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FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

EVALUATION OF ENDOSCOPE DISINFECTION METHOD

BEGÜM TOPUZ ÇİFTÇİ

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

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BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

ENDOSKOP CİHAZI DEZENFEKSİYON METODUNUN DEĞERLENDİRİLMESİ

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Begüm Topuz Çiftçi, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID 52011126, successfully defended the **thesis/dissertation** entitled "**EVALUATION OF ENDOSCOPE DISINFECTION METHOD**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my beloved husband Kürşad,

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ABBREVIATIONS

ASGH : American Society for Gastrointestinal Endoscopy
CFU/ml : Colony-Forming Units Per Milliliter
ERCP : Endoscopic Retrograde Cholongio Pancreatographyl
FDA : Food and Drug Administration
GI : Gastro-intestinal
HAIs : Hospital Acquired Infections
HCl : Hydrochloric acid
HLD : High Level Disinfection
HUM : Hydrocarbon Using Microorganisms
KCl : Potassium Chloride
MEC : Minimum Effective Concentration
NaOH : Sodium Hydroxide
OPA : Ortho-phthalaldehyde
PBS : Phosphate Buffered Saline
RPM : Root Per Minute
SGNA : Society of Gastroenterology Nurses and Associates
TSA : Triptych Soy Agar
TSB : Triptych Soy Broth
UV : Ultra Violet

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SUMMARY

EVALUATION OF ENDOSCOPE DISINFECTION METHOD

Begüm TOPUZ ÇİFTÇİ

Biomedical Engineering Programme

MSc Thesis

Advisor: Assist. Prof. Dr. Şükrü OKKESİM

Endoscopy is the instrument that assistances doctors to diagnosis gastroenterological syndromes in numerous ways. For the reason of endoscopic procedures are frequently applied, cleaning of it is certainly important to reduce the contamination of illnesses from one to another patient.

Term of sterilization is based on the cleaning process which is destruction of all microorganisms, fungi, bacteria also, bacterial spores. The term of disinfection is also destroying all kinds of microbial life from the surface but bacterial spores. Although these methods seems to be enough for adequate cleaning of endoscope, the structure of it is not suitable for using both ways to achieve cleaned surface due to its plastic made structure. Heat and corrosive chemicals destroys the surface of endoscope and these effects cause some damage on endoscope surface. Thus, corrosive parts are the best places for hidden bacteria if it is not cleaned enough.

In this thesis effectiveness of different chemicals which is using in HLD method as enzymatic, gluteraldehyde, peracetic acid, ethyl alcohol and their concentration values also, exposure times are examined on highly encountered nosocomial bacteria Pseudomonas aeruginosaATCC27856. After the contamination of endoscope with bacteria to detect the effectiveness of HLD method, dilution counting method was used

to get the bacteria numbers. Results show that gluteraldehyde is highly effective chemical for HLD. However, peracetic acid has not shown the same effectiveness even intensive concentration of it. On the other hand, enzymatic solution has not impressed the hygiene of the endoscope the reason of its removing effect on organic material. Additionally, for the positive control different concentrations of ethyl alcohol also examined due to its highly effective sterilization mechanism.

Keywords: High Level Disinfection, *Pseudomonas aeruginosa*, Endoscope Device.

FATIH UNIVERSITY - INSTITUTE OF BIOMEDICAL ENGINEERING

ÖZET

ENDOSKOP CİHAZI DEZENFEKSİYON YÖNTEMİNİN DEĞERLENDİRİLMESİ

Begüm TOPUZ ÇİFTÇİ

Biyomedikal Mühendisliği Programı
Yüksek Lisans Tezi

Danışman: Yrd. Doç. Dr. Şükrü OKKESİM

Endoskop cihazı, çeşitli sindirim sistemi rahatsızlıkları teşhisinde doktorlara yardımcı olmaktadır. Endoskopi işlemi çok sık uygulandığından dolayı bir hastadan diğerine hastalık bulaşmasının önlenmesi için temizliği son derece önemlidir.

Sterilizasyon terimi bir yüzey veya alandaki bütün mikroorganizmaların, mantarlar, bakteri ve bakteri sporlarının tamamıyla yok edilmesi anlamına gelmektedir. Dezenfeksiyon terimi de sporlu bakteriler haricindeki bütün mikrobiyal varlıkların yok edilmesi anlamına gelmektedir. Her ne kadar bu yöntemler endoskop cihazının etkin temizliği için yeterli gözükse de endoskop cihazı yapısı itibariyle her iki yöntem de uygun değildir..

Bu tezde, yüksek seviyeli dezenfeksiyon metodunda kullanılan enzimatik, gluteraldehid, perasetik asit kimyasallarının ve değişik konsantrasyonlarının etkinliği ve kimyasala maruz kalma süreleri sıklıkla karşılaşılan hastane enfeksiyonuna sebep olan *Pseudomonas aeruginosa*ATCC27853 bakterisi üzerinde denenmiştir. Bu bakteri ile kontamine edilen endoskop cihazına yüksek seviyeli dezenfeksiyon (HLD) işlemi uygulandıktan sonra bakteri sayılarının belirlenmesinde dilüsyonla sayım metodu uygulanmıştır. Sonuçlar gluteraldehid'in yüksek seviyeli dezenfeksiyon uygulaması için oldukça etkili olduğunu göstermektedir. Ancak, perasetik asit daha yoğun konsantrasyonlarında bile aynı etkiyi gösterememiştir.

Anahtar kelimeler: Yüksek Seviyeli Dezenfeksiyon, *Pseudomonas aeruginosa*, Endoskop Cihazı.

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Endoscope is a medical device that contains light source, air, and water and suction channels also; there is a special tip for biopsy tools. All narrow lumens and each flexible part of endoscopes have to be well cleaned after the operation to reduce the contamination risks [1].

There are many reports show that nosocomial infections transmitted from one to other patients after endoscopic procedures which help doctors to diagnose the gastrointestinal disorders such as presence of dysphagia, painful swallowing, and esophageal cancer suspicious, reflux, upper digestive tract bleeding and the presence of polyps in the stomach [2].

Some of the diseases are contaminating with the bacteria of *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Helicobacter pylori* even in some cases HIV the reason of the fact that inadequate reprocessing of endoscopes [3].

Medical equipment's are under high risks of nosocomial infections transmission from one patient to another. The reason of why Dr. H. Earl Spaulding classified all medical equipment as critical, semi-critical and non-critical items is that cleaning procedures of them are highly separated from each other [4].

For Spaulding classification items which penetrate the sterile tissue should be sterilized. The term of sterilization is the process of extinguishing all microbial forms from the surface or the area. Sterilization is can be applied with steam under pressure, ethylene oxide, hydrogen peroxide gas plasma sterilization agents etc [5].

On the other hand, semi-critical items like endoscopes which do not penetrate the sterile tissue should be high level disinfected at all. To prevent the contamination risks it is highly important to be applied adequate endoscope reprocessing. Disinfection as a

chemical process and cleaning as a physical procedure both steps are parts of the reprocessing endoscopes [6].

Physical and chemical cleaning are the steps of high level disinfection procedure which is been using in clinic. Physical cleaning process is based on removing the organic molecules from the endoscope surface with aldehyde free solution is called enzymatic. Enzymatic is the solution that contains enzymes, amylase due to remove organic structures such as lipids, blood, proteins from the surface [7].

Chemical procedure is based on cleaning with chemicals which contain different concentrations aldehyde, peracitic acid etc. Although these chemicals are effective to disinfect the area, they do not provide effective cleaning for endoscope's narrow lumens and tiny channels [6].

In the literature, there are many studies on reprocessing of endoscopes in different assumptions. Although, American Society for Gastrointestinal Endoscopy has a description for reprocessing procedure it has not achieved adequate successfully cleaning yet. In this thesis, at first we determined the description of endoscopes and its disinfection ways with comparing other methods from the literature studies and then we illustrated the effectiveness of chemical compounds in different concentration and their exposure times on resistant bacteria *Pseudomonas aeruginosa*ATCC27853.

1.2 Arrangement of the Thesis

This thesis is set up as follows:

_In the next chapter, information muscular system, definition and generation, recording process of electromyogram and mechanomyogram signals, MF in EMG and MMG signals and EMG-MMG signal processing are presented.

_In the third chapter, materials and methods of this thesis are represented significantly.

_In the fourth chapter, obtained results also, discussion with conclusions are represented.

CHAPTER 2

BACKGROUND INFORMATION

This section contains illustration of endoscope structure, main ideas of Spaulding classification, reprocessing steps are been using in clinics, definition of high level disinfection also, characters of nosocomial bacteria.

2.1 Upper Gastrointestinal System

Stomach is almost J/bag-shaped, first intra-abdominal digestive system organ that has mainly cardia, body and pylorus parts. In digestive system, eaten nourishments after it get wets with amylase enzyme in saliva in pharynx, go through the esophagus and then reach in to stomach. Stomach has two holes which one of it called cardia that connects the stomach to esophagus and the other's name is ostium pyloric which also makes a connection between stomach and duodenum as can be seen in Figure 2.1. Eaten and digested nourishment finally reaches to duodenum to end up the digestion [8].

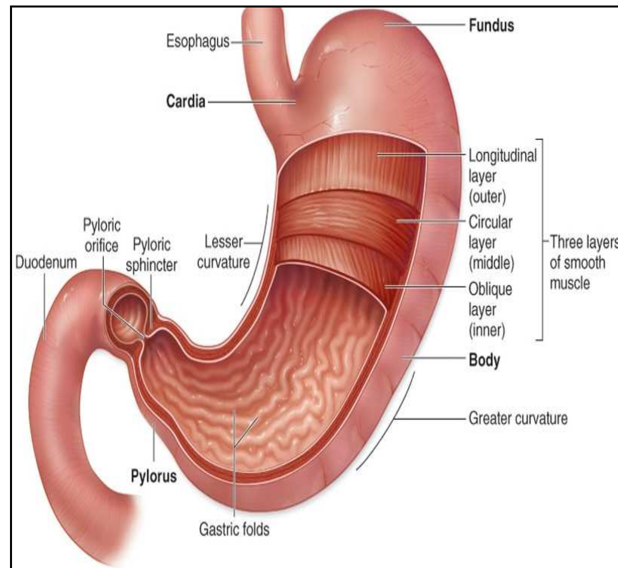


Figure 2.1 Anatomy structure of the stomach [9]

Interface of the stomach structure is covered with mucous membrane which protects the stomach walls from HCl acid that helps to food digestion. Also, stomach has 2 surfaces and 2 sides filled with oblique fibers and circular smooth muscles due to providing peristaltic movements every 15-20 seconds from cardia to pylorus.

Such a protective surface does not enough to prevent gastrointestinal disorders as well. These disorders occurs due to the fact that some physiological and psychological factors. Physiological factors are highly effective to increase of the illness impact. Many researches indicate that bacteria, germs and viruses originate the pathological illnesses. On the other hand, psychological factors such as stress, sadness, apprehension, desperation also reduce the disease factors due to decreasing immunological responds. Thus, diminishing of immune answers relates with occurrence of illnesses.

Even though, originates from physiological or pathological reasons *Helicobacter pylori* which cause gastritis, ulcer at the end stomach cancer spearheads the gastrointestinal disorders. Another pathological factor is nosocomial infections which originate from hospital reserves such as *Pseudomonas aeruginosa* bacteria, *Salmonella sp*. etc [10].

2.2 Gastro Endoscopic Device

Gastro endoscope is a medical device that assisting doctors to examine gastrointestinal disorders with its narrow tube which contains fibers in slender lumens and optical lens system goes through the digestive pathway [2].

First design of the endoscope was insufficient infrastructure with rough tube channel in front of the candle. Even though, Philipp Bazzini used reflection of light system in endoscope his invention had still hazardous ways for patient due to candle's poisoning oil. Kussmaul was a doctor in the middle of 19th century who improved Bazzini's endoscope design with adding first flexible tube the reason of the fact that earlier endoscope was very rough and hurting patients. That development made an approach as the first gastroscopy terminology is been used. Although, the design of endoscope device was theoretically enough, it was not adequate to performing in clinical trials. Due to the fact that designed flexible tube was sustaining the thickness and the risks of hurting patient. In 20th century American Doctor Logie Baird and his team made an endoscope design which achieves the adequate flexibility and a tinny lamp in the terminal point of the endoscope. Their clever invention method was based on optical system of submarines. Also, optic lens systems, cold light, mini color video cameras developed and took the significant place in clinic in latest of 20th century too [11].

Every nationality added some qualities such as Japanese scientist used little camera to get the images instead of using lens system as used before. With the camera system getting images with endoscope became much easier and functional than before.

Although, endoscope invention and development is almost recent novelty in clinic, it improved rapidly and helping doctors to diagnose the gastrointestinal disorders such as reflux, painfully swallowing, ulcer, esophageal cancer, upper track bleeding, painfully vomiting, polyps and their suspicious in stomach and duodenum with its light guide connector, light guide tube control body internal instrument channels which contains insertion tube and bending section in Figure 2.2.

There are different areas that endoscopic procedures are applied such as gastroscopy, colonoscopy, bronchoscopy, laparoscopy, arthroscopy, ERCP (endoscopic retrograde

cholongio pancreatographyl), cystoscopy etc. Main idea of all endoscopic applications is to see inside of the organs and channels [12].

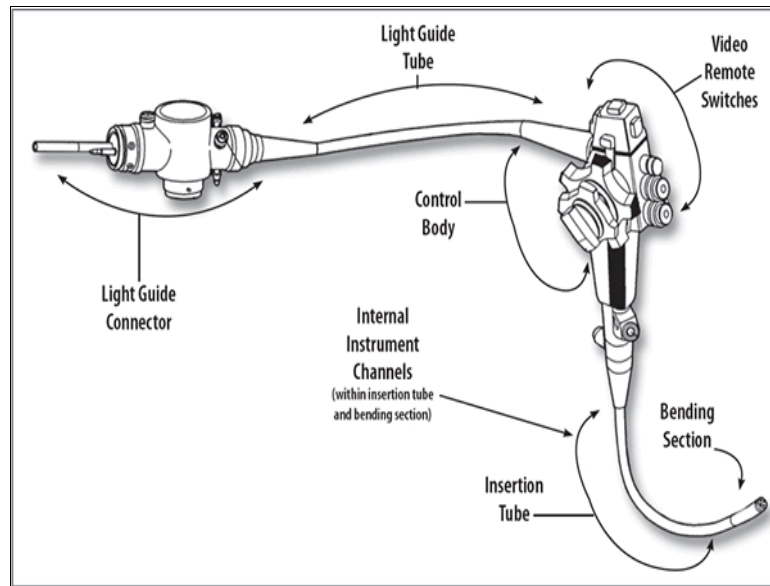


Figure 2.2 Endoscope Parts [13]

A modern endoscope has tubular lumen system which fibers go through in it, lens system to get the image, power supply which generates power for light bulbs, air and water channels inside of the lumen which helps doctors to get better view during the endoscopic procedure. Also, at the edge of the endoscope lumen there is a part that assisting to take biopsy from the tissue which can be seen in Figure 2.3.

Figure 2.3 Endoscope Tip Part-Light, Air and Water Suction

On the other hand endoscope lumen is made from non-plastic; non-metallic structure which obtains elasticity to the endoscope also, dynamic controller leads convenience to doctors during the application. For gastro endoscopes there are two types of endoscope device is been using in clinic which are named rigid and flexible endoscopes. Although, rigid endoscopes can be sterilized and seems to be more effective than flexible endoscopes – cannot be sterilized- due to their shape and adversity of enforcement makes rigid forms less preferable thus, flexible endoscopes has priority to be applied in clinic. With bending capacity, elasticity and easy usage of flexible forms appeal the endoscopic procedures day by day. It is well known that 46,5 million surgical applications and more invasive medical procedures were performed in United States and almost 5 million gastrointestinal endoscopic application are performing for every year [14].

2.3 Nosocomical Infections

Nosocomial infections are very significant and unavoidable problem in clinics for many years the reason of their multi drug resistance. A Nosocomial infection originates from hospital resources by smudging from one patient to another with hospital instruments such as bronchoscopy tools, respiratory equipment, and endoscopic materials. Elder people, children and new born babies, cancer patients are under the high risk of nosocomial infection due to their sapless immune system. Especially urinary catheter using patients should be very careful in order to caught urinary infection.

To highlighting the severity of the problem, rates and numbers of nosocomial infections of intensive care unit in United States and effected organisms illustrated in Table 2.1. Although, antibiotics are made for bacteria, they became more resistant than before day by day. That is why hospitals are counting risky area the reason of including bacteria rate comparing with other places.

Hospital's one of the infection risk department is endoscopy units the reason of the fact that non-sterilized structure of endoscopes cause some gastrointestinal pathogens which are shown in Table 2.2, constitutes by transmissions the bacteria from one to another patient after endoscopic application as many studies indicated that.

National Nosocomial Infections Surveillance System Report, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

*The total number of nosocomial infections in intensive care units during a recent 8-year time period was 92,454.

Table 2.1 Nosocomial Infections Rates in Intensive Care Unit [15]

	Bloodstream	Pneumonia	Urinary Track
Pathogen	Number	Number	Number
Enterobacter spp.	1,083	4,444	1,56
E.coli	514	1,725	5,393
Klebsiella pneumoniae	735	2,865	1,891
Heamophilus influenzae		1,738	
Pseudomonas aeruginosa	841	6,752	3,365
Staphylococcus aureus	2,758	7,205	497
Staphylococcus spp.	8,181		
Enterecoccus spp.	2,967	682	4,226
Candida albicans	1,09	1,862	4,856
Other pathogens	3,774	12,537	8,075
Total number*	21,943	39,81	30,701
Total %	23,7	43,1	33,2

E.coli, Pseudomonas aeruginosa, Enterobacter spp. Salmonella spp. are highly encountered bacteria types which cause gastrointestinal disorders such as painfully swallowing, severe vomiting, dearie etc. Also, one of the common bacteria which is not in the Table 2.2 is *Helicobacter pylori* that cause ulcer, gastritis even stomach cancer if cannot prevented in early stage of disease.

To prevent the contamination risks and get infected endoscopic reprocessing should be done meticulously and efficaciously. Although, reprocessing of endoscope is highly important, technician's motivation and cautiously performing are also have effective role of endoscope cleaning. Moreover than reprocessing, arrangement of which equipment should be disinfected or sterilized is the most important constituent.

Table 2.2 GI Tract Pathogens Potentially Encountered During GI Procedures [16]

GI Track Bacteria	Other GI Track/Bloodborne Pathogens
Clostridium difficile	Norovirus
Enterococcus/VRE	Hepatisis B
Enterobacteriaceae(CRE)	Hepatisis C
E.coli	HIV
Proteus spp.	
Klebsiella spp.	
Enterobacter spp.	
Serrotia spp.	
Salmonella spp.	
Citrobacter spp.	
Shigella	
Yersinia	
Staphylococcus spp. (including MRSA)	

2.4 Spaulding Classification

Every medical instrument has different physical characteristics to determining their resistivity under different conditions. Comparison of the refractory materials with nondurable instruments, prearrangement of cleaning process is extremely significant for decontamination units in clinic. Due to application of inappropriate decontamination procedures may be the root of problematic, unwanted results.

