ANTIMICROBIAL EFFECT OF SLOW RELEASE CHLORINE DIOXIDE DISINFECTANT, IN COMPARISON WITH SODIUM DICHLOROISOCYANURATE

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DECLARATION

I, Joy Ikechi Ebonwu, declare that this dissertation is my own work.

It is being submitted for the degree of Master of Science in Medicine to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….. (Signature of candidate)

…… day of ……. 2010

DEDICATION

To my Parents, Late Sir & Lady R.A. Odimegwu

and my husband, Emmanuel Ebonwu.

ABSTRACT

The goal of infection control is to minimize the risk of exposure to potential pathogens and to create a safe working environment in which patients can be treated. Use of disinfectants in is an integral part of infection control. The rate of killing of microorganisms depends upon the type, concentration and time of exposure of the killing agent (disinfectant). Chlorinated compounds are frequently used in healthcare settings but chlorine dioxide has only been used in industries on a large scale. Aseptrol® is newly developed slow release chlorine dioxide and noncorrosive formula which can be used on a smaller scale basis. This study assessed the antimicrobial properties of Aseptrol® (48ppm and 24ppm) in comparison with previously used sodium dichloroisocyanurate containing formula, Presept® (10 000ppm).

Both disinfectants killed more susceptible bacteria, such as *Staphylococcus aureus, Pseudomonas. aeruginosa and Streptococcus mutans* within 30 seconds and proved to be fungicidal by killing *Candida albicans* within 30 seconds. Aseptrol® and Presept® killed less susceptible mycobacteria such as *Mycobacterium tuberculosis, Mycobacterium avium subsp. avium* and blood borne organism Hepatitis B virus within 30 seconds. Highly resistant *B. subtilis* spores were killed in 2 and 2.5 minutes by Aseptrol® and Presept® respectively.

Although manufacturers recommend that the disinfectant solutions should be prepared daily, when the shelf-life of prepared solutions stored in screw cap bottles was studied, the results showed that Aseptrol® can be effectively used for 27 day and Presept® for more than 37 days.

Chlorinated disinfectants, such as Aseptrol® and Presept®, have potential to be used as intermediate to high level disinfectants in medical and dental settings, where above test organisms are primary contaminants. It is also possible to use them as sterilants, where semicritical conditions are required. Aseptrol® has an additional advantage because it is noncorrosive and can be used on metal instruments.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATCC:	American Type Culture Collection
CDC:	Centre for Disease control
CFU/ml:	Colony Forming Units per millilitre
ClO_2:	Chlorine dioxide
CMV	Cytomegalovirus
DNA:	Deoxyribonucleic acid
dNTP:	Deoxynucleotide triphosphates
EBV	Epstein Barr virus
EDTA:	Ethylenediaminetetraacetic acid
EPA:	Environmental protection agency
FDA:	Food and Drug Administration
g:	Grams
HBsAg:	Hepatitis B surface antigen
HBV:	Hepatitis B Virus
HCV:	Hepatitis C Virus
HCW:	Health care workers
HIV:	Human Immunodeficiency Virus
HPC:	High positive control
HSV	Herpes simplex virus
ICU:	Intensive care unit

IU/ml:	International unit per millilitre
IV:	Intravenous
LPC:	Low positive control
MGIT:	Mycobacteria growth indicator tube
NaDCC:	Sodium dichloroisocyanurate
NAT:	Nucleic Acid Technology
NC:	Negative control
NHLS:	National Health Laboratory Service
NNIS:	National Nosocomial infections Surveillance
RNA:	Ribonucleic acid
Mg/l	Milligram per litre
Mn^{2+}:	Manganese
$MnSO_4$	Manganese Sulphate
MRSA:	Methicilin-resistant *Staphylococcus aureus*
PCR:	Polymerase Chain Reaction
PPM:	Parts per million
QS:	Quantitation standard
SD:	Standard deviation
SDA:	Sabouraud Dextrose agar
µl	Microlitre
WHO:	World Health Organization

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Health care personnel are constantly exposed to wide variety of pathogenic microorganisms that can cause infections. Many microorganisms are transmitted in medical and dental hospitals including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida species,* Mycobacteria, Hepatitis viruses and Human immunodeficiency virus (HIV) (Mims et al., 2004). Infection control is an integral part of patient care and hospital setting in order to prevent nosocomial infections (Larson EL, 1995). The goal of infection control is to minimize the risk of exposure to potential pathogens and to create a safe working environment in which patients can be treated. Disinfection is a process of removing or killing most, but not all, viable microorganisms. Sterilization and Disinfection are key processes in the control and prevention of hospital acquired infections, as well as being central to many areas of medical practice. Disinfectants containing chlorines, aldehydes, phenols, alcohols and heavy metals are available and regularly used in the hospital environment (McDonnell and Russell, 1999) and they all have advantages and disadvantages.

The rate of killing of microorganisms depends upon the type, time of exposure and the concentration of the killing agent. Efficacy of disinfectants is routinely tested against standard indicator microorganisms (Rutala et al., 2000, Brady et al., 2003, Raffo et al., 2007, Taylor et al., 1999). This study was undertaken to establish efficacy of two chlorine based disinfectants in a laboratory environment.

1.2 LITERATURE REVIEW

1.2.1 Nosocomial infections

Centre for Disease Control (CDC) defines nosocomial infections as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s), without any evidence that the infection was present or incubating at the time of admission to the acute care setting (Garner et al., 1988). It occurs 48 hours or more after admission and up to 48 hours after the patient has been discharged. Nosocomial infections can occur due to endogenous or exogenous source. Exogenous source is usually another patient, hospital environment and healthcare workers. The common nosocomial infections are surgical wound infections, respiratory tract infections, urinary tract infections and bacteraemia. They constitute a major fraction of the adverse events complicating hospital treatments and are critical threat to patients, especially in the Intensive Care Unit (Kampf et al., 1998). Patients treated in intensive care units (ICUs) are at increased risk to acquire such infections because the invasive devices, such as mechanical ventilators, catheters, often used in these patients create ports of entry for opportunistic bacteria and fungi (Gastmeier, 2004).

Ding et al., (2009) reported a high and relatively stable rate of nosocomial infections in the ICU of a tertiary hospital in China through year 2003–2007, with some differences in the distribution of the infection sites. The sources of these infectious agents are patients, healthcare workers and objects. The route of transmission is air, droplets, direct contacts, devices, food, blood products and intravenous (IV) fluids.

The consequences of hospital infections are serious illness and death, prolonged hospital stay (meaning increased cost), additional treatment with antimicrobials (meaning cost, drug toxicity and increased risk of development of resistance), and infected patient becomes a risk and a source of infection.

Prevention of hospital infections can be done by excluding the source of infection from the hospital environment, interrupting the transmission of infection from source to susceptible host and enhancing the host's ability to resist infection (Mims et al., 2004). Transmission interruption can be achieved by establishing infection control in the hospital. One of the key aspects of infection control is use of disinfectants.

1.2.2 Infection control in medical healthcare settings

There are standard precautions for health-care workers (HCWs) presented by Centre for Disease Control (CDC). These guidelines are step by step infection control measures that should be followed by health-care professionals. They describe hand washing, protective barriers, sterilization and disinfection, waste disposal and accidental injuries. Although requirement of hand washing was known as early as 1822, first CDC guidelines for hand washing were published in 1975 and 1985 (Steere and Mallison, 1975; Garner and Favero, 1986). Surgical procedures, blood transfusion, and frequent visits to different dialysis units are major risk factors for contracting viral hepatitis. The results of Karkar et al., (2006) clearly showed that isolation of patients and machines, together with strict adherence to infection control policies and procedures, result in a significant decline in the incidence and prevalence and better control of viral hepatitis transmission among haemodialysis patients. Infections have been linked to endoscopic procedures due to

inadequate cleaning and disinfection during the reprocessing of the instruments and their accessories (Nelson, 2003; Lisgaris, 2003). In hospitals, environmental contamination has been linked to transmission of some important nosocomial pathogens, including methicillin-resistant *Staphylococcus aureus* (Mulligan et al., 1993), vancomycin-resistant *Enterococcus species* (Bonten et al., 1996), and *Clostridium difficile* (Malamou-Ladas et al., 1983).

1.2.3 Infection control in dental healthcare settings

Dental care is a field of high priority regarding the risk of infections. The dentist has to consider every patient potentially infected. On the other hand, health-care workers are not only susceptible persons to infections but they can also be sources of infections. In order to prevent the nosocomial infections, the dentist has to ensure the hygienic protection of both the patients and the health-care workers. All the health-occupational measures have to be known and kept by the dental personnel. The health personnel have to be informed of the risk and how to prevent infections. The essential importance of hygiene, the role of protective equipment and all the duties connected with should be emphasized.

Dental patients and health-care workers may be exposed to a variety of microorganisms by dental instruments or by direct contact with blood or respiratory secretions. The most likely mode of transmission is droplets from infected patients. Typical contaminating microorganisms include Cytomegalovirus, Hepatitis B virus (HBV), Hepatitis C virus (HCV), Herpes simplex virus types 1 and 2, HIV and *Mycobacterium tuberculosis*. Pathogens may be transmitted by direct contact with blood, oral fluids, or other secretions; indirect contact with contaminated instruments, operatory equipment, or environmental

surfaces; or contact with airborne contaminants present in droplets. The precautionary measures are similar to medical settings except protective barriers are very important due to the aerosols that are created by the procedures. Use of disinfectant is similar in both setting. CDC has presented guideline for infection control in dentistry (Kohn et al., 2003) describing above measures.

The frequency of exposure to HBV is greater in dental care workers (Naqao et al., 2008). Hepatitis B infection is a significant hazard in the dental environment because the virus may be transmitted through contaminated dental instruments such as hand pieces (Deng et al., 2005). Several studies have shown that even low-level disinfectants are able to kill HBV, Hepatitis C virus (HCV) and HIV (Bond et al., 1983; Prince et al., 1993). Radiographic films can be another source of infections. A study done by Coogan et al., (2004) has shown that Presept, a sodium dichloroisocyanurate based disinfectant, can be effective in the disinfection of radiographic films and gloves. Dental impressions are also potential transmission routes for pathogenic microorganisms that infect the oral cavity and respiratory tract and chlorine based disinfectants can be effective in decontaminating impressions (Rweyendela et al., 2009). Routine disinfection of impressions has been recommended to protect clinicians, laboratory personnel and patients (Owen and Goolam, 1993).

1.2.4 Disinfectants and their role in infection control

Disinfection is a process that eliminates many or all pathogenic microorganisms on inanimate objects with the exception of bacterial spores. With prolonged exposure however, some disinfectants can kill spores. Disinfectants are often the only practical

means of rapidly disinfecting heat sensitive equipments at point of use. A prerequisite for a disinfectant is its effectiveness against the expected spectrum of pathogens. To be effective, a disinfectant must produce consistently high percentage kill levels on every test (Schwartz et al., 1996). Besides the broad spectrum activity, it should be fast acting, nontoxic, odourless and easy to use. It should also be active in the presence of organic material, leave residual effect, be stable, economically and environmentally friendly.

Disinfectants are selected according to their use in degree or level of contamination, which can be categorised as critical, semicritical and noncritical. In the hospital environment, all three areas are recognised. Critical category includes surgical instruments, cardiac and urinary catheters, implants and ultrasound probes used in sterile body cavities. Heat sensitive objects are either treated with ethylene oxide, hydrogen peroxide gas plasma or by liquid chemical sterilants. Aldehyde-based agents have been commonly used for high-level disinfection in most hospitals. Glutaraldehyde is by far the most used, especially for equipments, because of its broad spectrum activity and potency (Hernández et al., 2003). However, polymerisation, potential mutagenic and carcinogenic effects of glutaraldehyde is problematic (Espigares et al., 2003). Chlorinated compounds are corrosive to metals and cause irritation to skin and mucosa (Robinson et al., 1986; Zhang et al., 2008). Alcohols do not have broad-spectrum activity (Woo et al., 2002). Use of triclosan a phenol based disinfectant to disinfect catheters and vascular grafts, has been described by Kim et al., (2002) and Hernández-Richter et al., (2000).

Semicritical items are those that come in contact with mucous membranes or non-intact skin. Endoscopes, laryngoscope blades, rectal memometric catheters are some of the devices which should be free of all microorganisms but small number of spores can be

present. Chemical disinfectants such as glutaraldehyde, hydrogen peroxide can be used for this purpose. They should be rinsed with sterile water after disinfection to prevent the microbial contamination of tap water.

Noncritical items are those that come in contact with intact skin but not mucous membranes. These are bedpans, blood pressure cuffs, bed rails, linens, floors etc., which can be decontaminated with low-level disinfectants. Some of the disinfectants are described below.

1.2.4.1 Alcohol

Alcohols are bactericidal rather than bacteriostatic and generally kill bacteria in vegetative form, fungi, viruses and even mycobacteria but not bacterial spores. Ethyl and isopropyl alcohols are the most effective alcohols and they are used in hospital settings. Alcohols denature microbial proteins and are most effective at 60 to 90% solution made with water (Morton, 1950; Morton 1983; Ali et al., 2001). The use is limited because of the lack of penetration into protein-rich material and sporicidal activity.

1.2.4.2 Formaldehyde and Glutaraldehyde

Formaldehyde is used as a disinfectant and sterilant both in liquid and gaseous form. The aqueous solution is bactericidal, mycobactericidal, viricidal, fungicidal and sporicidal (Klein and Deforest, 1963; Rubbo et al., 1967). The use of formaldehyde is limited due to its carcinogenic property (OSHA, 1991), irritating fumes and the pungent odour.

Glutaraldehyde is a saturated dialdehyde used as a high-level disinfectant and chemical sterilant. It has an excellent biocidal activity even in the presence of organic material and it is noncorrosive. It is widely used in healthcare facilities to sterilize endoscopes, thermometers and rubber or plastic equipments. In dentistry, it is extensively used. Glutaraldehyde causes alkylation of sulfhydral, hydroxyl, carboxyl and amino groups of microorganisms, which alters ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and protein synthesis (Scott and Gorman, 2001). However there are reports showing glutaraldehyde resistant mycobacteria such as *M. chelonae, M. intracellulare* (van Klingeren and Pullen, 1993; Griffiths et al., 1997) and some fungi (Barbee et al., 1999).

1.2.4.3 Iodine and Iodophors

Iodine has been used as an antiseptic for years by health professionals. Iodophor is a combination of iodine and a solubilizing agent or carrier. The combination is more stable with sustained release of iodine, free of toxicity, irritancy and non-staining property. Iodine is able to penetrate the cell wall of microorganisms quickly and the lethal effect is due to the disruption of protein and nucleic acid structure and synthesis. Iodophors are bactericidal, mycobactericidal, viricidal but may require prolonged contact times to kill certain fungi and spores (Klein and de Forest, 1963; Sattar et al., 1983; Rutala et al., 1991). In dentistry, iodine is used as mouthrinse effectively to reduce plaque and gingivitis (Domingo et al., 1996; Maruniak et al., 1992).

1.2.4.4 Chlorine and chlorine compounds

Chlorine-releasing agents are well established as environmental disinfectants. Hypochlorites are the most widely used compounds and they are available in liquid or solid form. The most used chlorine compound is sodium hypochlorite, which is usually called household bleach. It has broad spectrum antimicrobial activity, does not leave toxic residues, is unaffected by water hardness, fast acting, inexpensive, removes dried or fixed microorganisms and biofilms from surfaces. The disadvantages are: it is inactive in the presence of organic matter, corrosive to metals, eye and skin irritant and relatively unstable.

Alternative compounds that release chlorine and are used in the healthcare setting include demand-release chlorine dioxide, sodium dichloroisocyanurate and chloramine T. These compounds retain chlorine longer, meaning they have prolonged bactericidal effect.

Chlorine dioxide (ClO_2), which is a gas at temperature above 11^{o}C, has long been known to have germicidal properties (Chen and Vaughn, 1990; Farr and Walton, 1993; Eleraky et al., 2002; Wilson et al., 2005) and has been used in large scale municipal and industrial water purification and wastewater treatment system. It is a water-soluble gas and exhibits rapid kill over a wide range of organisms. It works through oxidation and penetrates bacterial cell walls and reacts with vital amino acids in the cytoplasm of the cell to kill the organism. Chlorine dioxide has been proven to kill a diverse array of bacteria, viruses, algae, fungi and protozoa. It has a long history of use as a disinfectant and is accepted by the United States Environmental protection agency (EPA) drinking water division in water treatment applications. Compared to phenols, bleach, glutaraldehyde, quaternary

ammonium compounds and other disinfectants, it is the most effective biocide on hard surfaces (Tanner, 1989) and has been shown to have a degree of fungicidal and sporicidal activity in solution (Price and Ahearn, 1999; Weaver-Meyers et al., 2000).
Chlorine dioxide water treatment system is safe and efficient in controlling Legionella contamination of hospital water supplies (Srinivasan et al., 2003; Zhang et al., 2007).

Chlorine dioxide offers a variety of safety and environmental advantages over chlorine and many other commonly used antimicrobial agents and is four to seven times more effective as a biocide than chlorine at equivalent doses. Furthermore, it does not produce halogenated, carcinogenic by-products, such as trihalomethanes, dioxins and haloacetic acids, which may be produced when free chlorine is used. It maintains its efficacy over a wide pH range (1-10) while chlorine's effectiveness is significantly reduced above neutral or basic pH.

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The use of chlorine dioxide had previously been restricted to large-scale industrial applications because it could not be incorporated into a stable, easy-to-use powder and tablet form. The development of new delivery technology (Engelhard Aseptrol technology) that allows chlorine dioxide to be generated at the point of use, in smaller quantities, without special equipments, has opened the door for smaller scale uses. Aseptrol® is unreactive in dry form but produces chlorine dioxide only when exposed to moisture in ambient temperature or dissolved in water. When sealed in airtight containers, it can be stored and used when needed (Cochran M, 2005). The formula of Aseptrol®, incorporates stabilizing agents and anticorrosion compounds making them suitable for small-scale applications. The manufacturer of this product claims of numerous advantages

of usage in industrial setting. The efficacy of Aseptrol® in medical and dental hospitals has not been established.

Sodium dichloroisocyanurate (NaDCC) is a stable source of chlorine (Janácek and Lodin, 1999) used as a disinfectant. In small doses it is common in water purification tablets/filters. It can be used for disinfecting spillages of blood containing many pathogens, including, Human immunodeficiency virus (HIV) and Hepatitis B virus (HBV). It has the advantage of providing a higher concentration of available chlorine and is less susceptible to inactivation by organic matter (McDonnell and Russell, 1999). However, it is corrosive to metals and at higher concentrations, irritant to skin. Sodium dichloroisocyanurate is stable until dissolved, rapidly effective and slightly less damaging to surfaces and instrument components than sodium hypochlorite. However, like all other chlorine-releasing agents, the use of NaDCC in hospitals is usually limited to environmental surfaces because of its corrosiveness.

1.2.5 Disinfectant testing

Disinfectant testing is important to evaluate the efficacy of the preparation for specified clinical applications. The activity against indicator organisms, usually common pathogens, remains the most important factor in selecting an appropriate disinfectant. The choice is best made by evaluating the activity against key organisms and comparing this activity with other factors such as toxicity, material compatibility, stability of the compound, user safety, rapidity of killing, and cost (Fraise, 1999).

1.2.5.1 Microbial Susceptibility

Generally, different microorganisms vary in their susceptibility to disinfectants (Fig 1.1). Bacterial spores are the most resistant, followed by mycobacteria, and then vegetative form of bacteria. The lipid-enveloped viruses that comprise the HIV and HBV are readily inactivated by disinfectants as compared with small non-enveloped viruses e.g. poliovirus (Russell, 1998).

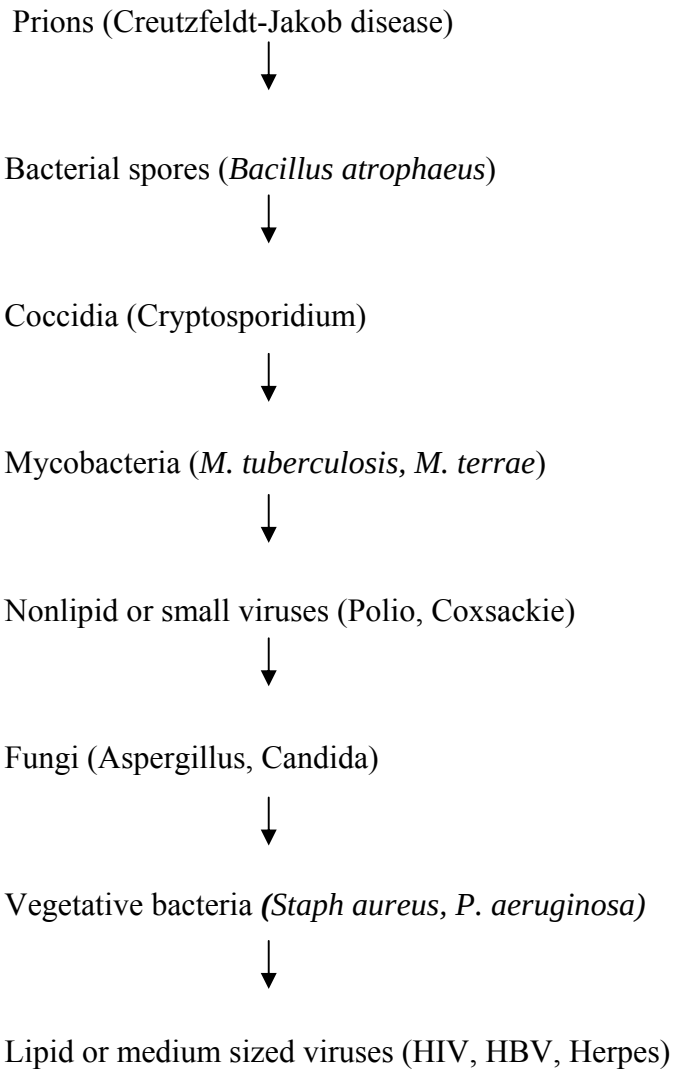


Figure 1.1: Decreasing order of resistance of microorganisms to disinfection and sterilization (Modified from Russell, 1998).

However, there are further variations within groups, with spores of *Bacillus subtilis* being more resistant than those of *Clostridium difficile* and Gram-negative organisms such as *Pseudomonas aeruginosa*, *Providentia spp* and *Proteus spp* being more difficult to inactivate (Russell, 1998).

1.2.5.2 Choice of Test Organisms

The Environment Protection Agency (EPA) recommends demonstration of activity against *Salmonella choleraesuis, P. aeruginosa* and *S. aureus* by all agents registered as hospital disinfectants (Centres for Disease Control, 2003). In general, microorganisms representing different groups and organisms generally found in the hospital environment are used for disinfectant testing. The following organisms have been specified and used as test organisms to test disinfectant efficacy for different clinical applications: *Streptococcus mutans, Staphylococcus aureus, Candida albicans, Escherichia coli, Salmonella choleraesuis, Pseudomonas aeruginosa, Mycobacterium bovis, Bacillus subtilis* spores, Hepatitis B virus, and Duck Hepatitis B virus. The European Committee for Standardisation (cited by van Klingeren, 1995) recommended the use of at least one Gram-positive and one Gram-negative organism

1.2.5.3 Testing Procedures

There is no specific universally accepted disinfectant testing procedure that has been developed. It is acceptable that available tests may be modified for specific purposes with regard to test strains, contact times, interfering substances (soiling, organic matter, hard water) and test surfaces (van Klingeren, 1995). The procedure can be designed according

to the type of area, in which the disinfectant will be used, what organisms is expected, suitable exposure time periods etc. The conditions of contact between the disinfectant and the organism have a significant impact on the activity of the disinfectant (Fraise, 1999). Many factors affect the efficacy of disinfectant, such as, hardness of water, pH of the solution, diluent and presence of organic material and therefore the tests have to take these into account.

A simple procedure, called suspension test method, can be used, where rapidity of biocidal activity of a disinfectant can be determined in the form of percentage kill or a reduction factor in a specific contact time period. In this method, known quantity of organisms and the disinfectant are mixed in a liquid form and the solution is neutralized at the end of the contact time period and the solution is cultured (quantitative) for the surviving organisms. The percentage kill is calculated using challenged organisms and surviving organism count. Being an in vitro test, suspension test is useful in determining whether the examined preparation possesses antimicrobial activity during the specified contact time. It is however a poor indicator of the efficacy of the disinfectant under practical circumstances, especially with regard to bacteria attached to surfaces (Reybrouck, 1991).

Another useful method is the surface or carrier test. Surface tests were developed to mimic the conditions of actual use of the disinfectant and hence assess the efficacy of the disinfectant in practice and the influence of interfering factors. In this method, the bacterial suspension is applied to a carrier surface and the disinfectant is then applied on the contaminated surface, followed by neutralisation after a specified contact time and sub-culturing to determine the antimicrobial activity.

1.2.6 Indicator organisms

Microorganisms representing different groups and organisms generally found in the hospital environment are used for disinfectant testing. At least one gram positive and one gram negative organism should be included (European Committee for Standardisation). Depending on what situation the disinfectant is recommended to be used, test organisms should be selected. If the disinfectant is used in a hospital environment, types of organisms generally found in hospitals should be used as test organisms. Some of the organisms found in hospital environment are discussed.

1.2.6.1 *Staphylococcus aureus*

Staphylococcus aureus are gram positive cocci in clusters and cause both common and uncommon infections, such as abscesses of many organs, endocarditis, food poisoning and toxic shock syndrome. They are carried by healthy individuals, in their nose or on the skin. They are always present in the hospital environment, on hospital patients and on the hands of hospital staff. Methicillin-resistant *S. aureus* (MRSA) has become an important pathogen leading to hospital-acquired infections, which often leads to major morbidity and mortality. The principal mode of transmission for MRSA is transfer of the organism from a carrier or infected patient to uninfected patients by the hands or clothing of staff (Wang et al., 2001).

1.2.6.2 *Psuedomonas aeruginosa*

Non-fermentative Gram negative rods such as, *P. aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia,* are opportunistic pathogens responsible for nosocomial infections. To better prevent nosocomial infections related to Gram negative non-fermentative rods, the control of the aqueous hospital environment, the strict application of hand disinfection and the investigation of potential cross-transmission in the hospital setting are needed (Berthelot et al., 2005). *P. aeruginosa* is one of the most important gram-negative pathogens causing infections in ICUs. According to the US National Nosocomial Infections Surveillance (NNIS) system report, *P. aeruginosa* ranks among the top five pathogens associated with wound, pulmonary and urinary tract infections (NNIS, 2004). *P. aeruginosa* also plays a significant role as a cause of ventilator-associated pneumonia (Zanetti et al., 2003). Trautmann et al., (2005) cited that approximately one fifth to one third of *P. aeruginosa* strains detected by screening cultures in the ICU were present on admission. The remaining fraction was apparently acquired newly in the ICU and cross transmissions were identified in between 8% and 50% of these newly acquired colonization or infections. Hospital staff was speculated to be a vector in these cases. *P. aeruginosa* has been cultured from the hands of hospital personnel and it can be implicated in the nosocomial transmission (Moolenar et al., 2000).

1.2.6.3 *Streptococcus mutans*

S. mutans are gram positive cocci in chains. They are viridians group of streptococci, commonly found in oral cavity as commensals. These bacteria are implicated in dental caries and are common contaminants in dental clinics. Although they are not commonly

used as an indicator organism for disinfectant testing, they can be used in order to establish the efficacy and use of the disinfectant in dental settings.

1.2.6.4 *Candida albicans*

C. albicans is unicellular, oval yeast usually endogenous in origin. Normal healthy individuals carry this yeast in their mouth, gastrointestinal tract and female genital tract. In South Africa, a study showed that 81% of HIV positive patients and 63% of HIV negative people carry *C. albicans* in their mouths (Patel et al., 2006). Colonization of this yeast can become a predictive value for the onset of nosocomial infection in elderly and immunocompromised individuals (Fanello et al., 2006). Transmission from patient to patient has also been suggested by Fanello et al., (2006). The major infections caused by this yeast are oropharyngeal and vaginal candidiasis, as well as systemic infections. The efficacy of many disinfectants is tested against this organism.

1.2.6.5 *Bacillus subtilis*

B. subtilis are gram positive, spore forming rod. They are saprophytic, environmental organism. They are non-pathogenic bacteria; however they have become popular in the disinfectant testing because of the spore formation. Spores are the dormant living stage of organisms and are difficult to eliminate. If a disinfectant is efficient in killing spores, it will definitely kill any vegetative form of organisms. Sodium dichloroisocyanurate, a chlorine based disinfectant, is an effective disinfectant against many vegetative form of organisms but was ineffective against bacterial spores in a hospital environment (Block C, 2004).

1.2.6.6 Mycobacteria

Mycobacteria are acid-fast bacilli which are commonly found in the environment. Many species of this genus are pathogenic to humans, animals and birds. They are very difficult to eliminate with common disinfectants because of the lipid content of their cell wall. Bactericidal disinfectants are generally considered excluding mycobacteria, unless they suggest mycobactericidal activity. *Mycobacterium tuberculosis,* the causative agent of tuberculosis, is generally transmitted by the airborne route. However, because of its resistance to drying, environmental surfaces may also act as potential vehicles of transmission. Improperly decontaminated flexible fibreoptic endoscopes, resuscitation and lung-function equipment and ventilators have also been implicated in the transmission of mycobacterial infection (Sattar et al., 1995). A study by Griffiths et al., (1999) showed that chlorine dioxide and higher concentrations of sodium dichloroisocyanurate (NaDCC) are rapidly mycobactericidal and glutaraldehyde was found to be a slow mycobactericide. The result also showed that the clinical isolate of *M. avium-intracellulare* was much more resistant than *M. tuberculosis* to the disinfectants.

1.2.6.7 Hepatitis B virus

Hepatitis B virus (HBV), a small circular, partially double-stranded DNA virus of approximately 3,200 base pairs, belongs to the family Hepadna viridae. The only known hosts for HBV are humans. It can cause acute, as well as asymptomatic disease. Asymptomatic disease can remain as a chronic infection, where the person becomes a carrier and a potential source of transmission (Mims et al., 2004). Chronic HBV infection can lead to liver cirrhosis and hepatocellular carcinoma (Gamen and Prince, 2004). The

transmission can occur through percutaneous or mucosal exposure to blood and other body fluids from infected person. Blood contains the highest HBV titres of all body fluids and is the most important vehicle of transmission in the healthcare setting. The most common marker of HBV infection is the presence of HBV surface antigen (HBsAg).

Hepatitis B is a major public health problem worldwide. Approximately 350 million to 400 million people throughout the world are chronically infected (Lee, 1997; WHO, 2000). Nosocomial infections caused by the Hepatitis B virus in patients during hospitalization and interventional procedures, as well as in health care workers, have been documented (CDC, 2003; Comstock et al., 2004). Infection control, including the usage of viricidal disinfectant is necessary in order to prevent this transmission. Transmission of human Hepatitis B virus (HBV) by plasma concentrates from donated blood has been reduced by screening and inactivation procedures. However, concern persists over nosocomial infections that could be acquired through inadequately disinfected equipment or accidental exposure of personnel to blood or other body fluids from HBV-positive individuals. Disinfection particularly with heptoviricidal efficacy disinfectant is an important measure to prevent Hepatitis B virus (HBV) transmission by instruments. Germicidal chemicals are important to prevent spread of HBV through re-usable devices, as well as in the clean-up of blood spills (Sattar et al., 2001).

Study of viricidal activity of biocides is difficult because animals and tissue culture facilities are required. The real-time quantitative polymerase chain reaction (PCR) is useful for rapid screening of the killing potency of disinfectants. The ease of PCR and enhanced sensitivity make it more desirable (Wang et al., 2002). Quantitative detection of Hepatitis B virus (HBV) in serum or plasma has become the most direct and reliable

method for monitoring chronic hepatitis B. HBV DNA quantification assays should ideally be sensitive, specific, precise, reproducible, automated, rapid and accurately provide HBV DNA levels in international units per millilitre (Valentine-Thon,2002). The COBAS® AmpliPrep/COBAS® Taqman® HBV Test provides a high-throughput sensitive and reliable method for quantification of HBV DNA levels in the routine molecular laboratory (Ronsin et al., 2006; Chevaliez et al., 2008). It is a nucleic acid amplification test for the quantitation of Hepatitis B virus (HBV) DNA in human plasma. The test is based on two major processes: specimen preparation to isolate HBV DNA and simultaneous PCR amplification of target DNA and detection of cleaved dual-labelled oligonucleotide detection probe specific to the target. Known quantity of HBV particles can be challenged with the test disinfectant and the resultant surviving HBV particles can be detected using this technique.

1.3 AIM

The aim of this study was to ascertain the antimicrobial effect of slow release chlorine dioxide (ClO_2) disinfectant (ASEPTROL®) in comparison with chlorine releasing agent sodium dichloroisocyanurate (PRESEPT®) using standard indicator organisms**.**

CHAPTER 2: MATERIALS AND METHODS

2.1 DISINFECTANT

Efficacy of Aseptrol® and Presept® were tested in this study.

Aseptrol® (Waylor Trading and Logistics cc, South Africa) in tablet form was used to make the disinfectant solution. A 48ppm solution of ClO_2 was made by dissolving one 1.5g tablet of Aseptrol® in 2.5 litres of tap water according to the manufacturer's instructions. The mixture was left for 20 minutes to dissolve, followed by gentle mixing with a glass rod. A further 2-fold dilution of the disinfectant was prepared, to obtain 24ppm solution, by mixing 1 litre of 48ppm solution to 1 litre of tap water.

Presept® (Johnson and Johnson, South Africa) solution was prepared by dissolving seven tablets of 2.5g each in one litre of tap water, according to the manufacturer's instruction for use in blood spillage. The solution contained 10 000ppm available chlorine.
For each experiment, disinfectant solutions were freshly prepared, just before use.
The manufactures of these products could have taken into consideration the presence of chlorine in tap water.

2.2 CULTURES

The antimicrobial property of chlorine dioxide (Aseptrol®) and sodium dichloroisocyanurate (Presept®) was tested against *Staphylococcus aureus (*ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus mutans* (NCTC 1044), *Candida albicans* (ATCC 90028), *Bacillus subtilis* (ATCC 15244) spores, