250 J/m^2)	2min 44s (283 J/m^2)	5min 23s (440 J/m^2)	12min 54s (322 J/m^2)

S. aureus		Chamber 1 (LAMP)	Chamber 2 (LED1) (45mW)	Chamber 3 (LED2) (70 mW)
Cycle 1	Dose 1 (55 J/m^2)	36s (62 J/m^2)	1min 11s (97 J/m^2)	2min 51s (72 J/m^2)
Cycle 2	Dose 2 (65 J/m^2)	43s (74 J/m^2)	1min 24s (115 J/m^2)	3min 22s (85 J/m^2)
Cycle 3	Dose 3 (105 J/m^2)	1min 9s (118 J/m^2)	2min 16s (186 J/m^2)	5min 26s (136 J/m^2)
Cycle 4	Dose 4 (160 J/m^2)	1min 45s (181 J/m^2)	3min 27s (282 J/m^2)	8min 16s (207 J/m^2)

B. subtilis		Chamber 1 (LAMP)	Chamber 2 (LED1) (45 mW)	Chamber 3 (LED2) (70 mW)
Cycle 1	Dose 1 (350 J/m^2)	3min 49s (394 J/m^2)	7min 32s (616 J/m^2)	18min 4s (451 J/m^2)
Cycle 2	Dose 2 (470 J/m^2)	5min 8s (530 J/m^2)	10min 6s (826 J/m^2)	24min 16s (606 J/m^2)
Cycle 3	Dose 3 (790 J/m^2)	8min 37s (890 J/m^2)	16 min 59s (1388 J/m^2)	40min 46s (1018 J/m^2)
Cycle 4	Dose 4 (1200 J/m^2)	13min 5s (1351 J/m^2)	25min 47s (2018 J/m^2)	1h 2min (1546 J/m^2)

C. albicans		Chamber 1 (LAMP)	Chamber 2 (LED1) (45 mW)	Chamber 3 (LED2) (70 mW)
Cycle 1	Dose 1 (500 J/m^2)	5min 27s (563 J/m^2)	10min 45s (879 J/m^2)	25min 48s (644 J/m^2)
Cycle 2	Dose 2 (1000 J/m^2)	10min 54s (1125 J/m^2)	21min 29s (1756 J/m^2)	51min 36s (1288 J/m^2)
Cycle 3	Dose 3 (1500 J/m^2)	16min 21s (1688 J/m^2)	32min 13s (2633 J/m^2)	1h 18min (1931 J/m^2)
Cycle 4	Dose 4 (2000 J/m^2)	21min 48s (2250 J/m^2)	42min 58s (3512 J/m^2)	1h 44min (2575 J/m^2)

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7.3 Measurements of Irradiance

Table 7-1 Measures of irradiance inside the APOTECAchemo system. In bold, the critical points.

ID	POSITION	IRRADIANCE [W/m^2]
L_1	Loading Area, Base DX, in front of the lamp	5.181
L_2	Loading Area, Base center	2.63E-01
L_3	Loading Area, Base SX	3.521
L_4	Loading Area, half-height, SX	7.27E-01
L_5	Loading Area, half-height, DX	7.34E-01
L_6	Loading Area, half-height, centro	2.34E-01
L_7	Loading Area, high, center	2.71E-01
L_8	Loading Area, up, SX	7.03E-01
L_9	Loading Area, up, DX	4.99E-01
L_{10}	Loading Area, side DX, half-height	1.72E-01
L_{11}	Loading Area, side SX, half-height	4.34E-01
W_1	Warehouse Area, Base, front	**1.90E-02**
W_2	Warehouse Area, Base, DX	1.13E-01
W_3	Warehouse Area, Base, SX	3.51E-01
W_4	Warehouse Area, Base, back	4.16E-01
W_5	Warehouse Area, carousel, up, front	1.07E-01
W_6	Warehouse Area, carousel, down, front	6.98E-01
W_7	Warehouse Area, carousel, up, DX	6.87E-02
W_8	Warehouse Area, carousel, down, DX	1.36E-01
W_9	Warehouse Area, carousel, down, SX	3.41E-01
W_{10}	Warehouse Area, carousel, down, back	1.85E-01
W_{11}	Warehouse Area, carousel, up, SX	3.25E-01
W_{12}	Warehouse Area, carousel, up, back	1.40E-01
W_{13}	Warehouse Area, side SX, half-height	3.83E-01
W_{14}	Warehouse Area, side DX, half-height	5.65E-01
W_{15}	Warehouse Area, above reader	1.450
W_{16}	Warehouse Area, back panel, half-height, center-SX	1.063
W_{17}	Warehouse Area, back panel, half-height, center-DX	6.89E-01
P_1	Preparation Area, Base, front	1.10E-01

P_2	Preparation Area, Base, DX	2.49E-01
P_3	Preparation Area, Base, SX	7.67E-01
P_4	Preparation Area, Base, back	6.16E-01
P_5	Preparation Area, Base, back corner DX, in front of the scale	8.09E-01
P_6	Preparation Area, Base, back corner DX, behind the scale	6.49E-01
P_7	Preparation Area, above the scale	7.59E-01
P_8	Preparation Area, shaker, back	9.76E-01
P_9	Preparation Area, shaker, front	6.65E-01
P_{10}	Preparation Area, Base, back corner SX	8.46E-01
P_{11}	Preparation Area, Base, front corner DX	5.34E-02
P_{12}	Preparation Area, Base, front corner SX	2.29E-01
P_{13}	Preparation Area, above the sclae (white support)	1.31E-01
P_{14}	Preparation Area, under the grid DX	8.75E-02
P_{15}	Preparation Area, under the front grid	6.72E-01
P_{16}	Preparation Area, under the grid SX	**9.15E-03**
P_{17}	Preparation Area, under the back grid	**9.57E-04**
P_{18}	Preparation Area, ROBOT forward part	1.54E-01
P_{19}	Preparation Area, ROBOT anterior junction	**1.74E-02**
P_{20}	Preparation Area, ROBOT back part	**5.32E-02**
P_{21}	Preparation Area, ROBOT clamp	3.65E-01
P_{22}	Preparation Area, ROBOT back part, up	**2.94E-03**
P_{23}	Preparation Area, side SX, first shelf	1.363
P_{24}	Preparation Area, side SX, second shelf	**3.89E-02**
P_{25}	Preparation Area, side SX up	**2.70E-02**
P_{26}	Preparation Area, side SX back, up	**4.28E-02**
P_{27}	Preparation Area, side SX back half-height	2.86E-01
P_{28}	Preparation Area, side SX back, down	7.55E-01
P_{29}	Preparation Area, side DX front, down	1.48E-01
P_{30}	Preparation Area, side DX, shelf	**1.15E-02**
P_{31}	Preparation Area, back panel, dosing device, up	8.85E-02
P_{32}	Preparation Area, back panel, dosing device, down	6.14E-01
P_{33}	Preparation Area, back panel, dosing device, center	2.89E-01
P_{34}	Preparation Area, back panel, parte DX, down	4.52E-01
P_{35}	Preparation Area, back panel, parte DX, half-height	**3.36E-02**
P_{36}	Preparation Area, back panel, parte DX, up	9.45E-02
P_{37}	Preparation Area, waste hatch	**2.10E-02**

Table 7-2 Measures of irradiance inside the APOTECAunit system. In bold, the critical points.

ID	POSITION	IRRADIANCE [W/m^2]
L_1	Loading zone, base, DX (under barcode reader)	7.95E-01
L_2	Loading zone, base, center	2.70E-01
L_3	Loading zone, base, SX	7.62E-01
L_4	Loading zone, DX, above barcode reader	1.73E-01
L_5	Loading zone, up, DX	**1.91E-02**
L_6	Loading zone, half-height, SX (level of barcode reader)	2.971
L_7	Loading zone, up, SX	1.42E-01
L_8	Loading zone, side SX, half-height	1.01E-01
L_9	Loading zone, side DX, above barcode reader	1.55E-01
L_{10}	Loading zone, central, half-height (with support)	2.12E-01
L_{11}	Loading zone, central, up (with support)	8.02E-02
W_1	Warehouse area, base, central front	7.51E-02
W_2	Warehouse area, base, central DX	5.27E-01
W_3	Warehouse area, base, central SX	3.85E-01
W_4	Warehouse area, base, central back	3.04E-01
W_5	Warehouse area, under the grid, central	**3.68E-03**
W_6	Warehouse area, carousel, front, down	1.00E-01
W_7	*Warehouse area, carousel, front, up	2.731
W_8	Warehouse area, carousel, DX, down	1.37E-01
W_9	Warehouse area, carousel, DX, up	5.44E-02
W_{10}	Warehouse area, carousel, SX, down	1.44E-01
W_{11}	Warehouse area, carousel, SX, up	7.22E-02
W_{12}	Warehouse area, carousel, back, down	1.65E-01
W_{13}	Warehouse area, carousel, back, up	1.90E-01
W_{14}	*Warehouse area, carousel internal, front, down	1.008
W_{15}	*Warehouse area, carousel internal, front, up	5.087
W_{16}	*Warehouse area, carousel internal, DX, down	5.48E-01
W_{17}	Warehouse area, carousel internal, DX, up	1.72E-02
W_{18}	*Warehouse area, carousel internal, SX, down	6.38E-01
W_{19}	Warehouse area, carousel internal, SX, up	2.16E-02
W_{20}	*Warehouse area, carousel internal, back, down	1.426
W_{21}	Warehouse area, carousel internal, back, up	4.02E-02
W_{22}	Warehouse area, side front, SX, up	4.02E-01

W_{23}	Warehouse area, side central, DX, up	1.80E-01
W_{24}	Warehouse area, back, center-SX, half-height	1.02E-01
W_{25}	*Warehouse area, back, center-DX, half-height	3.600
P_1	Preparation area, base, front, center	1.66E-01
P_2	Preparation area, base, DX, center	3.12E-01
P_3	Preparation area, base, SX, center	1.65E-02
P_4	Preparation area, base, back, center	1.58E-02
P_5	Preparation area, front grid	4.24E-01
P_6	Preparation area, grid DX	1.18E-01
P_7	Preparation area, grid SX	**4.91E-03**
P_8	Preparation area, grid back	**5.16E-03**
P_9	Preparation area, front corner DX	8.46E-02
P_{10}	Preparation area, back corner SX	**2.08E-02**
P_{11}	Preparation area, front corner SX	2.40E-01
P_{12}	Preparation area, back corner DX, in front of the scale	5.42E-02
P_{13}	Preparation area, spigolo dietro DX, behind the scale	5.35E-03
P_{14}	Preparation area, above the scale	6.83E-01
P_{15}	Preparation area, shaker back	1.73E-01
P_{16}	Preparation area, shaker front	1.406
P_{17}	Preparation area, carter SX	3.68E-01
P_{18}	Preparation area, carter (near shaker)	2.507
P_{19}	Preparation area, robot, front, up	2.34E-01
P_{20}	Preparation area, robot, back (junction)	7.17E-02
P_{21}	Preparation area, robot, clamp	5.35E-02
P_{22}	Preparation area, robot, back, up	**3.20E-03**
P_{23}	Preparation area, side DX, down, center	3.27E-01
P_{24}	Preparation area, side DX, first shelf	5.68E-02
P_{25}	Preparation area, side DX, second shelf	**1.71E-02**
P_{26}	Preparation area, side SX, up	**5.19E-02**
P_{27}	Preparation area, side SX, half-height	**4.03E-02**
P_{28}	Preparation area, side SX, above capping device	2.78E-01
P_{29}	Preparation area, side SX, above capping device	5.74E-01
P_{30}	Preparation area, side SX, middle, center	**3.11E-02**
P_{31}	Preparation area, side SX, down, center	**4.77E-02**
P_{32}	Preparation area, back panel, above dosing device	
P_{33}	Preparation area, back panle, dosing device (center)	4.03E-01

P_{34}	Preparation area, back panel, under dosing device	6.92E-01
P_{35}	Preparation area, back panel, up-DX	7.87E-02
P_{36}	Preparation area, back panel, half-height -DX	2.88E-01
P_{37}	Basket of final products	**1.79E-02**