Dr. Earle Spaulding made a medical instrument classification and their decontamination process classification which eliminate the detriment of medical devices and prevent the contamination risks due to critical degree properties in 1939 and that clear and rational classification still protects its accuracy and conceded from many hospital infection units.

Medical devices are using in clinics such as surgical tools, cardiac and urinary catheters, implants and ultrasound probes used in sterile body cavity, which penetrates sterile tissue and vascular system are consuming as critical devices also, they need to be sterilized for Spaulding Classification.

On the other hand, there are some devices that is been using in clinic which do not penetrates sterile or intact tissue but mucous membrane such as respiratory therapy tools and anesthesia equipment, endoscopes, laryngoscope blades, esophageal manometer probes, cystoscopies and diaphragm fitting rings are all under semi-critical items category due to Spaulding Classification and they required at least high level disinfection applying with chemical disinfectant [17].

The other medical device types are used in clinic which have carrying the lowest risk degree are patient care items and environmental surfaces are taken into account as non-critical items under Spaulding Classification [18].

Bedpans, blood pressure cuffs and computers are under the classification of non-critical items which do not have any contact with non-intact skin or mucous membranes the reason of the fact that there is not any risk report for transmission of contagious agents to patients [19].

One of the other non-critical element category is non-critical environmental surfaces such as bed rails, bedside tables, food utensils also patient furniture and floors that are hypothetically minor transmission by infecting hands of by healthcare workers or by touching medical apparatus that in some way associates patients [20].

For Spaulding Classification, it is enough to be applied low level disinfection procedures for non-critical items even though to prevent their low risk degree of contamination [21].

To get better aspect of Spaulding Classification, definitions of sterilization, disinfection, high level disinfection, low level disinfections should be known as well.

2.5 Definition of Sterilization, Disinfection and High Level Disinfection Terms

Sterilization is definition of the process that eliminating of all microbial life such as bacteria including spore forms, viruses, and fungi from the surface or is with physical or chemical agents like steam under pressure, dry heat, ethylene oxide, hydrogen peroxide gas plasma and liquid chemical components in clinic [22].

One of the sterilization instruments is autoclave in Figure 2.4 that working principle is based on effecting microorganisms under standardized high pressure and temperature which under 134°C 3-3,5minutes in pre-vacuumed chamber, under 121°C 30-45

minutes none-prevacuumed chamber and 121°C 15minutes in pre-vacuumed chamber. The advantage of the autoclave is the economic way to sterilize instruments without toxic effects thus, safe for environment but there are some disadvantages and limitations of autoclave that heat and humidity sensitive materials such as plastic, also oily components like soft paraffin con not be sterilized with autoclave. That is the reason of endoscopes cannot be sterilized with autoclave due to its similar plastic material is not appropriate for being under high pressure and temperature. As an additional information that suffix -cide or -cidal are very common terms for expressing the meaning of exterminate or wipe out the microorganisms identified through the prefix. For occurrence, term of "germicide" means an agent which contains disinfectant and antiseptics that eradicates germs as pathological microorganisms.

Figure 2.4 Autoclave Device

The second sterilization instrument is dry heat sterilization with the device of Pasteur. Dry heat sterilization agent is invented by Luis Pasteur 1863. Principle of pasteur is based on sterilizing the mostly heat resistant but humidity sensitive materials under dry heat flow in standardized high temperatures which are 1 hour under 170°C, 2 hours under 160°C, 2.5 hours under 150°C, 3 hours under 140°C. The advantage of dry heat sterilization is the low expenses and easy usage for heat resistant material's even closed

pots, sterilization without drying process with controlling of only one heat parameter. However, dry heat sterilization is not appropriate for heat sensitive materials such as plastic, rubber and paper based materials due to dry heat applied temperature is always higher than their dissolving degree. As similar as autoclave, dry heat is not proper sterilization way for endoscopes too.

One of the other sterilization techniques is ethylene oxide sterilization way which gets the name from invisible, heavier then air and odorous has ethylene oxide gas compound. Although, ethylene oxide's biocidal property has founded in 1859 and used to sterilize medical aspects during World War 2, 100 % pure ethylene oxide gas has highly toxic and explosive futures. Due to reduce the toxicity of the gas and prevent the damage to patient and technician, ethylene oxide is using in form of combination with other gasses such as CFC, HCFC, CO_2. With these gas combinations, ethylene oxide gas sterilization can be proceed under low temperature that is highly lower temperature comparing autoclave, 37-55 °C with under appropriate pressure and humidity. Although ethylene oxide seems to be best way of sterilization technique with effective penetration quality, toxic properties and gas residuals in lumens after aeration make ethylene oxide sterilization less preferable in clinics due to poisoning effects on patients. To reduce the gas residual effects the equipment which was sterilized with ethylene oxide should be aerated at least 8 hours to remove the hidden gas particles also, if it is possible it aeration period should be take 24 hours too. However, the frequency of endoscopic applications and limits of endoscope devices in public hospitals reduces the aeration times due to their frequently usage. When the patient exposed to ethylene oxide gases, corrosion of lungs and other organs occurs in short term periods. On the other hand, the other chemical effect is eye irritation and damage of technicians. With the combustible and toxic properties ethylene oxide sterilization technique becomes inappropriate usage in clinics.

Control mechanisms of ethylene oxide sterilization are substantiates with physical control, chemical control and biological indicator which helps to technician if sterilization process is applied successfully. These control groups can be observed with physical parameters of the device and chemical indicators of chemicals also the biological indicators which properties is well known heat resistance bacteria *Bacillus subtilis* indicators. After observation of control mechanisms and achievement of

successful sterilization technician documents the results and all information's of sterilized device and the process.

One of the other effective sterilization methods is gas plasma sterilization method which working principle is based on plasma induction with interactions between electromagnetic field and some chemicals in cabin such as hydrogen peroxide or peracetic acid thus occurrence of free radicals, chemical precursors also, ultra violet light and biocidal effects of the plasma phages under low temperature conditions. For biological indicators *Bacillus subtilis* indicators are also using too as many sterilization techniques. High penetration capacity of plasma phase and sterilization effectiveness makes gas plasma sterilization method unique despite the fact that there are some limitations of methods usage on hydrogen peroxide absorptive structures like cellulose, nylon surfaces. On the other hand gas plasma sterilization technique is not appropriate for cupper-nickel compounds due to occurrence of catalytic burnings with live wires. One of the other detrimental area of gas plasma sterilization method usage is endoscopic devices the reason of the fact that organic sulfur and organic emulsions which gets reactions with hydrogen peroxide easily. These properties and cost effects of gas plasma sterilization make the sterilization method less unfavorable. In Figure 2.5 gas plasma sterilization device can be seen.

Disinfection is also a cleaning process that targets to destroy all or many of microorganism's bacteria, viruses and fungi with disinfection agents such as liquid chemicals or wet pasteurization technique but unlike the sterilization, disinfection has not sporicidal character. The significant difference between the high level disinfection and low level disinfection terms is based on the chemical exposure time degrees to the surface. For instance, to get the effect of disinfection exposure time of chemicals should be about 10-12 hours, to achieve the high level disinfection it is enough to be exposure less than other about 20 minutes 2% gluteraldehyde and to see the low level disinfection effect exposure time should be decline less than 10 minutes. Evidently, effects on bacteria numbers there will be difference from each other likewise, while the long exposure application kills many of microorganisms almost all, short exposure time application will reach to the destruction of some fungi, bacteria.

Figure 2.5 Gas Plasma Sterilization Device [23]

Another significant step for disinfection and even sterilization procedure is cleaning which mean the destruction of organic or inorganic materials from the surface by using water, detergent or enzymatic. Due to prevent the bacterial growth on organic and inorganic material cleaning process is highly important the reason of the fact that organic material is perfect area for bacterial growth with its lipid, carbohydrate, protein, nucleic acid structures which are the keystones for alive cells to produce the needed energy and hormones for regulation systems. Also, it is highly important that inorganic material on the surface for bacterial growth due to its source of water, acid, base, salt, ionic structure which con not be produced by bacteria that is why required to be obtained from the outside to regulate their bacterial metabolic actions.

Disinfectants are antimicrobial agents which should be applied inanimate surfaces and should not be applied to skin or any tissue due to prohibit injure of the tissue. The other part of germicide is antiseptics germicide agents which should be applied to skin not surfaces. As the clearness of the meaning, virucide refers to solution which kills viruses, fungicide refers to agent that kills fungi and sporicide means an agent which kills spores in literature.

For FDA (Food and Drug Administration) adequate high level disinfection definition with application of cleaning procedure is based on achieving 6-log_{10} kill of appropriate *Mycobacterium sp.* to prevent the contamination risk of infections [24].

There are some disinfection methods are been using in clinics for eliminating the damage of semi-critical items which cannot be applied sterilization techniques. Some of the chemicals are highly effective to achieve the disinfecting surfaces at least effecting vegetative forms of bacteria, viruses, fungi [25].

Alcohol is one of the chemical solutions that kill vegetative forms of bacteria, viruses and fungi due to devastating protein structures of them under diluted concentrations of absolute alcohol approximately 60% and 70%. Although, 70% ethyl alcohol affects vegetative bacteria rapidly easy aeration of the solution cause prolonged application problems which limits the destruction efficiency during disinfecting endoscopes are complicated instruments with narrow lumens and other parts [26, 27].

One of the other disinfection chemical solutions are chlorine and chlorine compounds which are widely using in water disinfection to prevent the water-borne diseases which sources of vegetative bacteria, viruses, mycobacteria, fungi at all [28].

Even though chlorine has powerful disinfection effects on many microorganisms its rigorous corrosive odor affects the operator who can be affected by its maximum concentration that causes a cancer [29].

One of the other chemical solution is been using in clinic called formaldehyde which is well known of its odor that cause corrosive damages even under 1 ppm and the suspicious of formaldehyde’s injuries to lungs that cause respiratory difficulties like asthma [30].

Even formaldehyde has germicidal, sporocidal, virucidal effects on microorganisms and using the disinfection of surgical instruments and preparation of viral vaccines as influenza, chemical corrosive properties detracts the utilization of the solution in clinic as well. On the other hand many studies indicate that effect time of formaldehyde is

longer than duration of gluteraldehyde which is widely using area in disinfection units [31].

Gluteraldehyde is one of the high level disinfection chemical solutions which are approved by FDA (Food and Drug Administration) due to its biocidal activity under base conditions pH 7.5-8.5 which is the best level to polymerization of gluteraldehyde molecules until 14 days to achieve the effective disinfection level with alkination agents as sodium bicarbonate [32]. After 14 days polymerization deactivates or blockages the vigorous site of gluteraldehyde molecules which leads less effective and even non-effective disinfection process [33].

Many study established that the 2% concentration more of it has extremely effective solution to abolish vegetative bacteria forms under 2 minutes, *Mycobacterium tuberculosis* bacteria, fungi and viruses under 10 minutes and obliteration of Bacillus and Clostridium spores approximately in 3 hours are identical operative accomplishments of it for the literature [34, 35].

Even results and studies showed that gluteraldehyde is the best way to performing high level disinfection it is highly toxic chemicals which is the reason of respiratory problems, eye nose irritation, and allergic dermatitis also pulmonary problems by corrosive effects for technicians and people who work with [36]. Due to reduce the hazardous effect of gluteraldehyde solution technician should use protective gloves, cloths and impervious mask for the odor also, aeration of the room should be controlled 8-12 hours to prevent the vapor of irritation gases [37].

One of the disadvantages of usage gluteraldehyde for disinfection is dilution rates of the chemical are increasing with the water remains in lumens come from cleaning procedure [38].

One of the other type of chemical is approved by FDA is hydrogen peroxide chemical solution which is a stable chemical under properly conditions and has a widely using area and preferable for the disinfection of ventilators, fabrics, endoscopes and contact lenses due to hydrogen peroxide’s destruction effects on bacteria, yeasts, fungi, viruses, spore forms of bacteria also, no need to be activated while manual and reprocessing applications during reuse time 21 days [39-44] [45].

On the other hand, usage of hydrogen peroxide as a high level disinfectant and chemical sterilizer it is used for disinfection of drainage bags due to reduce the risks of blood carrying contamination which was successful way to inserted hydrogen peroxide to drainage bags but not enough to eliminate the catheter originated bacteria [46].

Although hydrogen peroxide has prevalent using area material compatibility to brass, zinc, copper, nickel are under suspicious and the toxic effects of solution should be considered as well.

Furthermore, FDA approved a solution which is 0.55 % concentration and 7.5pH clear pale blue chemical 1,2-benzenedicarboxaldehyde (OPA) as a high level disinfectant due to its sporicidal properties even though slow deactivation of spore germinations and excellent compatibility of the surfaces and the interaction of amino acids, protein layers of microorganisms easily and the effective disinfection at >5-log10 reduction in contradiction of of extensive variety of microorganisms also, gluteraldehyde resilient bacteria, mycobacteria and *B. atrophaeus* spores [47, 48].

Even the biocidal activity of OPA is directly related with the temperature and stability quality of the chemical also, no need for additional activation process raise the selection possibility of the solution in wide range of clinical areas [49].

Moreover, many studies indicated that even OPA has 14 days of reuse limits it under minimum effective concentration (MEC) disinfection process achieved completed 82 cycle of usage the solution at 0.3% concentration degree. Minimum effective concentration is the term of the lowest effective degree of the solutions concentration [50, 51].

As many chemicals and methods OPA has some disadvantages such as skin and cloth straining properties and the risk of carrying OPA remains in the channels due to prevent or eliminate the corrosive effect of solution which cause hypersensitivity in repeated exposures, instrument rinsing procedure which was indicated in a study results that 250ml water should across per lumens which demonstrated how important the rinsing procedure and the protective environment should be provided for technician as protective gloves, eye mouth protective masks even it has not significant odor notified as well also the protective clothes should be worn during the reprocessing both manually and mechanically application [50, 52, 53].

For the FDA approvals for OPA 5 minutes exposure time at 25 °C and 12 minutes exposure time at 20 °C are the appropriate parameters for achieving the effective high level disinfection. It is also should be known that OPA is more expensive than gluteraldehyde solution and it should be neutralizing with glycine 25grams/gallons for safety disposal to diminish the damage to sewage system [54].

One of the other disinfection agents is peracetic acid which can remove organic matter from the surface under low temperature 50-60 °C has extensively using area such as disinfection of endoscopes, arthroscopies, dental tools also surgical instruments except copper, brass, bronze, plain steel structures which are corroded by peracetic acid but the elimination of corrosive effect can be reduced by additive materials and some changing with the pH degree [55-57].

Denaturation of the protein bonds, enzymes also, cell permeability of the microorganism is the working principle of peracetic acid oxidation process thus, with yeasts, gram + and gram – bacteria are effected under 5 minutes exposure times and even with organic load 200-500 ppm of peracetic acid is enough to disinfect surfaces [58-60].

Moreover, in many study highlighted the information that all strains of Mycobacteria spores are effectively disinfected in 20-30 minutes without organic load conditions [61-63].

High cost expenses, single usage restrictions of the solution and the censoriously eroding effects on eye-skin compensations and limitation for only immersed instrument disinfection should be considered as well with the noble compatibility, rapidly sporicidal activity and non-stable properties when diluted concentrations are exceedingly significant [61].

Endoscope is a medical device that is accepted as semi-critical items in the world wide and as Spaulding classification and FDA approval each cleaning units acceptably know that should be applied the reprocessing after every single endoscopic procedure with high level disinfection technique due to reduce the contamination risks between the patients. Reprocessing with high level disinfection is quite different from other sterilization and disinfection methods for semi-critical items like gastrointestinal endoscopes, bronchoscopes due to their elaborate design and elusive material structure

requires scrupulous scrubbing and high level disinfection process which should be applied well accomplished and vigilant operator who had trained already and conforms the reprocessing rules unswervingly [64].

It is highly important that as many studies illustrated the failures of endoscope reprocessing procedure is mostly affiliated with operators less intention to pre-cleaning step of high level disinfection. Especially, microorganisms are found in narrow swivel lumens and suction channels which difficult accessibility points and convenient residences for hidden microorganisms.

Gluteraldehyde an automated usage and liquid sterilization chemicals as peracetic acid are commonly using compounds for endoscope reprocessing in United States of America. According to American Society for Gastrointestinal Endoscopy (ASGE) and Society of Gastroenterology Nurses and Associates (SGNA) and American College of Chest Physicians and multi-society guideline also FDA recommended the reprocessing under 4 steps which are cleaning, disinfecting, rinsing, and drying [64-69].

Each reprocessing steps have unique application instructions which should be considered step by step to clarify the importance of high level disinfection. Cleaning procedure is the head of reprocessing of endoscopes which is based on physical cleaning, brushing and rubbing exterior and interior of the implement with organic and inorganic coating subtraction enzymatic solutions that contains enzymes for lipids, proteins and blood etc [70].

It is important that leak testing is controlled which is the preeminent way of understanding the apparatuses substantial situation before the immersion of endoscope in to enzymatic [71, 72].

After the pre-cleaning procedure and after the technician also be sure of organic and inorganic layers are removed visually remains of detergent solutions should be removed from surfaces due to not to effect the disinfection step which is the second step of reprocessing of endoscopes [54].

During the disinfection procedure disinfectant reaching in all parts of lumens and endoscopes even tiny spots is the most significant mechanism in reprocessing the reason of the fact that bactericidal, virucidal, germicidal effects will be accomplished with these decontaminators which are FDA sanctioned high level disinfection solutions that

gluteraldehyde, peracetic acid, hydrogen peroxide with peracetic acid, hydrogen peroxide, ortho-phthalaldehyde (OPA) in different concentration [73].

The noteworthy application is also rinsing procedure before the disinfection step and after both consequence of rinsing waters carrying risky microorganism's factors. During the reprocessing all rinsing waters should be sterilized or filtered water to abolish the contamination risk from tap water which may carry many kinds of bacteria due to drainpipes cleanliness degree. Moreover, filtered water should also be controlled to diminish the bacterial contamination of the filter surface [69, 74].

After the removing disinfectants from endoscope channels to reduce the bacterial growth lumens and channels should be dried with hot air flow force after rinsing with alcohol which evaporates easily [75].

Reprocessing procedure can be applied manually or automated which is highly recommended from many societies and at the end of reprocessing steps high level disinfected endoscope storage in specific cabinet which has protected with heap filters due to aeration of insertion tube. For many health care organizations cultures of endoscopes should be controlled monthly doe to protect the decontamination level and monitoring the reprocessing applications [76].

As it is obvious that reprocessing of endoscope is not only about operator it is related with the concentration of disinfectants, their exposure times, enzymatic application way and all chemicals expiration dates and the quality of rinsing water are all combined to each other [77, 78].

CHAPTER 3

MATERIALS AND METHODS

In this section materials of thesis will be mentioned which are *Pseudomonas aeruginosa*, Gastro endoscopic device, appropriate medium for bacterial culture, incubator and shaker incubator, autoclave, 2% gluteraldehyde and 3% peracetic acid.

3.1 Materials:

3.1.1 Bacterial strains: *Pseudomonas aeruginosa*ATCC27853

According to the Centers for Disease Control and Prevention of America's study 2 million of people suffer from hospital acquired infections (HAIs) and almost 100.000 pernicious incidences are seen which are directly related with leaks of medical treatments, surgical operations and implanted medical devices also medical applications like endoscopic procedures, urinary catheters etc [79-82].

It is known that 200.000 case is originates from hospital acquired infections which are mainly originates from nosocomial infections annually in USA. According to the World Health Organization *Pseudomonas aeruginosa* is one of the nosocomial infection reason multi-drug resistance bacteria which character is gram – bacilli and aerobic human pathogen that cause inflammation and sepsis even it can be fatal if critical organs effected like lungs, urinary track, kidney [83-85].

The reason of high level resistance of *Pseudomonas aeruginosa* is the bio film constitution capacity which protects the microorganism from unwilling destructive environments like chemicals, heat even cold and up to 65-80% rate of nosocomial infections are originated by biofilms which impervious antibiotic anti-biotic cell membrane the reason of drug resistance.

Moisturized areas are most loved places for *Pseudomonas aeruginosa* the reason of the fact that medical instruments like gastro-endoscopes, bronchoscopes, and urinary catheters that cause cross-infections in hospitals and clinics are appropriate and widely countable places for grape shape and odor *Pseudomonas aeruginosa* which has pearlescent view and hidden special colors inside of it as can be seen in Figure 3.1. The identification of Pseudomonas types basically clarified based on the colors on the surface area which are originates from special pigments such as the fluorescent, which gives green color and all Pseudomonas species has that material the other color given ingredients are red-brown color produces pyorubin and specialized structure for only *Pseudomonas aeruginosa* is pycocyanin which gives green-blue view to the surface where it placed which can be even artificial places environment.

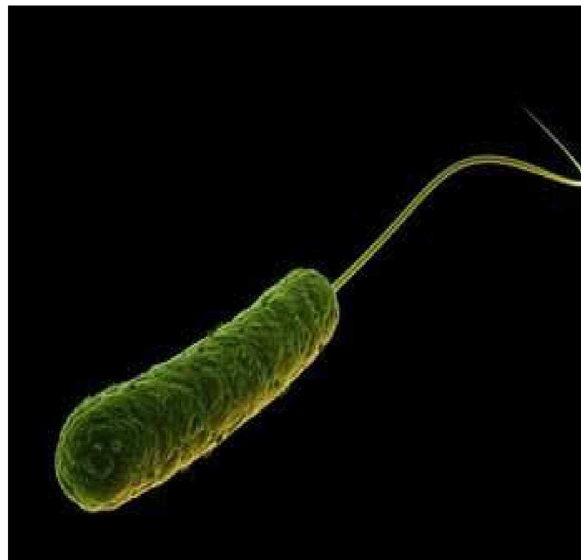


Figure 3.1 Pseudomonas aeruginosa [86]

Besides of the shape and color identification, destruction of hemoglobin at the medium represents the existence of *Pseudomonas aeruginosa* due to white areas on the blood mediums and beta hemolysis occurrence can be seen in Figure 3.2 as well.

Figure 3.2 *Pseudomonas aeruginosa* beta-hemolysis blood agar [87]

On the other hand milk is also perfect surface for growth of *Pseudomonas aeruginosa* due to the nutrient ingredients of it and the reason of greenish colors on the milk is also *Pseudomonas aeruginosa* bacteria the structure of florescent and pycocyanin. Also, a Pseudomonas bacterium is known as hydrocarbon using microorganisms (HUM). With the highlighters' of these information's *Pseudomonas aeruginosa*ATCC27853 is used during this thesis study due to illustrate the resistance of the bacteria and to clarify the importance of nosocomial infections for endoscopic applications.

3.1.2 Gastro-endoscopic Medical Device Properties

In this thesis, GIS Series endoscope (Upper Gastrointestinal Fiberscope Series) which has 10.5mm outer diameter and 1030mm working length also, 1350mm total length was

used which is a flexible endoscope type that has good bending capacity lumens with easy hand usage apparatus as can be seen in Figure 3.3.

Figure 3.3 Gastro-endoscopic device

3.1.3 Laboratory Equipment's

As all cultural studies refrigerator, incubation cabin, shaker incubator, -80 freezer, autoclave, centrifuge device, laminar flow, shaker are used in this thesis study too. Also, for bacterial incubation 50ml and 15ml disposal tubes are chosen. For the bacterial growth of triptych soy agar and triptych soy broth mediums are used as well. Unique different material is special endoscope washing basin is used high level disinfection cycles of the study.

As a high level disinfection solutions 2%gluteraldehyde and 3% peracetic acid also aldehyde free enzymatic is used for pre-cleaning the reason of mostly preferred effective chemicals in clinic.

Enzymatic is an aldehyde free liquid, limpid yellowish chemical solution which contains enzyme mixtures includes amylase, protease, and lipase. On the other hand there are three chemical compounds which are monoethyleneglycol, benzalkolyumchlorid, alcohol ethoxylate too. Although enzymatic solution is not

flammable material, wearing of protective cloths are suggested by the company as well. Also, to reduce the irritation risks wearing plastic or rubber glows and for the characteristic odor respiratory precautions are suggested too. Enzymatic solution is a stable chemical under appropriate conditions and convenient solution of rust inhibitor for non-ionic active surfaces. Also, enzymatic solution should be diluted before sending drainage system due to prevent the underground water system soiled. On the hand, it should be noticed that enzymatic solution expiration is until 24 hours due to its losing cleaning impression.

2%gluteraldehyde solution is a colorless liquid chemical solution which has toxic odor and corrosive effects on respiratory system, eyes and skin. If the contact with this solution surface should be washed with water and aeration of the room should be controlled. Although gluteraldehyde is a stable chemical solution it should be remove from hot places or flammable structures. Also, 2%gluteraldehyde solution is highly toxic for microorganisms in the water. Due to prevent the destruction of water life system gluteraldehyde solution should be inactivated with 6 gram glycine or sodium bisulfide per liter before throwing in to drainage system. Due to the instructor of solutions, 2%gluteraldehyde solution should be replaced in 15 days due to its misplacing disinfection efficiency.

3% peracetic acid is also liquid colorless chemical solution which has characteristic odor and corrosive effects on skin and respiratory system. According to the instructor protective cloths, specialized aeration filter masks should be used while working with peracetic acid. Also, aeration of the room should be well organized to prevent the toxic effect on respiratory system. As usually peracetic acid should not send away to drainage system however, it is not important for this study the reason of the fact that diluted concentrations of peracetic acid will be used during the study. Also, 3% peracetic acid should be replaced after 24 hours due to not the loose the disinfection effect.

3.2 Methods

In this thesis *Pseudomonas aeruginosa*ATCC27853 gram negative bacteria is used for contamination of endoscope with one and single colonies due to compare the effectiveness of chemical solutions which are used for disinfection and cleaning. Method's time schedule is represented in next Figure 3.4.

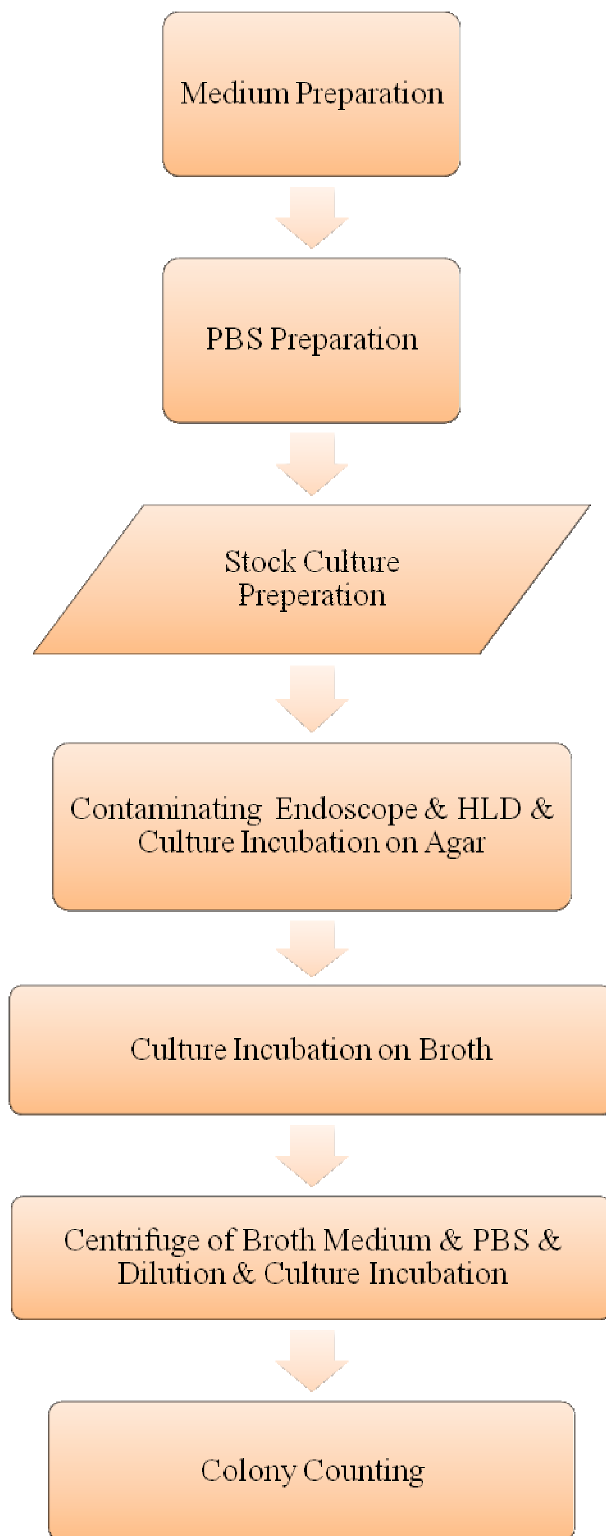


Figure 3.4 Time schedule of thesis study

In the Figure 3.4 every block represents 1 day which is the illustration of every single day application during this study. To not to lose time during the study broth and agar mediums should be prepared before the experimental stage. After the preparation of mediums they should be stored at the refrigerator for 24hours before used. After the preparation of PBS experimental stage can be proceed as well. Detailed processes are highlighted in next titles.

3.2.1 Medium Preparation

Before the contamination of endoscope to not to lose time during the study it is important to prepare triptych soy agar and triptych soy mediums are self-organized from stock cultures due to reduce the cost expenses of ready-mediums. For the Triptych soy agar preparation 40 gram culture medium is homogenized with 1000ml distilled water and autoclaved at 121 °C approximately during 20 minutes.

On the other hand autoclaved process takes an hour to raise the temperature and sterilization process also decrease the temperature. After the autoclave became unlock sterilized liquid culture medium is divided into portions on empty petri dishes which are sterilized under UV light about 20 minutes before the placement of medium. After the preparation of triptych soy agar medium petri dishes stored 24hours inside of the laminar flow chamber due to get cool and eliminate the occurrence of vapor top of the petri dishes. The next day of the medium preparation all petri dishes are stored in refrigerator at 6 °C.

To be get ready to the high level disinfection trials and to not to lose time triptych soy broth liquid medium should be prepared too which is need for *Pseudomonas aeruginosa*ATCC27853 cultural growth increasing and dilution process too. As the agar medium TSB medium preparation is almost same which steps are 30 gram TSB culture medium is homogenized with 1000ml distilled water and autoclaved at 121°C for 20 minutes too. Moreover, the basic difference between preparation of TSA and TSB is after sterilized liquid form of TSB there is no petri dishes spilled process. TSB stores in its own glass bottle due to eliminate the contamination risk the reason of if the contamination occurs medium cannot be used again. After the sterilization process of TSB medium and getting cooler it should be stored in the refrigerator too as same conditions as TSA.

3.2.2 Phosphate Buffered Solution Preparation

The phosphate based saline buffered solution helps to keep bacterial number unchanged without killing them but not to providing suitable place for growth. For the preparation of phosphate buffered solution 8 gram NaCl, 0.20gram KCL, 1.44 gram Na2HPO4 and 0.24 gram KH2PO4 is used in this thesis. All compounds are homogenized in 800ml distilled water with magnetic fish which is turning around with magnetic force to helping faster homogenization. After the homogenization solution should be filled until 1 liter with distilled water. pH of the solution is arranged in 7.4 with NaOH and NaCl. After the preparation of solution and removing magnetic fish out of the solution it should be autoclaved at 121°C for 20 minutes PBS solution is ready. These properties of solutions are the best futures *for Pseudomonas aeruginosa*ATCC27853.

3.2.3 Stock Culture Preparation

Bacteria CFU/ml can be calculated with extraction last CFU/ml from the first CFU/ml numbers. To calculate the bacteria colony forming unit and to evaluate the disinfectants efficiency stock culture preparation is highly important. During this study streak plate method is used for isolated cultures. Streak plate culture methods are the technique that dilution of bacteria concentration and reveal the isolated colonies.

Due to get the pure culture stocked bacteria under -80 °C is cultured with streak plate technique and then incubated 37°C incubator for 24 hours. During the streak plate cultured application only sterile or sterilized loops are used due to prevents the contamination risks. After the incubation bacteria colonies have greenish color and grape shape on the medium as seen in Figure 3.4 which is the pure colonies of *Pseudomonas aeruginosa*ATCC27853. Only one colony is contaminated with endoscope and nearby colony's CFU/ml is calculated due to get the efficient numbers but calculating of bacterium colony counting method is going to be highlighted in Colony Counting method section.

Figure 3.5 *Pseudomonas aeruginosa*ATCC27853 colonies

3.2.4 Contamination of Endoscope

To eliminate the infection risk all protective cautions are noted during this study which is wearing protective nitrile gloves and protective cloths also, aeration of the laboratory was under the control.

One of the other issues that were considered that was highly important to prevent infectious remains in the endoscope channels due to decline the risk of infection risk only endoscope’s tip part which contains lenses, forceps, and air suction and water channel part is contaminated with *Pseudomonas aerginosa*ATCC27853 gram negative bacteria during this thesis study.

The reason of getting comparable results while contamination process at first multi colonies and then single bacterial colony was used due to see the differences between bacterial numbers. The results of this trial are shown in Table 4.1 in results part.

As a contamination material sterile swabs was used but the suspicious of declining bacterial number swab using is canceled and then contamination of endoscopes are performed directly especially to the forceps point which has a small gap on edge. During this study between the contamination and disinfection processes there is no holding period for dehydration of bacteria.

3.2.5 Preparation of High Level Disinfectants and Enzymatic

In this thesis study we bought our endoscope washing basin and disinfectants which are aldehyde free enzymatic, %2 gluteraldehyde and 3% peracetic acid from one of the company which working area specialized in Hospital Infection Control Products, Disinfection and Hygiene Products, antiseptics for skin and hand, Industrial Cleaning Products and Genetic Laboratory Devices and Products. Also, hand-skin antiseptics and all instructions of solutions are provided by this company during this study. Also, in this study all pre-cleaning procedures applied without brushing and scrubbing due to showing only chemicals efficiency on 1 bacteria colony.

According to usage instructions enzymatic solution can be used for manual application and ultrasonic devices in different concentrations and different exposure times. We obey the instructions only for manual application usages. It is suggested that 0.5% concentration of enzymatic is appropriate application during 5 minute is the appropriate usage way for 1 liter. After the contamination of endoscope with *Pseudomonas aeruginosa*ATCC27853, 0.5% of enzymatic is prepared as adding 5 ml enzymatic solution to 1 liter distilled water for the cleaning process of high level disinfection method and contaminated endoscope immersed into the basin and waited for 5 minutes.

After cleaning with enzymatic, endoscope is washed with distilled water for 3 minutes due to remove the detergent from the surface and then immersed in 2%gluteraldehyde solution which is ready to use solution for 15 minutes in different endoscope washing basin. After chemical cleaning endoscope washed with dilution water due to remove the chemicals from the endoscope and culture sample which is the remain of one water drop is taken into triptych soy agar medium be incubated doe to illustrate the efficiency of high level disinfection. This was the high level disinfection which 2%gluteraldehyde is used in this process.

Also, to compare the different chemical solution's effect 3% peracetic acid is also prepared with 30ml of enzymatic for 1liter which is suggested concentrations according to the using instructors for manual applications. After the same processes of contaminating the endoscope and cleaning with enzymatic endoscope and washing period's endoscope immersed into 3% peracetic acid for 10 minutes. After the disinfection step with 3% peracetic acid endoscope washed with fresh distilled water

too to eliminate the chemical residues from the endoscope. And then the same taking bacteria culture to the triptych soy agar procedure is applied too.

3.2.6 Culture incubation on TSA and TSB

One water drop taken in to agar medium is cultured and streak plate technique is applied and incubated under 37 °C for 24 hours. After the incubation process if there is a bacterial growth it is seen in oval yellowish colonies on the dishes as seen in Figure 3.5 with characteristic smell of *Pseudomonas aeruginosa*ATCC27853. Colony growth illustrates the failure of disinfection method and if there is not any colony occurrence that means disinfection procedure was proceed successfully.

Figure 3.6 *Pseudomonas aeruginosa*ATCC27853 colonies

To get the bacteria colony numbers one colony should be placed in TSB medium to widen the one colony and make it to be countered. Due to increase the aeration of broth medium incubation process applied in shaker incubator for 24 hours under 37°C. We incubated single bacteria colony in 5 ml triptych soy broth medium for every broth medium cultured during this study.

After 24 hours of incubation process, broth medium turns blurry yellow color from unblemished yellow one. Bacterial growth existence causes indistinctness in broth medium.

To separate the medium from bacterial colonies solution should be centrifuged in 3000rpm (root per minute), in 4°C for 10 minutes. Cool area is important due to not to support the bacterial growth again.

After the centrifugation process bacterial remains which has white residues lay in tip of the tube and medium is separated from bacteria at all. The remains are the widened 1 bacteria colony which has still uncountable bacteria inside of it. To prevent the bacterial growth and not to kill them also, broth medium should be removed from the tube. Removing procedure can be proceed by spilling it carefully or automatic pipes can be helpful to not to destroy the bacterial residues.

After the removing broth medium phosphate saline buffered solution should be added as same ml as preferred while adding broth medium on the bacterial residues. We added 5ml PBS as same as broth volume and homogenized with shaker and the solution became white fuzzy view.

3.2.7 Dilution of Bacteria for a Viable Count

The bacterial population can be determined with different techniques which are directly microscopic examination and viable growth cells on plates of agar. Direct microscopic account is not as sensitive as viable counting method event its rapid performing. Advantage of the viable counting is after the counting method one bacterium can be seen naked eye due to its growths and divides on agar medium. These colonies help to determination of the colony numbers in the suspension. The non-diluted bacterial suspension is uncountable thus, to get the countable bacterial number and to determine the colony numbers in the solution serial dilution method should be applied.

During the study we used triptych soy broth medium for dilution solutions. 1 ml of bacteria suspension should add into 9 ml medium blanks which total volume is reached 10 ml and then 1 ml of solution from first medium blank should add into second 9 ml medium blank and the series goes on like this. At the end bacterial suspension is diluted in 10^{-1} rates for every blank. Dilution blanks is illustrated in Figure 3.7.

Spread plate method is the viable count of diluted method that we used during this study. Spreading 0, 1 ml of diluted cell suspensions on the surface of the agar plate with L shaped glass rod bent which is sterilized with flame for every usage. After the spread

plate method application all agar plates incubated under 37 °C for 24 hours. It is expected to meet with uncountable colonies for the first diluted blanks however, approximately 20-30th diluted blanks' incubated colonies become countable then.

To get the colony forming unit of the suspension diluted numbers can be calculated for 1ml suspension. For instance, if we diluted the suspension for 6 times it means that the suspension is diluted at 10^{-6}. If the countable colonies are 35 for the 6th incubated plate agar it means that in 0,1 ml there is 35 colonies so, in 1 ml there are 350 colony is existed but the solution is diluted as 10^{-6} thus, our suspension colony forming unit is 350.10^{6} equals to 35.10^{7}. Data and results of this thesis will be explained in result section with table charts.

Figure 3.7Dilution blanks for serial dilution method

CHAPTER 4

RESULTS

In this thesis, we try to evaluate the high level disinfection methods of gastro endoscope with different aspects such as the concentration of high level disinfection chemicals and + controls of these chemicals, +control chemicals effects on different concentrations, exposure times of these chemicals and efficiency of suggested usage times of these solutions by comparing the exposure rates and all parameters' disinfection efficiency on the nosocomial infection source bacteria *Pseudomonas aeruginosa*ATCC27853. There will be comparable Tables of these parameters which are going to be explained step by step.

Ethyl alcohol is an effective chemical solution that has a widely using area in clinics for disinfection applications. We also used ethyl alcohol due to see the effectiveness of the solution on bacteria. On the other hand, even washing with ethyl alcohol is the one of the reprocessing step before the drying with air flow; we used ethyl alcohol to see the disinfection effects on *Pseudomonas aeruginosa*ATCC27853. Also, this study was also applied due to get the best concentration value of ethyl alcohol for disinfection applications.

First step of the reprocessing of high level disinfection is washing with enzymatic which helps to remove blood, urine, lipid structures from the endoscope surface. Moreover, cleaning step with enzymatic is applied manually with the assisting of technician who scrubs and brushes the endoscope even reprocessing application continues in automatic device. The reason of why we examined the enzymatic due to see the effectiveness of the solution on *Pseudomonas aeruginosa*ATCC27853 stand-alone too.

All exposure times and concentration values were arranged according to the instructors for use book. For instance, aldehyde free enzymatic which is the detergent for first step of reprocessing is suggested for manual application 0.5% for 5 minutes and for ultrasonic automatic devices 0.5 % enzymatic should be applied for 3 minutes. Also,

instructors for use book also suggest that concentration value and exposure time can be increased due to its required. Enzymatic solutions expiration hour is 24 hours which means every 24 hours solution should be replaced.

One of the other suggestions of the high level disinfection solutions is 2%gluteraldehyde solution. The 2%gluteraldehyde solutions' expiration date is 15 days which means solution should be replaced every 15 days. Also, other suggested information is solution can be replaced if dirty appearance comprised.

The other high level disinfection solution is 3% peracetic acid which is suggested manual application concentration is 3% for 10 minutes and for automatic washing device also 3% for 5 minutes. As enzymatic solution 3% peracetic acid solution should be replaced in 24 hours.

However, there is no suggested frequency of the usages for any solutions. Due to investigate the frequency usage efficiencies we also examined these solutions too.

As the beginning of this study we wanted to see the disinfection efficiency of well-known disinfectant ethyl alcohol used in clinics widely comparing in different concentrations and exposure times on single colony and multi colony as can be seen in Table4.1

The term of the "no bacterial growth" means that after the dilution method incubated plates were completely clear which can be identified by naked eyes. Also, there is not any bacterial growth after disinfection with 70% ethyl alcohol as can be seen in Figure 4.1..

Due to the Table 4.1 illustration, 70% of ethyl alcohol is very effective disinfectant even under low exposure time usages. Also, when we reduced the concentration of ethyl alcohol solution lost its efficiency even for single bacterium. The reason of why we made this experiment due to see the effectiveness of ethyl alcohol with different concentrations thus, we used 70% of ethyl alcohol for disinfecting the endoscope tip before every contaminating process and after the high level disinfection to prevent the contamination risk may occur from period studies. As in other words, sterilization with 70% ethyl alcohol is our guarantee for contamination leaks of the study as + control of the high level disinfection solutions.

Figure 4. 1 No bacterial growth dishes

On the other hand, this result also suggested that exposure time of the 70% ethyl alcohol which is 5 minutes is effective to disinfect the surface of the endoscope. Thus, total application of high level disinfection procedure time can be reduced under these circumstances. Reduction of the process period is highly important due to increasing the endoscopic application rates in a day. If the period between the reprocesses can be condensed, the endoscopic application rates will be escalates in clinics.

Table 4.1 Comparison of 70 % and 35 % ethyl alcohol in different exposure times for single and multi-*Pseudomonas aeruginosa*ATCC27853 colonies.

Percentages of Alcohol (CFU/ml)	70% Ethyl Alcohol	70% Ethyl Alcohol	35% Ethyl Alcohol
Exposure Times	10 min.	5 min.	10 min.
Single Colony	No bacterial growth	No bacterial growth	$2,865.10^{32}$
Multi Colony	No bacterial growth	No bacterial growth	

One of the other studies of this thesis is the exploration of the enzymatic solution's efficiency on *Pseudomonas aeruginosa*ATCC27853 bacteria due to notice if there is any helping effects on disinfection process. As it was mentioned before cleaning with enzymatic step is the first step of the reprocessing of endoscopes. Although, enzymatic is using as a cleaning detergent there is no disinfection effect on bacteria according to our results is shown in Table 4.2.

Table 4.2 Efficiency of 0.5% Aldehyde Free enzymatic on single and multi-colony of *Pseudomonas aeruginosa*ATCC27853

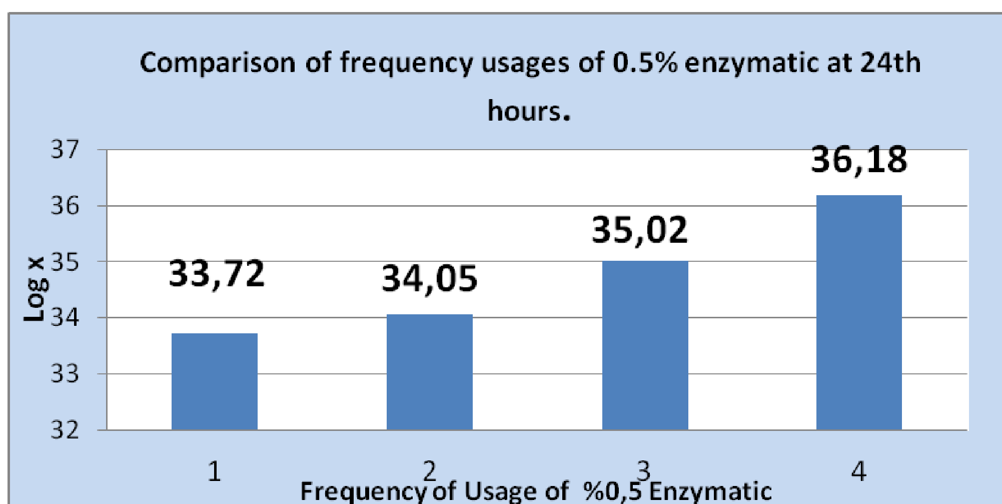
Percentage of Enzymatic (CFU/ml)	0.5% Aldehyde Free Enzymatic
Exposure Time	5 min.
Single Colony	10^{11} - 10^{15}
Multi Colony	$>10^{17}$

In this study the percentages of aldehyde free enzymatic and the exposure times were chosen according to the production companies suggested concentration of the solution. For manual applications company suggests 0.5% aldehyde-free enzymatic for 5 minutes and for ultrasonic devices 3minutes for the same concentration. Towards of the Table4.2 clarification that enzymatic does not affect the bacteria in disinfection way.

Although, enzymatic has 24 hours efficiency according to the industrial companies and instructor usage book, Table 4.3 shows the efficiency results of the frequency of using enzymatic in the 24th hour on *Pseudomonas aeruginosa*ATCC27853. On the other hand bacteria CFU/Ml is 2, 12.10^{37} without any chemical process.

Bestowing of the results given in Table 4.3 bacterial number increasing progressively at the end of 24th hour also, we can roughly say that bacteria can endure even can proliferate in the enzymatic solution which is cleaning detergent.

Table 4.3 Comparison of frequency usages of 0.5% enzymatic at 24th hours.



After the comparison of the frequency usage of enzymatic, we studied on 2%gluteraldehyde solution which is the most suggested high level disinfectant in clinics. The results and the efficiency of the 2%gluteraldeyhde were fascinating as can be seen in Table 4.4.

Table 4.4 Comparison of HLD with %2gluteraldehyde and CFU/mL single and multi-colony of *Pseudomonas aeruginosa*ATCC27853

	Without Disinfection	With 2% Gluteraldehyde
Single Colony(CFU/ml)	10^{12}-10^{15}	No Bacterial Growth
Multi Colony(CFU/ml)	>10^{18}	No Bacterial Growth

Gluteraldehyde which is 2% concentration form has effective disinfection effects on *Pseudomonas aeruginosa*ATCC27853. As also can be seen in Table4.4 after the high level disinfection with 2%gluteraldehyde there is no bacterial growth on the plates. Also, the term of “No Bacterial Growth” means that there is no bacterial remains on medium plates after high level disinfection with 2%gluteraldehyde.

As other studies we examined the expiration dates of the 2%gluteraldehyde solution too which are given in Table 4.5. Also, it should be known that bacteria CFU/ml is

2,12.10[37] without any chemical process too. The reason of the fact that we studied with 2%guluteraldehyde on 10th day is suggested expiration date is 15 days for the solution.

According to the instructors using book there is no frequency usage rate suggested for 2%gluteraldehyde however, when we applied high level disinfection with 2%gluteraldehyde solution for 5 times at 10th day we realized that chemical's disinfection effects continues successfully.

Table 4.5 Illustration of the efficiency of 2%gluteraldehyde solution during HLD at 10th day.

CFU/ml Frequency of usage	10th Day of the 2%Gluteraldehyde usage
1st application	No Bacterial Growth
2nd application	No Bacterial Growth
3th application	No Bacterial Growth
4th application	No Bacterial Growth
5th application	No Bacterial Growth

Another high level disinfection solution is peracetic acid which suggested effective concentration rate is 3% in clinics and industrial area. To evaluate the peracetic acid efficiency we also examined the 3% peracetic acid effects on *Pseudomonas aeruginosa*ATCC27853 for high level disinfection of endoscopes too.

Efficiency of 3% peracetic acid also the frequency of usage of the solution with expiration of hours is highlighted in next tables too.

Table 4.6 Comparison of HLD with %3 Peracetic Acid CFU/mL single colony of *Pseudomonas aeruginosa*ATCC27853

Disinfectant Concentrations	Without Disinfection	3% Peracetic Acid
CFU/ml	$5,34.10^{32}$	$2,05.10^{31}$

Although, 3% peracetic acid is a high level disinfectant which is widely preferred in clinics disinfection effect is highly unexpected due to the diminution of the bacteria colony forming units according to the Table 4.6. Even 3% peracetic acid reduced the bacteria numbers, there is a big amount of bacteria remains after the high level disinfection on endoscope tip.

Table 4.7 Comparison the effectiveness of HLD with % 3 peracetic acid after 24-48-120 hours later

Solution Concentration Hour	Without Disinfection CFU/ml	24 Hours Later	48 Hours Later	120 Hours Later
3% Peracetic Acid	$2,5.10^{31}$	$1,42.10^{32}$	$5,36.10^{28}$	Contamination

On the other hand, the expiration hours were suggested by the production company as 24 hours for a day high level disinfection process. As the information is given in the instruction 3% peracetic acid solution should be changed due to the obscure rate of the solution view. As the results can be seen in Table 4.7 we examined the efficiency of 3% peracetic acid for 24-48 and 120 hours due to its losing disinfection effect. Also, the colony forming units of without disinfection bacteria are inserted the Table 4.7 too.

The application of High Level Disinfection procedure after 120 hours incubated plated were contaminated and bacteria colonies were not countable. The reason of the occurrence of contamination ought to be the solutions lost efficiency.

Due to improve the high level disinfection method we tried to increase the concentration of peracetic acid after 24 hours and applied the same reprocessing steps for the endoscope after the contaminating with *Pseudomonas aeruginosa*ATCC27853. The tick concentration of peracetic acid was not used before for the high level disinfection procedure as far we investigated in the literature and the result of our trial is in Table4.8.

Table 4.8 Relationship between the frequency of usage and the efficiency of 6% Peracetic acid after 24 hours on *Pseudomonas aeruginosa*ATCC27856

Concentration/ Frequency	Without Disinfection	1st application CFU/ml	2nd application CFU/ml	3th application CFU/ml	4th application CFU/ml	5th application CFU/ml
6% Peracetic Acid	$2,12.10^{37}$	No Bacterial Growth	Too many to be counted	Too many to be counted	Too many to be counted	Too many to be counted

As a conclusion of Table 4.8 even the concentration peracetic acid increased it is not the effective disinfection solution for destruction of nosocomial infection origin bacteria *Pseudomonas aeruginosa*ATCC27853 due to the results of our independent study. Burdens of the study and future works will be illustrated in discussion part of the thesis. Also, too many to be counted dish is illustrated in Figure 4.2.

Figure 4. 2 Too many to be counted colonies

DISCUSSION

R. Knieler compared the efficiencies of enzymatic and gluteraldehyde on *Bacillus subtilis* or *Staphylococus aureus* contaminated sheep blood on the surface of metal plate in 2001 [6]. On the other hand, bacteriocidal efficiency, sporicidal efficiency, odor characteristics of the solutions are also illustrated. Also, Knieler's studies main idea to evaluate the physical cleaning efficiency only with gluteraldehyde and enzymatic based solutions. Knieler used serial dilution method also, we used too. The major difference with our study is we used real gastroendoscope device during this study instead of metal plate. Also, we evaluated different kinds of high level disinfection such as 3% peracetic acid. On the other hand, in our study exposure times of the solutions and expiration dates of the solutions are highlighted too.

According to these results of our study, aldehyde free compounds have no disinfection effects as enzymatic which are called detergents. Also, bacteria can survive and keep growing under enzymatic conditions as can be seen in result section Table 4.2. Moreover, if there is a patient who has contagious disease, illness can transport to next patient with enzymatic solution the reason of non-disinfection effects of the enzymatic. This is the unacceptable risks for hospitals, clinics especially for patients.

During the thesis study we did not achieve the same paper or study such ours. Although, limitations of similar study there are lots of study that focusing on the difficulties of endoscope reprocessing steps and focusing on the high level disinfection directions in H. Martiny, H. Floss, B. Zühlsdorf's study which is published in 2004 [7]. In this study, design of the endoscope, bioburdens of endoscopes and importance of qualified reprocessing system is also highlighted too.

In one of the study which was published in 2013 William A. Rutala et al. made an overview of the disinfection and sterilization in wide aspects [88]. In this study the properties of different chemical compounds and different methods for sterilization and disinfection were evaluated. Also, chemicals advantage and disadvantage futures highlighted too.

According to Rutala's study, critical degrees of the medical devices are highly important also; their disinfection or sterilization techniques are significant too. One of

the similar study is W. A. Rutala and D. J. Weber's study which is also focusing on the Spaulding Classification and methods of sterilization and disinfection in 1999 [5]. In this study, they evaluate the efficiency of different chemicals in different exposure times on *C.parvum, Helicobacter pylori, E.coli, Micobacterium tuberculosis*. One of the results of this study is after the high level disinfection application with peracetic acid, strains were still resistant to chemical solution.

Although, industrial suggested and widely preferred peracetic acid have not disinfection effect neither. Even it has small amount of decreasing effects on *Pseudomonas aeruginosa*ATCC27853 after the high level disinfection procedure bacteria can survive and contaminate next patient. Diluted concentrations of peracetic acid may not disturb the bacteria as well however; condensed forms of peracetic acid still cannot destroy the nosocomial bacteria either as shown in Table 4.8. Moreover, intensive concentration form of peracetic acid which is at 6% degree destroyed all bacteria on the surface however, when we checked the solution according to its frequency usage disinfection application was failed. In other words, intensive concentration of chemicals may not always the best solution for disinfection applications as can be seen in Table 4.8 too.

The fulfilment of the successful disinfection procedure was the high level disinfection with 2%gluteraldehyde even under contamination with multi-colony task. Also, even enzymatic does not any disinfection pressure on bacteria 2%gluteraldehye cleared whole bacteria remains from the endoscope surface. When the 2%gluteraldehyde solution is forced to disinfect after 10 days with running usages of high level disinfection, efficiency of disinfection was still performing.

Due to comparison of well-known chemical solutions of high level disinfection procedure 2%gluteraldehyde is extensively more successful than 3% peracetic acid even 6% concentrations of it.

Additionally, it should be known that, as a study group of this thesis, we visited one of the high level disinfection production companies who developed the automatic washing machine for endoscopes in Turkey. We illustrated the aspects of our study in several meetings. Moreover, company director also stated that they are very glad to have a chance to work with our department also they are pleased to have a chance to working with us. After multiple interviewing's we decided to improve this study with using high

level disinfection automatic washing device in further studies which can be supported with other projects.

CONCLUSIONS AND RECOMMENDATIONS

In this thesis, it was decided that to evaluate the high level disinfection methods and the widely using chemicals destructive efficiency on nosocomial infection origin bacteria *Pseudomonas aeruginasa*ATCC27853. With the efficiency of high level disinfection solutions concentrations frequency of usages are high lightened too due to clarify the information that given by the product companies recommendation. The reason of the fact that, even the chemical is suggested to be used for a day or 15 days, frequency of these solutions definitely effects the efficiency of high level disinfection. This study is highly important for specialized endoscopic application clinics where the application rates are very high centers. According to results charts, industrial product company should suggest the frequency usage rates to clinics and users.

In this study manual approach of the high level disinfection is illustrated even under non-real endoscopic procedures. One of the important issue that there is no organic or inorganic material used during this study only the bacteria colonies mostly one colony is contaminated little tiny piece of endoscope. One of the benefit of this study is we illustrated widely preferred chemicals which are on the stage of high level disinfection applications, without injected inside of the lumens only the surface of high contamination risk area.

It should be considered that will enzymatic solution destroy or remove the all organic and inorganic layers from the surface efficiently with manual or automated high level disinfection reprocessing? To get the knowledge of the enzymatic efficiency reprocessing should be applied with organic and inorganic matters in future works.

Additionally this study can be improved with using organic or inorganic structures to make a real endoscopic application picture or to arrange similar properties of stomach flora. However, due to the contamination of endoscope with organic and inorganic materials high level disinfection reprocessing should be applied automatically with specialized automatic washing machines.

On the other hand to improvement of this study can be applied with resistant bacteria not only *Pseudomonas aeruginosa*ATCC27853. Even though, studying with spore forms of bacteria will cause many problems such as resistivity to the solutions, producing biofilms.

To sum up the all informations, parts of this study needs to be considered as the illustration of evaluating high level disinfection methods not the improvement statements for endoscope reprocessing.

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