T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

COMPARING HYDROGEN PEROXIDE VAPORIZATION AND NEBULIZATION TECHNIQUES FOR ENVIRONMENTAL DISINFECTION

TUĞÇE MANAV

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

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THESIS ADVISOR
ASST. PROF. DR. ŞÜKRÜ OKKESİM

ISTANBUL, MAY / 2015

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

ORTAM DEZENFEKSİYONUNDA HİDROJEN PEROKSİT BUHARLAMA VE SİSLEME TEKNİKLERİNİN KARŞILAŞTIRILMASI

TUĞÇE MANAV

YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

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İSTANBUL, MAYIS / 2015

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Tuğçe Manav, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID **520113010**, successfully defended the **thesis** entitled "**COMPARING HYDROGEN PEROXIDE VAPORIZATION AND NEBULIZATION TECHNIQUES FOR ENVIRONMENTAL DISINFECTION**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Prof. Dr. Sadık KARA

Director

Date of Submission : 8 May 2015

Date of Defense : 25 May 2015

As I have never given up, I dedicate my thesis to Myself,

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May 2015 Tuğçe MANAV

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LIST OF SYMBOLS

~	Approximately
°	Degree
>	Greater
μ	Mean
μ	Micro
%	Percentage
σ	Standard Deviation

ABBREVIATIONS

aHP : Aerosolized Hydrogen Peroxide

CI : Confidence Interval

cm : Centimeter

CNC : Computer Numerical Control

CPU : Central Processing Unit

cP : centiPoise (Unit of Dynamic Viscosity)

°C : degree Celsius

h : Height

HAI : Hospital-Acquired Infection

HCAI : Healthcare Associated Infection

HPV : Hydrogen Peroxide Vapor

ICU : Intensive Care Unit

KHz : Kilohertz (10^3)

m^3 : Cubic Meter

MHz : Megahertz (10^6)

ml : Milliliter

mm : Millimeter

MRSA : Methicillin Resistant *Staphylococcus Aureus*

µm : Micrometer

µl : Microliter

NTD : No-Touch Automated Room Disinfection

OR : Odds Ratio

TSA : Triptych Soy Agar

TSB : Triptych Soy Broth

V : Volt

VRE : Vancomycin Resistant Enterococci

W : Watt

WHO : World Health Organization

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SUMMARY

COMPARING HYDROGEN PEROXIDE VAPORIZATION AND NEBULIZATION TECHNIQUES FOR ENVIRONMENTAL DISINFECTION

Tuğçe MANAV

Biomedical Engineering Programme
MSc Thesis

Advisor: Asst. Prof. Dr. Şükrü OKKESİM

Hospital-acquired infections also known as nosocomial infections are health problem and their importance has increased swiftly in recent years. Contaminated objects or surfaces in case of hand contact and air, directly or indirectly have a role in transmission and increase the risk of infection of subsequent patient. Routine cleaning methods by mopping with quaternary ammonium or chlorine-based compounds were inadequate at the rate of >50%. Among the reasons for the inadequate hygiene conditions, the unequal distribution of the disinfectant used, the inability to set the correct contact time, lack of education and time constraints are located. Automatic disinfection robots not only play an important role in the fight against pathogenic microorganisms, but also reduce the risk of new infections occur. These systems are hydrogen peroxide-based and affecting the broad spectrum event; even bacterial endospores and not carrying carcinogenic properties by contrast with alternative disinfectants. Researches continue to work on behalf of a safe and efficient exploration of these systems. Hydrogen peroxide vaporizing or aerosolizing are intended methods to provide disinfection and they are effective only in visible areas, not reach to the closed areas such as the drawer or bottom of the objects that are found in the environment. As a result of experiments which were conducted in 1:4 scale of actual size ICU miniature experimental setup, the technique of vaporizing hydrogen peroxide which was assumed best-in-today and hydrogen peroxide nebulizing by ultrasonic excitation which was formed the hypothesis of this thesis, were compared and found biological and chemical differences between them. Results were matched up with the hypothesis that is to say, fog phase of hydrogen peroxide which was obtained by ultrasonic excitation, can be reach to the all areas such as the drawer even closed when the sterilization bands' color change observed and have more lethal effect on *Pseudomonas aeruginosa* than vapor phase when the colonies counted at the end of each disinfection process.

Keywords: Environmental disinfection, nosocomial infection, hydrogen peroxide vaporizing, ultrasonic excitation, *Pseudomonas aeruginosa*, miniature ICU.

ÖZET

ORTAM DEZENFEKSİYONUNDA HİDROJEN PEROKSİT BUHARLAMA VE SİSLEME TEKNİKLERİNİN KARŞILAŞTIRILMASI

Tuğçe MANAV

Biyomedikal Mühendisliği Programı

Yüksek Lisans Tezi

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Hastane enfeksiyonu olarak bilinen nozokomiyal enfeksiyonlar birer sağlık sorunu olup son yıllarda önemi hızla artmıştır. El ile temas halinde kontamine olmuş nesneler, yüzeyler ve hava, doğrudan veya dolaylı olarak transmisyonda önemli rol oynamakta ve bir sonraki hastanın enfeksiyon riskini de arttırmaktadır. Kuaterner amonyum veya klor esaslı silme işlemleri ile yapılan rutin temizleme yöntemleri ile yapılan rutin temizleme yöntemleri >%50 oranında yetersiz kalmaktadır. Yetersiz hijyen koşullarının sebepleri arasında kullanılan dezenfektanın eşit dağılımının ve doğru temas süresinin ayarlanamamasının yanı sıra eğitim eksikliği ve zaman kısıtlaması yer almaktadır. Otomatik dezenfeksiyon robotları patojenik mikroorganizmalarla mücadelede önemli rol oynamakta, yeni enfeksiyon oluşma riskini de azaltmaktadır. Güvenli ve etkin bir keşif adına çalışmaların devam ettiği bu sistemler, bakteri endosporuna dahi etki eden geniş spektrumlu etkinlikte ve alternatif dezenfektanların aksine karsinojenik özellik taşımayan hidrojen peroksit tabanlıdırlar. Hidrojen peroksit buharlama veya aerosolleme yöntemleri ile dezenfeksiyon sağlamayı amaçlayan bu sistemler yalnızca görünür yüzeylerde etkili olup, çekmece gibi kapalı alanlara ve ortamda bulunan cisimlerin altına ulaşmamaktadır. 1:4 oranında minyatürize edilmiş yoğun bakım deney düzeneğinde yapılan deneyler sonucunda, günümüzde var olan tekniklerin en verimlisi olduğu varsayılan hidrojen peroksit buharlama ve bu tezin hipotezini oluşturan ultrasonik uyarım ile hidrojen peroksit sisleme teknikleri arasında kimyasal ve biyolojik farklar olduğu saptanmıştır. Sonuçlar hipotezi tutarlı kılmış, ultrasonik uyarım ile edilen hidrojen peroksit sis fazının, sterilizasyon bantları üzerindeki renk değişimi gözlemlenerek çekmece gibi kapalı alanlara ve ortamda bulunan cisimlerin altına ulaşabilmekte olduğu ve *Pseudomonas aeruginosa* bakterisi üzerinde daha fazla öldürücü etkiye sahip olduğunu göstermiştir.

Anahtar kelimeler: ortam dezenfeksiyonu, nozokomiyal enfeksiyon, hidrojen peroksit buharlama, ultrasonik uyarım, *Pseudomonas aeruginosa*, minyatür yoğun bakım.

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Infections that related to healthcare called as nosocomial infections or HCAIs or HAIs are major health problem. Some of the microorganisms are factors that colonized healthcare facility environmental surfaces especially frequent hand contact **[1,2]** and contagious objects, surfaces, and air can be either directly or indirectly involved in the transmission pathway as well **[10]**.

Epidemiologically important pathogens are MRSA, VRE, *Acinetobacter*, norovirus, *Clostridium difficile* **[4-7]** and *Pseudomonas aeruginosa* **[10]**. These organisms can continue to populate on inanimate objects for a long time **[19]**. Because of the survivability and antimicrobial agents resistivity of these pathogens, routine cleaning by wet mop technique with quaternary ammonium compounds or chlorine-based product **[72]** for high touch surfaces is not an extinctive method and usually have failure **[57,58]**. Inadequate environmental hygienic conditions are risk factors for developing infection **[68]** so cleaning flaws are a significant factor of outbreak **[25]**. Sufficient distribution of the active matter **[23]**, ensuring correct contact time for the microbial reduction **[24]**, contamination of cleaning solutions or materials **[26,27]**, lack of training, education and time to do job properly **[25]** are another critical drawbacks of conventional cleaning. As mentioned in the literature, the highest infection rates are in ICU patients **[41]**, roughly three times higher than another place in hospitals **[50]**.

Automated disinfection systems in removal of the pathogenic microorganisms that colonize in the environmental media, plays a significant role in the fight against nosocomial infections. Besides, it is reported that these automated systems feature in a reduction in the rate of new infections acquisitions throughout the hospital **[14]**.

Researches maintain to explore safe and effective way of the disinfection automated systems. For this objective, hydrogen peroxide is privileged antiseptic by reason of its essential benefits which are broad spectrum effectiveness, that involves impact against even bacterial endospores, deficiency of peripheral toxicity following exact degradation into water and oxygen, residual free, surface erosiveness and non carcinogenic to human in contrast with alternative disinfectants **[12]**. Terminal room disinfection with hydrogen peroxide has been recommended to decrease the frequency and level of contamination on environmental surfaces because of conventional cleaning of room surfaces by environmental services personnel is inadequate **[11,20,23-27]**. NTD systems have been developed to improve terminal room disinfection in order to decrease the risk of a subsequent patient admitted to the room developing infection or colonization with any multidrug resistant organism.

In recent years, different types of NTD systems are currently used in clinical healthcare settings which are HPV and aHP systems **[3]**. Biocidal influence of both methods on healthcare related pathogens and used disinfectant of hydrogen peroxide is residual free and not cause health or safety apprehensions are profits although the implementation length of time require for specialized equipment and well-trained staff, rooms fully discharged from people due to obstruct daily cleaning and needs certain parameters are mentioned as losses in the prior literature **[1]**.

In this research, we desire to shed light on whether the hydrogen peroxide particle size that is effective only on the apparent surface or it does not reach closed or indoor areas like drawer and closet and the strength of the particle is insufficient in order to provide adequate penetration rather than the well known disadvantages of disinfection method of HPV and aHP **[30]**. Our study, atomizing the size of hydrogen peroxide with ultrasonic excitation in order to reduce particle size in minimal level, by this means increasing the percentage of penetration and disinfection with diminished particle size and with high condensation of liquid chemicals is also aimed to saving at the same time.

As it is stated in the literature, ultrasonic excitation for atomization produces very small droplets which resulting size distributions are very narrow and droplet diameter is controlled by ultrasonic frequency **[31]** also minimal available range of size

distributions in resultant particles **[37]**. It is likely to shape particles whose median diameter enlarges from 0.3 µm to 0.4 µm by use of atomizers that are operating roughly 1.8 MHz **[89]**. The magnitudes of the droplets depend on the frequency of the ultrasonic wave and the specifications of the liquid. Ultrasonic fountain that is atomization with high frequency between 300 KHz to 3 MHz happens and atomization is from above the fountain forming a fine, stable fog **[89]**. Medical nebulizers which are examples of fountain atomization and have popularity of atomizing by ultrasonic excitation **[32]**, were unsuitable for delivery of viscous fluids since nebulization was intermittent or completely ceased at >1.92 cP (centiPoise) **[98]** due to this reason, hydrogen peroxide reaches the condition of the liquid specifications based on the compatibility of hydrogen peroxide, this technique can be an alternative for disinfection.

After all, when it comes to experimental process, cell culture dish that includes *Pseudomonas aeruginosa* colony, is placed in the hand-crafted 1:4 scale of actual size ICU experimental setup. First of all, the environment is tested via hydrogen peroxide vaporizing. After the test process, detecting the bacteria that left behind is determined. Then, the new phase is started via hydrogen peroxide nebulizing which is run with also cell culture dishes that includes new cultivated bacteria and rest of the procedure is the same as previous. In this context, hydrogen peroxide vaporization and hydrogen peroxide nebulization techniques are used as disinfection of contaminated areas and surfaces. In consequence of this experiment, an opportunity is grabbed to compare not only lethal effect but also distribution force by using sterilization band act as a chemical indicator that indicates color change when expose to hydrogen peroxide. As a result of this, the answer of which technique performed better influence on which surfaces is revealed easily. Based on this comparison and analysis, the following hypothesis can be derived: “The areas and surfaces which are disinfected with hydrogen peroxide nebulization by ultrasonic excitation achieve higher elimination and deep clean in contrast with hydrogen peroxide vaporization.”

1.2 Arrangement of the Thesis

This thesis is organized as follows:

The next chapter starts with elaborating the theoretical framework. In that chapter definitions and background information on the proposed concepts are given as nosocomial infection, impact and frequency of nosocomial infection, patient risk factors, epidemiologically important pathogens and mode of transmission, environmental cleanliness and limitations of cleaning, no-touch automated room disinfection systems and ultrasonic excitation for disinfection.

Chapter three served as materials and method of this thesis and includes methodological approach of the proposed study. This includes justification and argumentation for the methodological choices that were made.

Finally, the study concludes with chapter four, obtained results and discussion are presented. Furthermore, a conclusion is drawn, followed by recommendation.

CHAPTER 2

BACKGROUND INFORMATION

This section contains definition of nosocomial infection; patient risk factors; impact and frequency of nosocomial infection; epidemiologically important pathogens and their mode of transmission; environmental cleanliness and limitations of cleaning; no-touch automated room disinfection systems and targeted aim that becomes evident based upon ultrasonic excitation due to lack of these systems.

2.1 Nosocomial Infection

Infection is the spreading over and reproduction of pathogenic microorganisms which can be able to produce disease in body tissues, notably that causing local cellular damage based on vying metabolism. In this connection, nosocomial infection also called hospital-acquired infection (HAI) or healthcare associated infection (HCAI) can be described as an infection gained in hospital that develops during the course of healthcare treatment and invite remarkable patient illnesses and deaths, prolong the hospital stays, and require additional diagnostic and therapeutic interference **[39]**.

2.2 Impact and Frequency of Nosocomial Infection

Nosocomial infections are worldwide issue that increased morbidity and mortality so induce significant burden both for the patient and for public health **[41]**.

Most countries have inadequate controlling systems for HCAIs and results from studies clearly indicate that each year, a vast number of patients are influenced by HCAIs around the world **[68]**. According to World Health Organization (WHO) program for the control of hospital infections in 1987, over 1.4 million people worldwide suffer from transmissible complications obtained in hospital **[42]**.

Patient safety studies, which were published in 1991, find out drugs, nosocomial infections, and surgical complications are the most frequent types of adverse events which influence hospitalized patients **[43,44]**. In parallel to those studies, the Institute of Medicine reported that these events affect almost 2 million patients each year only in the United States, resulting in 90,000 deaths and a computed $4.5–5.7 billion per year in extra costs for patient care **[45]**. As recently reported in 2013, 99,000 deaths and cost $28 to $45 billion estimated annually by reason of the HCAIs **[3]**.

The maximal contributor to cost is the escalated length of stay for infected patients **[47,48,49]**. Elongated stay not only enhances direct costs to patients or payers but also indirect costs due to the lost of work. The rationale behind of lost work referred to as the enhanced use of drugs, the need for separation, and the use of extra laboratory and other diagnostic studies also promote to costs **[41]**.

As mentioned in the literature, the highest infection rates are in intensive care unit (ICU) patients **[41]**. According to Weinstein (1998), nosocomial infection ratio in adult and pediatric ICUs are roughly three times higher than another place in hospitals that means infection directly related to treatment in ICUs **[50]**. The distribution of microorganism kinds isolated especially at the ICUs in a period of four years is shown in Figure 2.1 **[87]**.

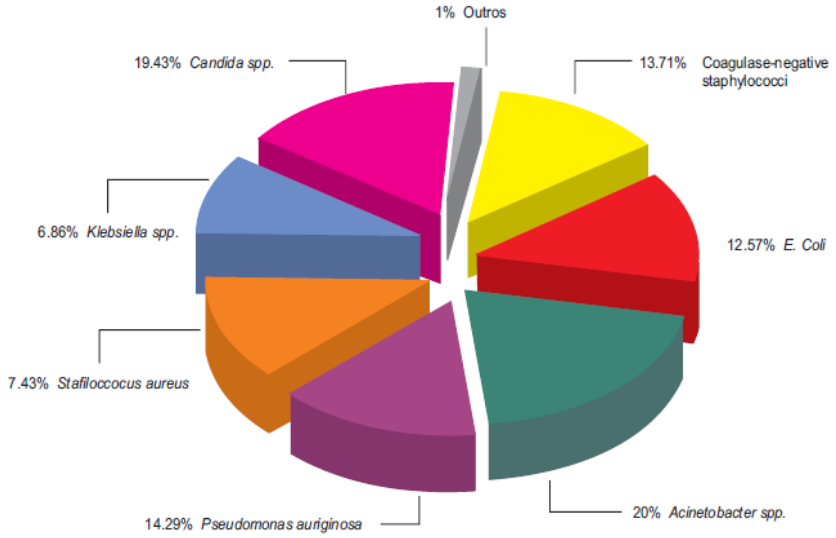


Figure 2.1 Distribution of microorganisms at the ICU **[87]**

2.3 Patient Risk Factors

Scientific review of 1,022 outbreak study demonstrate that the individual patient, medical equipment or devices, the hospital environment, the healthcare personnel, contaminated drugs, contaminated food, and contaminated patient care equipment are the most common sources of contagious agents causing nosocomial infection **[46]**. According to Prevention of HAIs Practical Guide, nosocomial infections can be transferred to the society via deinstitutionalized patients, staff, and visitors. In addition to this, if organisms which are causing infection are resistant, they may cause burden of illness in the community **[41]**.

According to Collins (2008), patient's endogenous flora as remnant bacteria staying on the skin, mucous membranes, gastrointestinal tract, or respiratory tract which may be hard to overcome so room touch surfaces, equipment, and medications that have become contaminated and carry risk factor. Immunocompromised patients due to old age or underlying diseases utilize extrinsic and intrinsic devices and more time for experience to exogenous microorganisms, improper use of invasive devices and antibiotics and extended length of stay which means prolonged hospitalization are also risk factors to fast microbial colonization **[39]**. In addition to all these, cross infection which means a microorganism gained from another patient, is also a patient risk factor

for developing infection because previous patient had been infected with pathogens has been posed a risk for the next patient. Pathogens may contaminate objects, devices, and materials which subsequently contact impressible body sites of patients under the crowded conditions within the hospital, frequent transfers of patients from one unit to another **[8-10]**.

Besides, insufficient environmental hygienic conditions and waste disposal; weak infrastructure; insufficient equipment; understaffing; overcrowding; weak knowledge and application of basic infection control measures; lack of procedure; are determinants of HCAIs **[68]**.

2.4 Epidemiologically Important Pathogens and Mode of Transmission

Characteristics of the microorganisms which are resistance to antimicrobial agents, subsistent lethality, and inoculums of morbific supplies yield spearheading to infection so many different bacteria, viruses, fungi and parasites may cause nosocomial infections **[41]**. There exist many studies in scientific literature which sheds light on the importance between environmental contamination and transmission of pathogens. Contaminated environmental surfaces promote transmission of pathogens to patients based on including the ability of pathogens to remain viable on a variety of dry environmental surfaces because of antimicrobial agents' resistivity.

It should be noted that most of the prior literature is highlighted on healthcare associated pathogens, including methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), *Acinetobacter*, norovirus, and *Clostridium difficile* **[4-7]** and *Pseudomonas aeruginosa* **[10]**. As it can be seen from Table 2.1, these organisms are able to survive in healthcare environments **[4]** for hours to days or in some cases for months **[19]** and they may give cause for disease outbreaks.

By reason of this differences of time interval is because of the surface type. For instance, it takes for one week to two months on countertops but for more than seven days on fabric. On the other hand, on dry polyvinyl chloride surfaces the time interval is from seven days to four months and for a few days to more than three months on plastic surfaces **[51-54]**.

Type of bacterium	Duration of persistence (range)
Acinetobacter spp.	3 days to 5 months
Bordetella pertussis	3 – 5 days
Campylobacter jejuni	up to 6 days
***Clostridium difficile* (spores)**	5 months
Chlamydia pneumoniae, C. trachomatis	≤ 30 hours
Chlamydia psittaci	15 days
Corynebacterium diphtheriae	7 days – 6 months
Corynebacterium pseudotuberculosis	1–8 days
Escherichia coli	1.5 hours – 16 months
Enterococcus spp. including VRE and VSE	5 days – 4 months
Haemophilus influenzae	12 days
Helicobacter pylori	≤ 90 minutes
Klebsiella spp.	2 hours to > 30 months
Listeria spp.	1 day – months
Mycobacterium bovis	> 2 months
Mycobacterium tuberculosis	1 day – 4 months
Neisseria gonorrhoeae	1 – 3 days
Proteus vulgaris	1 – 2 days
Pseudomonas aeruginosa	6 hours – 16 months; on dry floor: 5 weeks
Salmonella typhi	6 hours – 4 weeks
Salmonella typhimurium	10 days – 4.2 years
Salmonella spp.	1 day
Serratia marcescens	3 days – 2 months; on dry floor: 5 weeks
Shigella spp.	2 days – 5 months
***Staphylococcus aureus*, including MRSA**	7 days – 7 months
Streptococcus pneumoniae	1 – 20 days
Streptococcus pyogenes	3 days – 6.5 months
Vibrio cholerae	1 – 7 days

Table 2.1 Persistence of clinically relevant bacteria on dry inanimate surfaces **[19]**

Coughing, sneezing, talking and suctioning, which are routes of transmission, tend to emergence of respiratory droplets which includes microorganisms. Airborne spread is referred to as small particle size microorganisms remain hanged in the air for a long time, they can expand to other people by this way.

In this case, contaminated food, water, medications, solutions, devices or equipment are the common transmission vehicles **[39]**. Moreover, infections may be obtained from a contaminated inanimate object or substances or air via another human source which is called as cross infection.

In the American journal of medicine in 1991, approximately 20% to 40% of health care associated infections have been predicated on cross infection via the hands of health care staff **[2,3]** either direct patient contact or indirectly from touching contaminated environmental surfaces in patient's rooms **[40]**.

As represented by Otter et al. (2011), pathogen transfer from an affected patient to host occurs mostly through the hands of healthcare workers, but contagious objects, surfaces,

and air can be either directly or indirectly involved in the transmission pathway as can be illustrated in Figure 2.2 **[10]**.

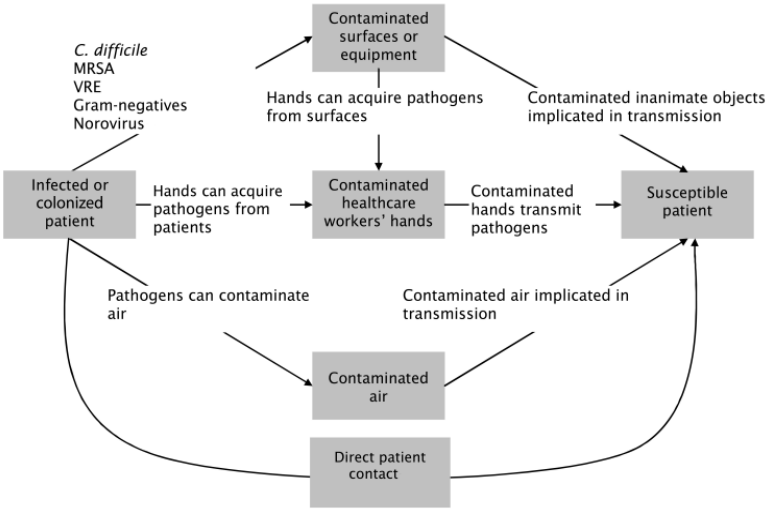


Figure 2.2 Generic transmission routes **[10]**

2.5 Environmental Cleanliness and Limitations of Cleaning

In 1999, Dancer (1999) said "Hospital cleaning is the *'Cinderella'* of infection control" **[25]**.

The term "cleaning" is the removal of all foreign material such as soil or organic material from objects and it is normally carried out with water, detergents or enzymatic products. Environmental cleanliness especially in hospital is requirement of preventing pave the way for transmission potential pathogens.

As it has been mentioned in many scientific literature, cleaning or disinfection of the environment can decrease transmission of healthcare associated pathogens. According to Boyce (2007), the role of contaminated environmental surfaces in transmission of healthcare associated pathogens is also reinforced by the fact that cleaning or disinfection can minimize the incidence of healthcare associated colonization or infection **[5]**. Main interferences that used to control health care associated infections include observation **[59,60]**, separation of patients with infectious diseases **[55]** or multidrug resistant pathogens **[61]**, suitable skin antisepsis before invasive routines and hand hygiene by medical staff **[62]**, and proper disinfection. In addition to what is mentioned previously, sterilization of medical devices and environmental surfaces take

into consideration in this context. **[63,64,65]**. Because of these reasons, the preciousness of cleaning comes to the forefront one more step.

The efficacy of surface disinfection and cleaning in reducing microbial loads and their dissemination with routine cleaning which is a wet mop technique with quaternary ammonium compounds or chlorine-based product **[72]** usually have ineffectualness and when combined with a detergent, also encourage spore formation **[57,58]**. Surroundings of patients recontaminate swiftly, but cleaning fluids also a vehicle of expands of bacteria from one patient to another. The ratio of using disinfectant in fluids for cleaning ward furniture may be potential for cross infection and this requires extra observations in high risk areas such as ICUs and neonatal units.

Pathogens contaminate surfaces commonly touched by patients and healthcare workers that means high touch surfaces. These surfaces are as follows; bedside rails, blood pressure cuffs, television remote control devices, bedside tables, toilet seats, toilet rails, toilet dressers, door handles and intravenous pumps. Routine cleaning method for high touch surfaces is not an extinctive method by itself in order to completely get rid of pathogens from contaminated surfaces because of the survivability and resistivity of antimicrobial agents of health care associated pathogens.

Many cleaning solutions are seriously contaminated and potentially may redeliver organisms into areas where they were not previously **[27]**. Manual cleaning is not only unsatisfying efficacy in removing microorganisms but also occupational health and safety risk for cleaners as well because of prolonged exposure to disinfectants **[72]**. Therefore, enhanced methods of disinfecting are required for elimination of pathogens from the hospital environment **[5]**.

As indicated existing literatures' authors, cleaning flaws were a significant factor of outbreak **[25]**. To minimize the rate and level of contamination of environmental surfaces and medical equipment in hospital area, routine disinfection with a germicide which is an agent that demolishes microorganisms [**71]**, well advised **[66]** but regrettably, routine cleaning of surfaces by environmental services personnel and medical equipment by nursing staff is frequently inadequate **[67]**. Existing studies in each of the 23 acute care hospitals have demonstrated adequate environment cleaning is

frequently lacking because more than 50% area not disinfected as can be seen in Figure 2.3 **[11]**.

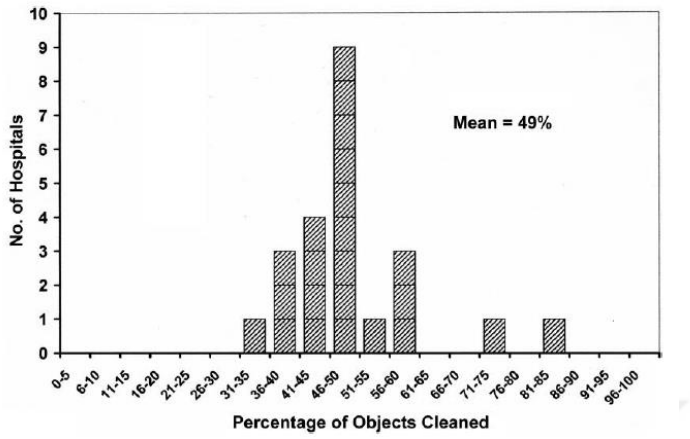


Figure 2.3 Overall percentage of high risk objects determined to have been cleaned in each of the 23 acute care hospitals **[11]**

Antiseptics are antimicrobial which are implemented to the skin or mucous membranes to lessen the microbial flora. Various nosocomial outbreaks have proceeded from insufficient antisepsis, lack of intrinsic antimicrobial activity, a resistant pathogen, an incorrect choice, over dilution, or the use of a contaminated antiseptic. Disinfectants are carried out to inanimate objects to demolish harmful microorganisms. The insufficient disinfection of medical devices or environmental surfaces may consist from the same reasons of antiseptics, additively an inadequate duration and lack of contact between the disinfectant and the microbes **[26]**.

Dettenkofer et al. (2005) pointed out use of liquid disinfectants are not effective against; pathogens can spread other surfaces or may harm equipment, especially electronics, and may corrode metals **[20]**. Adequate distribution of the active agent **[23]**, ensured correct contact time for the microbial reduction **[24]**, contamination of cleaning solutions or materials **[26,27]**, lack of training, education and time to do job properly **[25]** are another serious obstacles of conventional cleaning. As informed in 2005, 236 articles retrieved and none of these studies showed only cleaning with detergent lower the infection rates associated with routine disinfection of surfaces; especially floors. **[20]**. According to Barker et al. (2004), detergent-based cleaning fails to eliminate pathogens,

and even after the use of a combined detergent formulation, contamination still be appointed up to 28% on the surfaces **[56]**.

According to Linley et al. (2012), oxidizing agents in the medical arena ought to be applicable for hard surface disinfection, having broad spectrum activity, which includes efficacy against bacterial endospores, being lack of environmental toxicity following their complete degradation, non corrosive and scentless **[12]**. Among the various disinfectants that perform most of the aforesaid requisites are hydrogen peroxide and its compounds in that good efficiency in terms of disinfection **[70]** and might well to substitute currently used substances that have toxicological standpoint, such as chlorine and its derivatives **[20]**.

Taking everything into account, hydrogen peroxide is favored antiseptic due to its main advantages that are broad spectrum activity, which includes efficacy against even bacterial endospores, lack of environmental toxicity following complete degradation into water and oxygen, residual free, surface corrosiveness and non carcinogenic to human when compared with other disinfectants **[12]**.

On the other side, Liochev et al. (1999) point out that, hydroxyl radical and other oxygenated species can act as potent oxidizing agents, reacting with lipids, proteins and nucleic acids so it is easy to comprehend antimicrobial effects of hydrogen peroxide **[13]**. Last but not least, most of the previous studies indicate that hydrogen peroxide is particularly interesting for its application in liquid but also vaporized form for antisepsis and for the disinfection of surfaces.

2.6 No-Touch Automated Room Disinfection Systems

Powerful cleaning and disinfection via conventional methods responsible for correctly choose and formulate a convenient agent and distribute the agent to all surfaces for required contact time. Advancement of conventional methods based on alteration of human behavior, which is mostly troublesome. The use of newly developed no-touch automated room disinfection (NTD) systems obtains an option. Automated systems have been embraced greatly in other areas of healthcare in order to minimize human fault such as robotic surgery and many aspects of critical care like ventilators **[3,73-75]**.

Dr Robert Weinstein commented on the future of infection control and wrote: 'Given the choice of improving technology or improving human behavior, technology is the better choice' **[50]**.

NTD systems are gaining acceptance as a useful tool for infection prevention and control. These systems enable to ensure accurate distribution and contact time, process repeatability, improve the level of disinfection and reduce the increased risk from the prior room occupant. Passaretti et al. (2013) compared NTD with standard disinfection methods and found that NTD was associated with a 64% reduced risk of patients acquiring any multidrug resistant organisms **[14]**. French et al. (2004) asserted that NTD is more effective than manual cleaning for removing environmental microbial contamination, for example by MRSA **[18]**. The study was demonstrated by Boyce et al. (2008) **[22]** and Manian et al. (2010) **[21]**, a reduction in C. difficile infections following the use of NTD also an association with a significant hospital wide reduction in the incidence of VRE.

Based upon the recent literatures that claim hydrogen peroxide-based NTD has recently increased in popularity for disinfection of hospital wards and for the terminal or discharged room disinfection **[15-17]**. These systems have important differences in their active agent, delivery mechanism, efficacy, process time and ease of use. With such kind of systems, disinfectant can reach many areas as distinct from routine cleanings but need physically cleaned of dirt and debris in that these systems can only be used for terminal room disinfection means room must be emptied of people so they do not take the place of conventional disinfection methods, in short running together. Available NTD systems include hydrogen peroxide vapor systems (HPV) and aerosolized hydrogen peroxide (aHP).

Vaporous disinfection contains the implementation of a disinfectant in the vapor/gas phase to disinfect enclosed spaces or sensitive equipment.

The impressiveness of the vaporous disinfection process is attached to concentration of disinfectant, duration of exposure, rate of reaction, temperature, humidity, and the nature of the contaminated material **[79]**.

Vapor phase fumigants which are formaldehyde, chlorine dioxide, and hydrogen peroxide, were used. Formaldehyde and chlorine dioxide vapors are efficient in

eradicating microorganisms, but their use is associated with potentially toxic end products that need particular disposal techniques **[81]**. In consequence of the handicaps of formaldehyde and chlorine dioxide vapors, hydrogen peroxide vapor becomes crucial among those for disinfection procedure. Several types of hydrogen peroxide-based room disinfection systems have become an important and obtainable to the market.

HPV systems have been used mostly for disinfection of pharmaceutical applications **[77,78]** and this method has more lately been performed for the disinfection of animal rooms, as an option to formaldehyde because the vapor breaks down into water and oxygen, the procedure has none of the environmental concerns and leaving no harmful by products associated with formaldehyde. **[76]**. Slow acting, long exposure times, difficult residue removing, toxic, carcinogenic, strong irritant and requires high humidity for efficacy are other reasons for not to choose formaldehyde **[76]**.

HPV systems which showed in Figure 2.4 **[82,83]**, deliver a heat generated vapor of 30-35% aqueous hydrogen peroxide solution through a high velocity air stream and generates particles 1 μm in diameter to achieve homogenous distribution throughout an enclosed area **[29]**. In contrast to HPV systems, aHP systems are using of spray technique of liquid disinfectants is a relatively common method for disinfecting patient rooms **[80]** which are showed in Figure 2.5 **[84,85]**, are aerosolizing a solution containing a lower concentration of hydrogen peroxide solution with <50 ppm silver **[28]** typically 5-6% and composed of particles range 8-12 μm, in diameter **[29]**.

Figure 2.4 HPV technologies **[82,83]**

In 1972, the efficacy of spraying is published and concluded that had no role in the terminal disinfection of hospital rooms by Centers for Disease Control and Prevention

National Nosocomial Infections Study **[80]**. Spraying techniques that is aerosolized relatively large particles of disinfectant that is not completely dispersed throughout the enclosed space **[29]**.

Aerosolized hydrogen peroxide achieves >3log$_{10}$ mean destruction of microorganisms **[86]** however a system using hydrogen peroxide vapor has been demonstrated to completely inactivate >6log$_{10}$ *Geobacillus stearothermophilus* spores contained in biological indicators in patient rooms **[23]**.

Figure 2.5 aHP technologies **[84,85]**

Reliable biocidal efficacy of both systems on healthcare associated pathogens and used disinfectant of hydrogen peroxide is residual free and not give rise to health or safety concerns are advantages but application length of time need for specialized equipment and trained personnel, rooms completely evacuated from people because of the unbecoming daily cleaning and requires precise parameters are expressed as disadvantages in the existing literature **[3]**.

In addition to them, hydrogen peroxide is effective on the apparent surface but does not reach ‘hook line and sinker’ means that particle size that is effective on the apparent surface or it does not reach closed or indoor areas like drawer and the strength of the particle is insufficient in order to provide adequate penetration rather than the well known disadvantages of disinfection method of HPV and aHP **[30]**.

If hydrogen peroxide particle size in diameter is smaller than the current state, this means increasing the percentage of penetration and disinfection with minimized particle size and with high condensation of liquid chemicals is also aimed to saving at the same

time. For this reason, we need to generate very small droplets which resulting size distributions are very narrow and droplet diameter is able to be controlled.

2.7 Ultrasonic Excitation for Disinfection

The phenomenon referred to as ultrasonic excitation has its references in late 19th century acoustical physics **[92]**. This method can be used to atomization of the liquids by generating very small droplets which resulting size distributions are very narrow and droplet diameter is controlled by ultrasonic frequency **[31]** also minimal available range of size distributions in resultant particles **[37]**.

An aerosol generated by beam of ultrasound near the surface of a liquid. The dimensions of the droplet based on the frequency of the ultrasonic wave and the properties of the liquid. It is possible to form particles from liquids whose median diameter extends from 0.3 μm to 4 μm by using atomizers which are functioning around 1.8 MHz **[89]**.

Atomizers can be used to test ventilation efficiencies**.** In 2003, Lozano et al. remarked that the use of ultrasonic atomizing devices is becoming increasingly popular for a number of applications, for instance in domestic humidifiers, or medical nebulizers **[32]** where small droplets are released for inhalation **[97]**. The respiratory therapy literature (e.g., Spearman et al., 1982, Kacmarek et al., 1985) contains numerous descriptions of the application of nebulizers employing ultrasonic transducers **[34,35]**.

Ultrasonic atomization offers three substantial characteristics which are a finely controlled particle size, stately atomization and an easily modification the atomization rate. There are two modes of ultrasonic atomization of liquids which are layer atomization with low frequency and fountain atomization with high frequency. When ultrasonic fountain occurs, atomization is in the upper part of the fountain forming a fine, stable fog **[89]**.

A transducer is any device used to transform energy from one form to another as can be seen in microphone, which transforms the sound waves generated by a voice or instrument, to the electrical impulses in the form of manned sound. In case piezoelectric

transducers are used to transform the electrical charges into energy, in a word, the "piezoelectric" literally means that electricity occasioned by pressure **[93]**.

Vatansever et al. (2012) mentioned that piezoelectric effect exists in two domains as follows; direct piezoelectric effect and inverse piezoelectric effect, respectively. Direct piezoelectric effect converts mechanical energy to electrical energy which is also known as generator/transducer effect. On the other hand, the inverse piezoelectric effect transforms electrical energy to mechanical energy which is also known as motor/actuator effect **[33]**. Krautkrämer et al. (1990) implied that the inverse piezoelectric effect is used in production of ultrasonic sound waves **[36]** so this effect over a liquid is an acoustic pressure wave the result is the atomizing of the liquid then fog is formed **[38]** with correct piezoelectric excitation.

Ceramic piezoelectric transducer which is manufactured from titanium for good acoustical properties, high tensile strength, and excellent corrosion resistance showed in Figure 2.6 **[90]**, convert electrical energy into mechanical energy by way of produce high frequency sound waves over the range of human hearing. The size of particle that is generated by ultrasonic sound waves can be reached to minimal level with frequency dependent available range of size.

Figure 2.6 Ceramic piezoelectric transducer **[90]**

Electrical input is received from the transducers in the form of a high frequency signal, from a power generator and converted into juddering motion at the same frequency. Titanium cylinders amplify the motion and increase the vibration at the atomizing surface. The liquid absorbs some vibrational energy, which is transformed into standing waves called as capillary waves that is evinced in Figure 2.7, form a grid pattern in the liquid on the surface **[92]**.

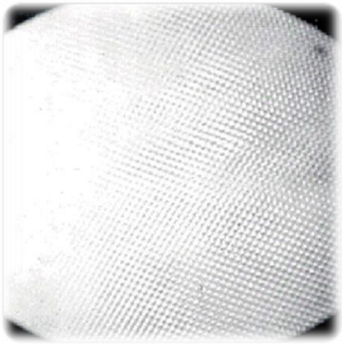


Figure 2.7 Capillary waves **[92]**

For estimation of the droplet size of an ultrasonically generated aerosol is based on the assumption that standing waves which are capillary waves are formed at the surface of the liquid. Lines at the surface are pulled apart and cause the formation of droplets. Atomization has perfect advantage that the droplet size depend the frequency of the ultrasonic excitation and the nature of the liquid. The characteristic of droplet size distribution depends on the function of diameter and the droplet diameter depends on the function of frequency **[89]**.As showed in Figure 2.8 **[92]**, when the liquid is vibrated in a direction to its surface, capillary waves have a period which is double that of the starting vibration **[88]** and it has measurable magnitude **[89]**.

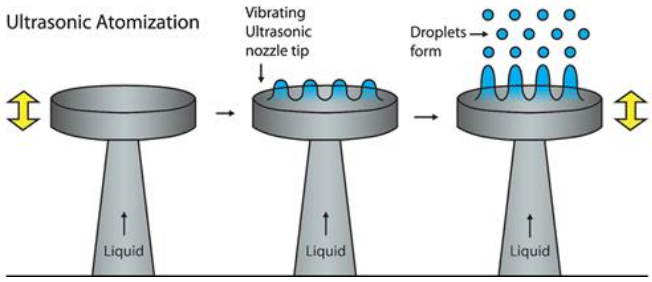


Figure 2.8 Ultrasonic atomization stages **[92]**

Particle size that can be controlled by depending on the frequency value is a key contributing cause in order to selection of ultrasonic excitation method. Another significant cause for selection of this method, ultrasonic excitation is obtained by nebulizer device which is also produce atomization by the time the ultrasonic field exceeds a certain threshold value. This value depends on the viscosity of the liquid **[89]**.

Normally, nebulizer device works with water and the viscosity of water is 1.002 cP at 20^{o} C showed in Figure 2.9 **[95]**. However, hydrogen peroxide is also formed into fog state by ultrasonic excitation because of the close enough viscosity value with water is 1.245 cP at the same temperature which is represented in Figure 2.10 **[94]**.

In order to confirm this fact the following statement can be given; "nebulizers were unsuitable for delivery of viscous fluids since nebulization was intermittent or completely ceased at >1.92 cP **[98]**". As is evident from here, hydrogen peroxide can be experienced as of disinfection by ultrasonic excitation. Thus, insufficient particle problem that is one of the disadvantages of NTD systems using for existing environment disinfection is eliminated and as a result of this, more powerful penetration with smaller particles is provided. As it is indicated the prior literature, HPV systems are much better compare to aHP systems because of particle size difference and HPV achieves more destruction on microorganism. We are planning to get better of HPV system in terms of attaining much better penetration and destruction with much smaller number of particles by using ultrasonic excitation.

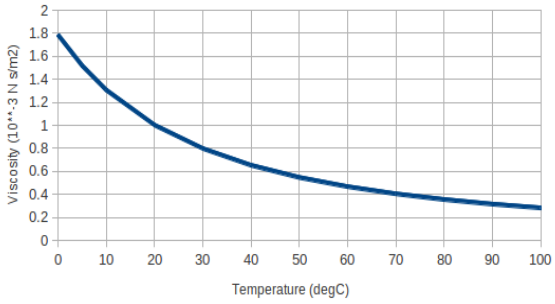


Figure 2.9 Viscosity of water **[95]**

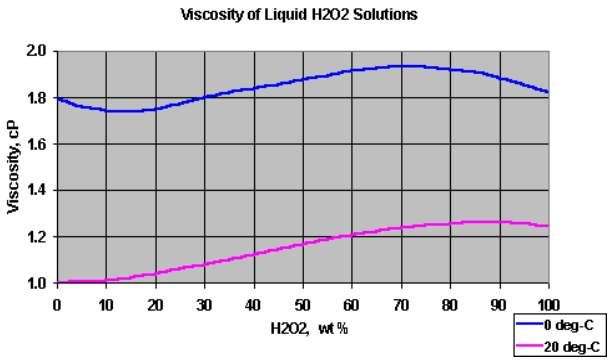


Figure 2.10 Viscosity of liquid hydrogen peroxide **[94]**

CHAPTER 3

MATERIAL AND METHOD

3.1 Materials

Experimental setup equipments and laboratory equipments used in this research were listed in Table 3.1.

Table 3.1 Experimental setup equipments and laboratory equipment

Experimental Setup Equipments	Laboratory Equipments
120cm*120cm*70cm (h) Acrylic Glass	*Pseudomonas aeruginosa*ATCC27853
ICU Miniatures; Sickbed, Bed-Side Monitor, Overbed Table	Tryptic Soy Agar Tryptic Soy Broth
Heating coil	Cell Culture Dishes
Nebulizer	Autoclave
Fan & Aspirator	Refrigerator
Aqueous Hydrogen Peroxide Solution	Incubator
Thermohydrometer	Cell Scraper
Sterilization Band	Bunsen burner

3.2 Method

This section includes methodology which consists of, construction period of experimental setup, bacterial contamination and disinfection scenarios (hydrogen peroxide dilution, vaporizing hydrogen peroxide by heating coil, atomizing hydrogen peroxide by nebulizer), bacterial counting process before and after scenarios and comparison distribution force of vapor and fog by sterilization band.

3.2.1 Construction Period of Experimental Setup

There are three major reasons why we prefer intensive care unit are as follows; nosocomial infection ratio in ICUs are roughly three times higher than another place in hospitals **[50]**, high touch surfaces carry high risk factors mostly in ICUs **[5]** and concentration of patients more susceptible to infection in ICUs **[42]**. Because of these reasons we planned and decided to study on intensive care unit for the comparison of disinfection efficiency.

The experimental procedure of this study is implemented in a custom made transparent acrylic glass experimental case with a custom made miniature ICU instruments rather than a real hospital ICU. The rationale behind of using custom made equipments is to eliminate the disadvantages and obstacles of real hospital environment, and also in order to approach real hospital conditions at most as well.

We do not make any demand on using real ICU at any hospital because the scarcity of ICUs in hospitals. When we consider the hospital environment, there is always high volume of circulation. Even if we start this experiment at hospital, there is a time limitation because this experiment extends over time which means the experiment process takes nearly more than a month.

In addition to this, there can be some factors that affect the experiment negatively such as uncontrollable or unpredictable staff actions. Besides, we do not wish to increase idle time cost of the hospital.

On the other hand, using custom made miniature ICU provides unique benefits compare to the real hospital ICUs. These benefits can be stated as follows; easy to use and

control, immediate intervention, rapid and effortless to cleanliness/ventilation, ergonomic and modular design, easy to monitor and convenience to transportation.

In order to take advantage of those benefits, we had limited alternatives in decision making of experimental setup cover material; glass and acrylic glass respectively. Acrylic glass was preferred for this study because of competitive advantage. To clarify this fact, acrylic glass is lighter, more drillable and shapeable by lathe, more endurable for chemical and more heat tolerant than glass. These features are the main essentials that were claimed at the production stage of the experimental setup.

Before the realization of experimental setup, various prototypes are prepared in terms of visualization of scale, size and sample. Several options are created for the measurement of the experiment setup. The optimal alternative which means the most suitable size for field of study is shined amongst others. Therefore, the exact size of the experimental setup is determined as 120*cm*120cm*70cm (h) which is also specified in the following sections as well. Prototype of the experimental setup is designed with Google SketchUp which is a 3D modeling computer program that allows simulate a model easily like terrain. The final prototype is demonstrated in Figure 3.1.

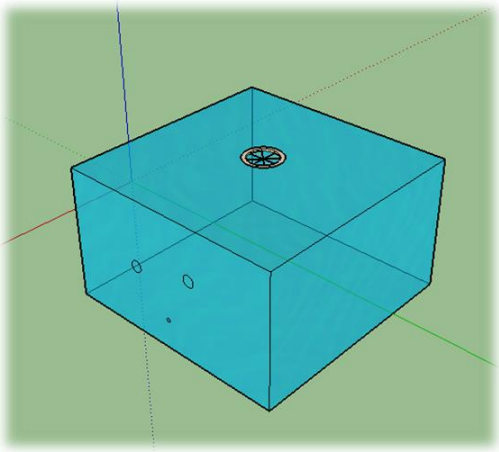
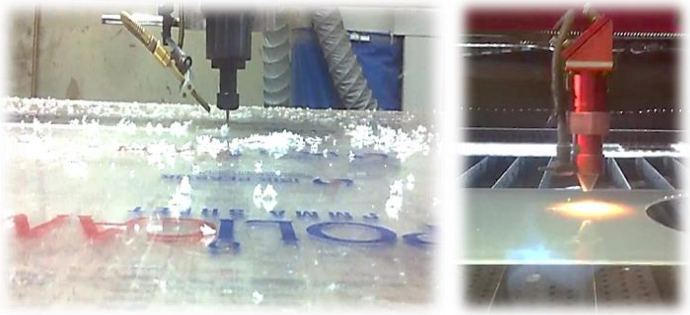


Figure 3.1 Prototype of experimental setup

In consequent of the prototypes, fully custom made experiment mechanism is made of transparent acrylic glass which is polymethylmethacrylate-based thermoplastic material as it is represented in Figure 3.2.

Figure 3.2 Experimental setup

Correspondingly, the fraction process of acrylic glass is performed as of sizes 120cm*120cm*70cm (h) by Partner Techno CNC router as can be seen in Figure 3.3 (a), which has high-speed data processing and also parameters can be entered easily with Turkish control program called as Type Edit 2007. After the fraction phase, the acrylic glass plaques which are attentive to be dry and dust-free are glued with special adhesive named as “chloroform” and as a result of this process, miniature ICU room is created almost 1 cubic meter (m^3).



(a) (b)

Figure 3.3 (a) Partner Techno CNC router and (b) Laser CNC machine

Thereafter, one of the lateral surfaces of acrylic glass plaques were punctured as three round holes by laser CNC machine as it is seen in Figure 3.3 (b), which uses 15 V CO_2 laser. The two of these round holes for the use of two fans which are used to provide with intent to distribution of gas or fog phases of hydrogen peroxide on an equal basis

during the disinfection process of the air tight environment. These two fans are the conventional 12 V Central Processing Unit (CPU) fans as it is shown in Figure 3.4, are corded to modified adaptors which have input 220 V and output 12 V.

The last one is for hose connection that is used to purpose of transferring gas or fog phases of hydrogen peroxide during the disinfection procedure of the hermetic environment as it is represented in Figure 3.5.

Figure 3.4 Sides facing inward and outward of fans

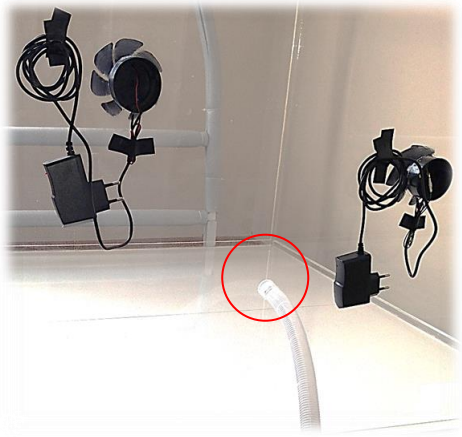


Figure 3.5 Hose connection

With the same laser CNC machine, at the cover part (ceiling) of the experimental case is drilled for hole of aspirator as can be seen in Figure 3.6, which is used to ventilate the enclosed environment at the end of process. Electrical connection of aspirator is made

manually which means connection of plug, cable and on-off button are made functional by hand and it works with 220 V electricity.

Figure 3.6 Sides facing inward and outward of aspirator

In order to avoid any spread of gas or fog phases of hydrogen peroxide, that will be forthcoming from the chimney of the aspirator, to the operating environment during the ventilation process, an aluminum flexible pipe is provided to evacuating to the window as can be seen in Figure 3.7 (a). A suitable tap is produced on the outward side chimney of aspirator in order to prevent any air inlet or outlet to the enclosed environment through the disinfection process which is represented in Figure 3.7 (b).

Figure 3.7 (a) An aluminum flexible pipe and (b) A suitable tap

After the case that act as a “room” was prepared, hand-crafted 1:4 scale of actual size miniatures manufactured with using different materials (glass, wood, plastic, and

textile) as can be seen in Figure 3.8. These miniatures consist of four pieces which are sickbed 25cm*54cm*20cm (h), bed-side monitor 4cm*12cm*10cm (h), sliding mechanism of bed-side monitor 18cm*64cm*50cm (h) and overbed table 10cm*17cm*20cm (h) were placed into the room as it is shown in Figure 3.9 and 3.10.

Figure 3.8 1:4 scale of actual size miniatures

These miniatures consist of four pieces which are sickbed 25cm*54cm*20cm (h), bed-side monitor 4cm*12cm*10cm (h), sliding mechanism of bed-side monitor 18cm*64cm*50cm (h) and overbed table 10cm*17cm*20cm (h) were placed into the room as it is shown in Figure 3.9 and 3.10.

Figure 3.9 Sickbed, bed-side monitor, sliding mechanism of bed-side monitor and overbed table

Figure 3.10 Miniature ICU

3.2.2 *Pseudomonas aeruginosa* Contamination

As in Figure 3.11, *Pseudomonas aeruginosa* is a prevalent bacteria which causes nosocomial infection because of multi-drug resistance feature **[10]**. Another feature is gram negative bacilli that has been described to persist at high humidity **[104]** and longer than gram positive bacilli **[102]**. This bacterium is aerobic human pathogen that causes inflammation and sepsis. In fact, it can be fatal even if critical organs effected like lungs, urinary track and kidney **[103]**.

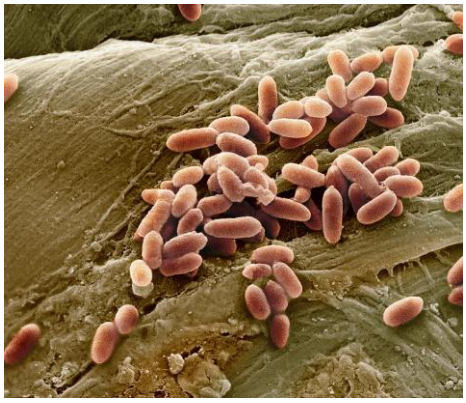


Figure 3.11 *Pseudomonas aeruginosa* bacteria, SEM **[105]**

A recent prospective cohort study specified that prior room occupancy by a patient colonized or infected with *Pseudomonas aeruginosa* is an undeniable risk factor. To be more precise, this is the first evidence from an endemic setting that contaminated surfaces contribute to the transmission of gram-negative (Table 3.2). Nseir et al. (2011) compared the relationship between patients who did acquire the pathogen and patients who did not acquire the pathogen that is listed below the "Variables" row **[99]**.

Table 3.2 Impact of the infection or colonization aspects of the previous room occupant on the gain of *Pseudomonas aeruginosa* by following occupants of the same room **[99]**

Reference	**Nseir et al [99] (2010)**
Setting (Study design)	ICU, France (12-month prospective cohort study)
Findings	Acceptance to a room before occupied by a *Pseudomonas aeruginosa*–positive patient was related to gain of these pathogens
Variables	Prior room occupant with *Pseudomonas aeruginosa*
Acquired	25.6% of 82
Did not acquire	14.9% of 429
Percentage difference	41.7
Adjusted ratio 95% CI (Confidence Interval)	OR (Odds Ratio): 2.3 (1.2–4.3)

In a prospective study in medical surgical ICUs were used serial surveillance cultures and serotyping to detect the endemic sources of *Pseudomonas aeruginosa* and discovered that approximately 40% of patients were colonized and the two thirds of these carriers were positive at the time of admission to the unit **[100]**. In prospective studies in oncology unit were also confirmed that admission colonization with *Pseudomonas aeruginosa* was as frequent as nosocomial acquisition **[101]**.

Lay emphasis on *Pseudomonas aeruginosa* in previous topic, is used during this thesis study due to illustrate the resistance of the bacteria while making comparison between hydrogen peroxide vapor and fog in the belief of clarifying the importance for environmental disinfection.

Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) are growth medias for culturing of *Pseudomonas aeruginosa.* They were prearranged and kept as stock in order to reduce the costs and loss of time.

4 gram of Tryptic Soy Agar culture medium was homogenized with 100 ml distilled water and autoclaved at 121 °C approximately 20 minute during this procedure. After autoclave became unlock sterilized liquid culture medium is divided into two allocations and embedded in empty cell culture dishes as can be seen in Figure 3.12. This process was performed next to Bunsen burner in order to protect sterilization. After the liquid in the dishes frosted, the dishes were placed in the refrigerator which was adjusted at +4 °C.

Figure 3.12 Cell culture dishes that are filled liquid TSA culture medium nearby bunsen burner

TSB liquid medium was made ready for *Pseudomonas aeruginosa* cultural growth increase and dilution processes. As the agar medium, TSB medium preparation was almost the same which was 3 gram of TSB culture medium homogenized with 100 ml distilled water and autoclaved at 121 °C 20 minute as well. The main difference between the preparation method of TSA and TSB, sterilized liquid form of TSB was not embedded in cell culture dishes. TSB stored in its own glass due to avoid potential contamination risk. The logic behind of this, if any contamination arises in medium, it cannot be used again. After the sterilization procedure of TSB medium, getting cooler and stored under the same conditions as TSA in the refrigerator.

In order to obtain the purest bacteria, *Pseudomonas aeruginosa* that was taken from freezer at -80 °C was waited almost half an hour into the ice until the strain decomposed. Then, *Pseudomonas aeruginosa* bacteria was taken and expanded to cell culture dish that contains TSA with using streak plate technique through the instrument of disposable cell scraper and incubated by incubator for 24 hours at 37°C. This cell culture dish was used as main stock as can be seen in Figure 3.13. Every counts involve 10^9 colony in this main stock.

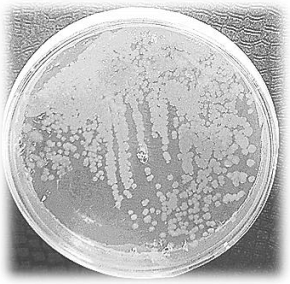


Figure 3.13 *Pseudomonas aeruginosa* main stock

3.2.3 *Pseudomonas aeruginosa* Disinfection

In the existing literature, consumption of aqueous hydrogen peroxide solution for disinfection is 6 milliliters (ml) per cubic meter **[17].** Also, created ICU room for this study is almost 1 cubic meter in consequence of this, the amount of aqueous hydrogen peroxide solution that is used for experiment process is arranged as 6 ml. Different concentrations of aqueous hydrogen peroxide solution which were 5% and 30%, were used to compare the impact level.

Before the disinfection proceeding, the suitable humidity condition was achieved with following steps; first of all, the current humidity was checked by Weewell digital thermohydrometer, which is temperature and humidity meter as demonstrated in Figure 3.14 in order to monitor the changes.

Then, the experimental setting was heated by Blyss 500 W mini fan heater as in Figure 3.15, afterward the heater off and the surface was aerated. As a result of this which is dehumidification procedure, the level of humidity was reduced in terms of conditioning process. Thereafter, the experimental setting was checked again by the same digital thermohydrometer right after the dehumidification process.

Figure 3.14 Digital thermohydrometer

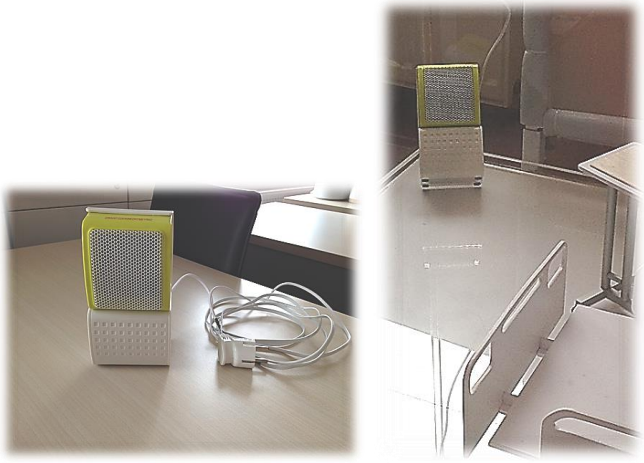
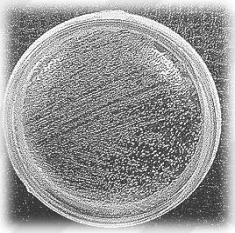
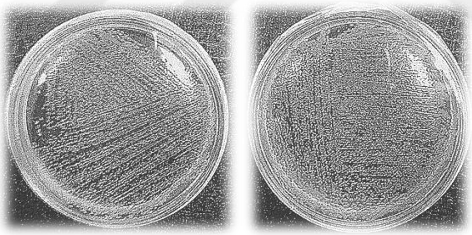


Figure 3.15 Mini fan heater

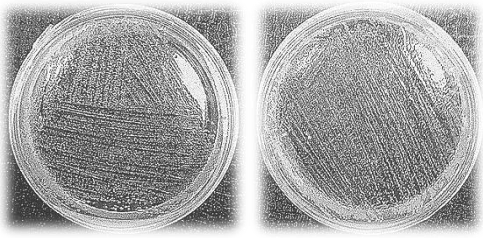
A colony from main stock was taken and placed into a centrifuge tube that contains 1000 µl TSB by pipette. At every turn, 10 µl was taken from centrifuge tube and diffused to five separate TSA culture medium and put into the incubator at 37°C to wait for 24 hours. On the following day, one of these five culture mediums (Figure 3.16 (a)) was separated as stock, Figure 3.16 (b) and (c) were entreated with vaporization and Figure 3.16 (d) and (e) were entreated with nebulization separately. The reason of using two separate dish for each transaction, was different waiting-period of disinfection process which were 5 minute and 15 minute. At the beginning of each experiment, new colony was taken from main stock, dissolved in 1000 µl TSB and new culture mediums were prepared for new transaction.



(a) Stock



(b) , (c) For vaporization



(d) , (e) For nebulization

Figure 3.16 Five separate *Pseudomonas aeruginosa* culture mediums

One of these cultured cell culture dish was placed in the room and the sterilization band was also located in the drawer which was closed in half simultaneously as can be seen

in Figure 3.17. Sterlization band as in Figure 3.18, was used to perform comparison for distribution force of different phases which were hydrogen peroxide vapor and fog. Due to this sterilization band that changes color when interacts with hydrogen peroxide, the comparison of which phase might be reach how far and depth was fulfilled.

Figure 3.17 Positioned cell culture dish and sterilization band

The nebulizer as in Figure 3.20, was activated to atomize hydrogen peroxide to gain fog. As can be seen in Figure 3.19, modified nebulizer with scaled container was used to estimate the expended hydrogen peroxide in time domain. Gauge of scaled container was sticked on the ultrasonic transducer portion of the nebulizer.

Lastly, knob of the nebulizer was settled on the scaled container and kept time. Noted down that, how much hydrogen peroxide was spent and become fog in approximately how many minute. Later on, scaled container was taken down from nebulizer and knob of the nebulizer was settled on its placement as in Figure 3.20.

To prevent any damage and receive optimum efficiency of the nebulizer's piezoelectric sensor, entire reservoir was filled with topfull of hydrogen peroxide at the beginning of each experiment. When conditioning process was completed after 5 minute to spent 6 ml hydrogen peroxide, the nebulizer was shut down and ambient fog was kept waiting in furtherance to allow disinfection process was occurred. Different waiting-period of

disinfection process which were 5 minute and 15 minute were experienced to compare and gain the best outcome. Until ambient hydrogen peroxide fog was deflated visibly, aeration process was performed roughly 5 minute. At the end of the process, sterilization band and cell culture dish was taken from the room for analyzing disinfecting quality via observing any color change and counting alive microorganisms.

Figure 3.18 Sterilization band

Figure 3.19 Modified nebulizer to calculate elapsed time for hydrogen peroxide consumption

Figure 3.20 Nebulizer

Figure 3.21 Modified heating coil to calculate elapsed time for hydrogen peroxide consumption

Figure 3.22 Heating coil

Whereafter the nebulization procedure, before starting the vaporization experiment, the experimental setting was heated again by mini fan heater, afterward the heater off and the surface was aerated based on the dehumidification procedure, the level of humidity was reduced. Other cultured cell culture dish was placed in the room and the sterilization band was also located in the drawer which was closed in half simultaneously.

The heating coil as in Figure 3.22, was activated to vaporize hydrogen peroxide.

Keeping time for consumption of hydrogen peroxide was also applied with heating coil as like as the nebulizer. For this reason, a partition was opened to be placed a scale on the heating coil. Then, the scale was placed on the heating coil and it was glued solidly as can be seen in Figure 3.21. After that, it was calculated how long took to decrease of hydrogen peroxide and decreasing amount within.

Later on, as shown in Figure 3.22, a cap which has hose connection was mounted on the new heating coil. The same disinfection steps, that made obtaining fog by hydrogen peroxide atomization method, was applied for obtaining hydrogen peroxide vapor by heating coil in the disinfection process as well.

When conditioning process was completed after 3 minute to spent 6 ml hydrogen peroxide, the heating coil was shut down and ambient vapor was kept waiting in furtherance to allow disinfection process was occurred. Different waiting-period of

disinfection process which were 5 minute and 15 minute were experienced to compare and gain the best outcome. Until ambient hydrogen peroxide vapor was deflated visibly, aeration process was performed roughly 5 minute. At the end of the disinfection process, sterilization band and cell culture dish was taken from the room for analyzing disinfecting quality via observing any color change and counting alive microorganisms.

3.2.4 Counting Process of *Pseudomonas aeruginosa*

A colony that was received via micropipette from entreated dishes with hydrogen peroxide vapor and fog and they were stirred in centrifuge tubes with 1000 µl TSB and

this dilution procedure was repeated 5 times by taking 10 µl from each ones to obtain the clear number of colonies. The last mixture was diffused to a new TSA culture medium and it was put to the incubator at 37°C to wait for 24 hours. The same process was applied to a colony that was taken from dish reserved as a stock

CHAPTER 4

RESULTS

In consequence of long-term viability on inanimate objects and antibiotic resistance of epidemiologically important pathogens, routine cleaning methods are inadequate about efficacious distribution of the active matter and ensuring correct contact time for microbial reduction and thus an automatic disinfection systems are still investigated and developed. In this research, we try to take a decision of the most efficient automated disinfection technique by comparing present and ostensibly the best way which is hydrogen peroxide vaporizing and our hypothesis dependent choice of hydrogen peroxide nebulizing. The resistance of *Pseudomonas aeruginosa* to hydrogen peroxide vapor and hydrogen peroxide fog phases was tested in 1:4 scale of actual size ICU miniature experimental setup.

In this section, lethal effect with colony counts and distribution force with sterilization band color variations of both hydrogen peroxide vaporizing and hydrogen peroxide nebulizing techniques were demonstrated in following tables. Also humidity and temperature values of the experimental setup before transaction and after transaction were noted.

TSA culture mediums with *Pseudomonas aeruginosa* were transacted with vaporization or nebulization and two separate dishes were used for each transaction in order to compare different waiting-periods' impact on bacterial destruction which were 5 minute and 15 minute disinfection. At the beginning of each experiment, new culture mediums were prepared with new colony, for new transactions and one of these culture mediums was separated as stock for analogy, at the beginning of each experiments.

When dehumidification process was achieved, cultured cell culture dish and the sterilization band was placed in the room and the environment was ready for conditioning process. Conditioning process of vaporization was completed after 3 minute, disinfection process was occurred in different waiting-periods. Finally, aeration process was performed roughly 5 minute. At the end of the disinfection process, sterilization band and cell culture dish was taken from the room for examining.

Before starting the nebulization experiment, dehumidification process was achieved again. This once, conditioning process of nebulization was completed after 5 minute, disinfection process was occurred in different waiting-periods. Finally, aeration process was performed roughly 5 minute and at the end of the disinfection process, sterilization band and cell culture dish was taken from the room for examining again.

At the end of the experiment, a colony that was received from entreated dishes with hydrogen peroxide vapor and fog and the dilution procedure was applied 5 times to obtain the clear number of colonies. The last mixtures were diffused to a new TSA culture mediums and they were put to the incubator. Same procedures were carried out for stock. Bacterial colony count stock results were showed in table 4.1.

Aqueous hydrogen peroxide solution for disinfection by each vaporizing and nebulizing techniques was used up 6 ml and varied concentrations which were 5% and 30%, were used to compare the impact level which were stated in table 4.4 and 4.8 with mean (μ) of 7 experiments' bacterial colony count outcomes. The standard deviation (σ) values were also displayed in table 4.4 and 4.8 for the statistical relationship between these 7 experiments.

As we can see in table 4.4 and 4.8, when the μ values were compared between hydrogen peroxide vaporization and hydrogen peroxide nebulization techniques for disinfection process performed with 5% or 30% aqueous hydrogen peroxide solution, hydrogen peroxide nebulization was showed more destruction on the *Pseudomonas aeruginosa* than hydrogen peroxide vaporization. The effect of the different concentrations of aqueous hydrogen peroxide solution on disinfection were compared but not observed significant differences between them.

Varied disinfection times; 5 minute and 15 minute were expressed and also compared their impacts on bacterial colony count outcomes were showed in table 4.4 and 4.8. When the different waiting-period of disinfection process were compared, 5 minute disinfection was more efficient with respect to kill *Pseudomonas aeruginosa.*

The humidity and temperature conditions were saved by checking digital thermohydrometer before and after disinfection procedures that were specified in table 4.2 and table 4.3 for transaction with 5% aqueous hydrogen peroxide solution and table 4.6 and 4.7 for transaction with 30% aqueous hydrogen peroxide solution.

Color changes on the sterilization bands as can be seen in table 4.5 and table 4.9 were evinced distribution force, in other words validation results about disinfection with 5% and 30% aqueous hydrogen peroxide solution for both vaporizing and nebulizing techniques, therefore we can observe the transactions which were done with nebulization processes have more color change than vaporizing techniques. Columns of 15 minute disinfection in both tables 4.5 and 4.9, were showed color variations but the most obvious color change was seen in table 4.9 which was expended 30% aqueous hydrogen peroxide solution.

Table 4.1 Bacterial colony count stock results

5% Aqueous Hydrogen Peroxide Solution

$409.\ 10^9$	$1107.\ 10^9$	$439.\ 10^9$	$419.\ 10^9$	$408.\ 10^9$	$427.\ 10^9$	$407.\ 10^9$	
							$\mu: 516{,}5714.\ 10^9$ $\sigma: 260{,}6184.\ 10^9$

30% Aqueous Hydrogen Peroxide Solution

$219.\ 10^9$	$1074.\ 10^9$	$599.\ 10^9$	$471.\ 10^9$	$413.\ 10^9$	$438.\ 10^9$	$419.\ 10^9$	
							$\mu: 519.\ 10^9$ $\sigma: 269{,}1115.\ 10^9$

Table 4.2 Humidity and Temperature values of experiental setup *before* each disinfection with 5% aqueous hydrogen peroxide solution

	5 Minute Disinfection		**15 Minute Disinfection**	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	Humidity&Temp.	**Humidity&Temp.**	**Humidity&Temp.**	**Humidity&Temp.**
1	28% 23.3°C	23% 24.3°C	25% 22.3°C	22% 25.3°C
2	31% 23.4°C	33% 23.1°C	32% 22.6°C	33% 26.8°C
3	30% 22.5°C	38% 23.6°C	36% 22.6°C	33% 33.2°C
4	25% 35.6°C	26% 32.2°C	25% 33°C	29% 30.8°C
5	33% 27.4°C	29% 30.8°C	23% 38.7°C	31% 28°C
6	29% 23.7°C	35% 27.9°C	31% 36.9°C	35% 31.5°C
7	31% 22.7°C	38% 24.7°C	30% 29.2°C	38% 28.7°C

Table 4.3 Humidity and Temperature values of experiental setup *after* each disinfection with 5% aqueous hydrogen peroxide solution

	5 Minute Disinfection		**15 Minute Disinfection**	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	Humidity&Temp.	**Humidity&Temp.**	**Humidity&Temp.**	**Humidity&Temp.**
1	40% 23.7°C	29% 24.1°C	45% 22.9°C	37% 23.7°C
2	46% 24.2°C	45% 22.5°C	54% 22°C	39% 25.7°C
3	45% 22.8°C	50% 22.8°C	56% 22.3°C	39% 34.1°C
4	41% 33.2°C	35% 28.6°C	47% 28.9°C	34% 33.5°C
5	38% 27.5°C	36% 32.7°C	42% 33.6°C	47% 24.9°C
6	45% 24.5°C	45% 26.9°C	50% 34.3°C	54% 29.2°C
7	45% 23.7°C	50% 26.1°C	54% 28.5°C	57% 26.4°C

Table 4.4 Bacterial colony count results after each disinfection with 5% aqueous hydrogen peroxide solution

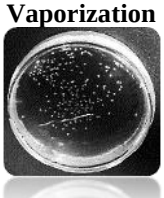
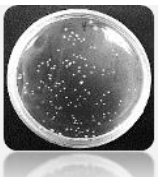
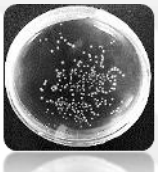
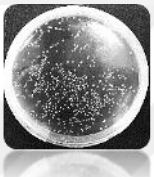
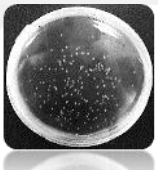
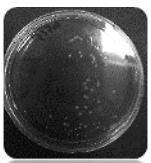
	5 Minute Disinfection		15 Minute Disinfection	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	 **306.10^3**	 **77.10^3**	 **233.10^3**	 **54.10^3**
	 **336.10^3**	 **156.10^3**	 **283.10^3**	 **145.10^3**
	 **336.10^3**	 **160.10^3**	 **364.10^3**	 **192.10^3**
	 **252.10^3**	 **94.10^3**	 **384.10^3**	 **210.10^3**
	 **265.10^3**	 **137.10^3**	 **156.10^3**	 **72.10^3**
	 **232.10^3**	 **152.10^3**	 **495.10^3**	 **254.10^3**
	 **150.10^3**	 **55.10^3**	 **138.10^3**	 **43.10^3**
μ	**268,1429.10^3**	**118,7143.10^3**	**303,3333.10^3**	**152,6667.10^3**
σ	**66,05157.10^3**	**42,415.10^3**	**138,8376.10^3**	**82,09182.10^3**

Table 4.5 Sterilization band results after each disinfection with 5% aqueous hydrogen peroxide solution

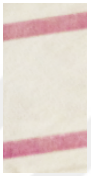
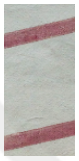
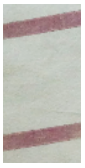
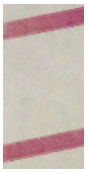
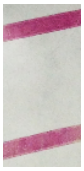
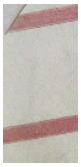
	5 Minute Disinfection		15 Minute Disinfection	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
1				
2				
3				
4				
5				
6				
7				

Table 4.6 Humidity and Temperature values of experiental setup *before* each disinfection with 30% aqueous hydrogen peroxide solution

	5 Minute Disinfection		**15 Minute Disinfection**	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	Humidity&Temp.	**Humidity&Temp.**	**Humidity&Temp.**	**Humidity&Temp.**
1	30% 22°C	34% 23.7°C	32% 25.4°C	34% 23.7°C
2	35% 21.6°C	38% 25.2°C	35% 24.9°C	38% 26.1°C
3	33% 21.7°C	40% 21.9°C	38% 22.3°C	47% 23.2°C
4	29% 23.5°C	35% 24.6°C	33% 21°C	33% 26.7°C
5	30% 22.1°C	34% 21.5°C	33% 20.7°C	35% 20°C
6	32% 21.5°C	31% 25.4°C	32% 22.6°C	33% 24.5°C
7	27% 31.9°C	28% 32.6°C	23% 38.7°C	31% 28°C

Table 4.7 Humidity and Temperature values of experiental setup *after* each disinfection with 30% aqueous hydrogen peroxide solution

	5 Minute Disinfection		**15 Minute Disinfection**	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	Humidity&Temp.	**Humidity&Temp.**	**Humidity&Temp.**	**Humidity&Temp.**
1	40% 22.9°C	39% 23.2°C	49% 25.1°C	49% 23.2°C
2	49% 22.7°C	49% 24.5°C	56% 25.2°C	53% 25.6°C
3	46% 21.9°C	50% 21.5°C	56% 22.3°C	52% 27.7°C
4	38% 23.1°C	43% 24.3°C	50% 21.5°C	46% 25.2°C
5	40% 22.4°C	42% 20.4°C	49% 21.4°C	47% 19.4°C
6	44% 22.3°C	39% 24.3°C	53% 22.2°C	49% 22.3°C
7	41% 34°C	35% 29.4°C	42% 33.6°C	47% 24.9°C

Table 4.8 Bacterial colony count results after each disinfection with 30% aqueous hydrogen peroxide solution

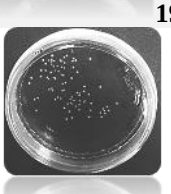
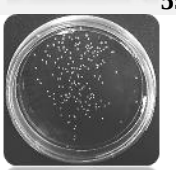
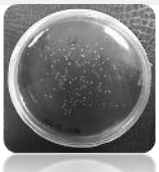
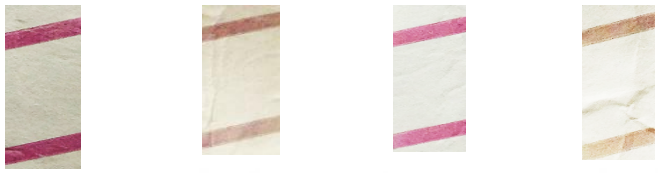
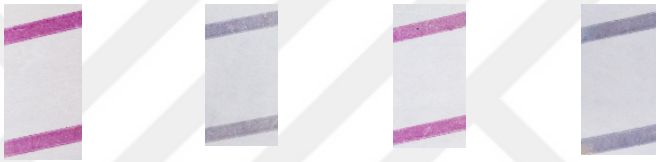
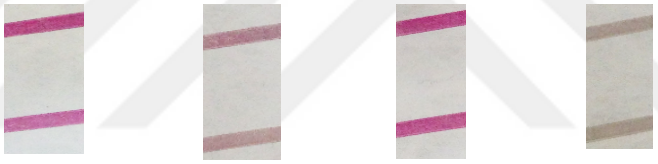
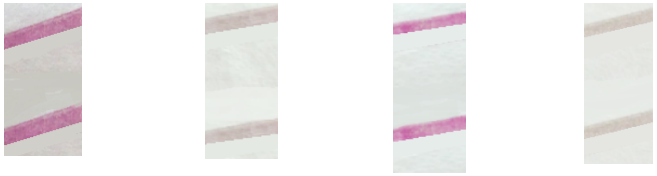
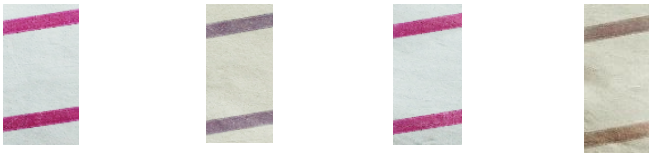
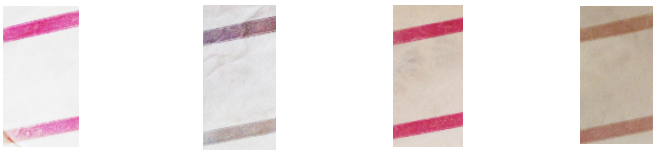
	5 Minute Disinfection		**15 Minute Disinfection**	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
	 **105.10³**	 **55.10³**	 **125.10³**	 **62.10³**
	 **633.10³**	 **173.10³**	 **368.10³**	 **196.10³**
	 **179.10³**	 **169.10³**	 **548.10³**	 **415.10³**
	 **419.10³**	 **196.10³**	 **596.10³**	 **367.10³**
	 **167.10³**	 **91.10³**	 **180.10³**	 **119.10³**
	 **314.10³**	 **188.10³**	 **394.10³**	 **275.10³**
	 **138.10³**	 **95.10³**	 **343.10³**	 **327.10³**
μ	**279,2857.10³**	**138,1429.10³**	**364,8571.10³**	**251,5714.10³**
σ	**190,9806.10³**	**56,26849.10³**	**173,2093.10³**	**130,94.10³**

Table 4.9 Sterilization band results after each disinfection with 30% aqueous hydrogen peroxide solution

	5 Minute Disinfection		15 Minute Disinfection	
	Vaporization	**Nebulization**	**Vaporization**	**Nebulization**
1				
2				
3				
4				
5				
6				
7				

DISCUSSION

Nosocomial infections are major health problem as a result of microorganisms that colonized healthcare facility environmental surfaces. These microorganisms can continue to populate on inanimate objects for a long time **[19]** and their survivability can show resistivity of routine cleaning by wet mop technique with quaternary ammonium compounds or chlorine-based product **[72]**. The only way to do the best environmental hygienic conditions is sufficient distribution of the active matter **[23]**, ensuring correct contact time for the microbial reduction **[24]**, keep away from contamination of cleaning solutions or materials **[26,27]**, eliminate lack of training, more education and do not lose time to do job **[25]**. All these features remain deficient in conventional cleaning methods and ICUs are the most impressed areas from this situation roughly three times higher than another place in hospitals **[50]**.

NTD technologies have shown up during the recent years for the disinfection of hospital environmental area that may not be disinfected sufficiently by conventional methods. There are several hydrogen peroxide-based NTD technologies on the market. Hydrogen peroxide is an influential disinfectant which is not harmful to the environment because it breaks down to water and oxygen and leaving no toxic end products. We respect hydrogen peroxide disinfection with automated systems an important and necessity method in environmental disinfection of rooms which may previously engaged by patients positive for epidemiologically important pathogens and they also have a feature in a reduction in the rate of new infections acquisitions throughout the hospital **[14]**.

Different types of NTD systems are currently used in clinical healthcare settings which are HPV and aHP **[3]**. These systems have diversified characteristics such as working principles, by heating liquid hydrogen peroxide and generates vapor and also by spraying liquid hydrogen peroxide; hydrogen peroxide concentrations, %30 aqueous hydrogen peroxide solution for vaporization and %5 aqueous hydrogen peroxide solution for aerosolization; particle sizes of final phases; vapor phase in 1 μm and aerosol phase 8-12 μm; and finally the effects on microorganisms, $>6\log_{10}$ mean destruction of microorganisms by vaporizing and $>3\log_{10}$ mean destruction of

microorganisms by aerosolizing. In addition to all these, hydrogen peroxide does not reach ‘hook line and sinker’ means that particle size that is effective on the apparent surface or it does not reach closed or indoor areas like drawer and the strength of the particle is insufficient in order to provide adequate penetration **[30]**. Our hypothesis was, if hydrogen peroxide particle size in diameter is smaller than the current state, this means increasing the percentage of penetration and disinfection with minimized particle size and with high condensation of liquid chemicals is also aimed to saving at the same time. For this reason, we need to generate very small droplets which resulting size distributions are very narrow and droplet diameter is able to be controlled. So then, we were atomized the size of hydrogen peroxide with ultrasonic excitation and droplet diameter was controlled by ultrasonic frequency that were operating roughly 1.8 MHz to gain particles whose median diameter around 0.3 µm to 0.4 µm **[89]**. We were simulated medical nebulizer which is example of fountain atomization and have popularity of atomizing by ultrasonic excitation **[32]**. The specifications of the liquid directly affect the magnitudes of the droplets and when the viscosity is >1.92 cP, nebulization is intermittent or completely ceased **[98].** In addition to this, hydrogen peroxide can be also formed into fog state by ultrasonic excitation because of the convenient viscosity value which is 1.245 cP **[94]**. Thus, insufficient particle problem that is one of the disadvantages of NTD systems using for existing environment disinfection, was eliminated and as a result of this, more powerful penetration with smaller particles was provided. We were planned to get better disinfection systems in terms of attaining much better penetration and destruction with much smaller number of particles by using ultrasonic excitation.

We were tested two different types of hydrogen peroxide-based environmental disinfection systems' impression on the *Pseudomonas aeruginosa.* The main difference between the two systems was the formation of the vapor or fog phase of hydrogen peroxide conclude that, vapor based hydrogen peroxide disinfection which is allegedly the most efficient NTD system in present-day, was showed inadequate ability to reach ‘hook line and sinker’ based on the color changes in the sterilization bands and also showed to kill bacteria much less than nebulizing technique based on the comparisons of µ values.

Hydrogen peroxide vapor in gaseous form from 5% and 30% aqueous hydrogen peroxide solution, likewise nebulization were created a fog from 5% and 30% aqueous hydrogen peroxide solution were attempted one by one. Each process in different concentrations were displayed separately in the result part, thereby the role of the different concentrations on disinfection were compared in the sense of effectiveness.

With reference to table 4.4, when the μ values were compared between vaporization and nebulization techniques for disinfection process performed with 5% aqueous hydrogen peroxide solution, hydrogen peroxide nebulization was more lethal on the Pseudomonas aeruginosa than hydrogen peroxide vaporization ($118,7143.10^3 < 268,1429.10^3$ in 5 minute disinfection and $152,6667.10^3 < 303,3333.10^3$ in 15 minute disinfection). Also in table 4.8, when the μ values were compared between hydrogen peroxide vaporization and hydrogen peroxide nebulization techniques for disinfection process performed with 30% aqueous hydrogen peroxide solution, hydrogen peroxide nebulization was more lethal on the Pseudomonas aeruginosa than hydrogen peroxide vaporization ($138,1429.10^3 < 267.10^3$ in 5 minute disinfection and $251,5714.10^3 < 364,8571.10^3$ in 15 minute disinfection). The effect of the different concentrations of aqueous hydrogen peroxide solution on disinfection were compared but not observed significant differences between them.

Disinfection was carried out along with two different waiting-periods which were 5 minute and 15 minute in order to gain the best outcome. Each process with different waiting-periods were displayed separately in the result part, thereby the role of the exposure duration on disinfection were compared in the sense of effectiveness. According to comparison of table 4.4 and 4.8, in both concentrations that were 5% and 30%, 5 minute disinfection was more efficient than 15 minute because the duration of 15 minute was enhanced the humidity, thus contrary to expectations, the amount of bacteria were totally increased.

At the beginning of each experiment, a colony was taken from the stock and dissolved in 1000 µl TSB. At every turn, 10 µl were taken and difused into five separate cell culture dish and put into the incubator at 37°C to wait for 24 hours. On the following day, a dish was separated as stock, other four dishes were used in different transactions.

At the start of the experiment, one of these cultured cell culture dish was placed in the room and the sterilization band that was used as distribution force analogy as validation, was also located in the drawer which was closed in half simultaneously.

In the existing literature, it was stated that 6 ml of hydrogen peroxide was consumed per cubic meter **[17]**. Subsequently, we were designed our experimental setup in a cubic meter and used as 6 mL of hydrogen peroxide in each experiment. Due to the previous researches about environmental disinfection that were made in an actual room conditions **[76,106]**, we were adapted every step of the disinfection process, into our own experimental setup.

Firstly, we were performed the first step was dehumidifying. As indicated in the literature, at the beginning of each experiment the humidity value was reduced as possible. Vaporizing and nebulizing techniques were implemented individually after dehumidification process.

According to estimates which were done before, found that how long 6 ml of hydrogen peroxide was spent by our modified devices; heating coil and nebulizer. Therefore, in the second step, we were used this duration as a conditioning period. Heating coil was taken 3 minute to spent 6 ml hydrogen peroxide, in nebulizer this duration was 5 minute. These periods should be standardized while working at normal room conditions on the basis of cubic meter.

Ventilation step was sustained until hydrogen peroxide vapor or fog was completely discharged from the experimental setup through aspirator. This period was taken approximately 5 minute. After ventilation process was completed sterilization band and cell culture dish was taken for analyzing disinfecting quality.

Each colony was obtained from the treated dishes and diluted five times. At every turn, 10 µl was taken and diffused to the new dishes. They were put into the incubator at 37°C to wait for 24 hours. In the next day, tables were created by counting the results of dishes. Five times dilution was more appropriate for clear colony count but it was given the cause of the duration and material waste. If <5 µl was given at every turn, five times dilution was not required because of the rare colony.

Similar statements were made in sterilization bands. At the end of the each process, they were collected and observed their color changes. According to table 4.5 and 4.9, the hydrogen peroxide nebulizing via ultrasonic excitation was suggested a strong and homogenous distribution in comparison to vaporizing. Nebulization processes are more powerful for deep cleaning so this was the validation result about disinfection with 5% and 30% aqueous hydrogen peroxide solution for both vaporizing and nebulizing techniques. Columns of 15 minute disinfection in both tables 4.5 and 4.9, were showed color variations but the most obvious color change (pink to indistinct) was seen in table 4.9 which was expended 30% aqueous hydrogen peroxide solution.

CONCLUSIONS AND RECOMMENDATIONS

According to Volchek et al. (2005) the trenchancy of disinfection process is allied to concentration of disinfectant, duration of exposure, rate of reaction, temperature, humidity, and the sort of the contaminated material **[79]**. Starting from these features, experiments were conducted, proper temperature and humidity were determined, be knowledgeable with the best exposure time of disinfection but not obtained sufficient information about the disinfectant concentration. Therefore we were decided to increase the rate of retries. By increasing the number of experiments, we will able to develop this research with regards to exhibit the hydrogen peroxide nebulization by ultrasonic excitation's difference clearly.

As a result of committed comparisons, in a view of sterilization bands, 15 minute disinfection in each vaporizing and nebulizing techniques may seemed more efficient, but bacterial colony counts were showed 15 minute disinfection was unaffected by the reason of increased humidity proportionally to increased disinfection duration and high humidity is a favorable environment for the reproduction of bacteria. Considering all of these, warm air circulation should be provide to prevent the rise of humidity, during the disinfection process.

Another case that is related to humidity, when the average of ambient conditions of cell culture dishes that were had maximum bacterial death were taken in order to provide efficient disinfection, it was found that the humidity condition should be ~30% and temperature condition should be ~25°C for flawless beginning.

HPV systems generate vapor by heating liquid disinfectant. For this reason it may cause degradation of chemical structure that provoke its working on destruction in negative way means that affected adversely. But in nebulization, liquid disinfectant turns into fog by only ultrasonic vibrations so provide more effective results.

In conclusion, the answer of which technique performed better influence on which surfaces is revealed easily. Based on this comparison and analysis, the following hypothesis can be derived: "The areas and surfaces which are disinfected with hydrogen

peroxide nebulization by ultrasonic excitation achieve higher elimination and deep clean in contrast with hydrogen peroxide vaporization."

According to Boyce et al. (2008), by virtue of the costs of HPV disinfection systems have high cost than traditional cleaning methods and healthcare facilities are needed the cost-effectiveness of HPV disinfection systems **[22]**. The hydrogen peroxide nebulization technique not only have the most lethal effect on microorganisms, but also have low price because of that's all we need is: piezoelectric transducer so as to nebulize hydrogen peroxide.

We can say that in addition to the Dancer's sentence (1999) "Hospital cleaning is the *'Cinderella'* of infection control" **[25]**; "Hospital cleaning with nebulization will be the *'Superman'* against superbugs".

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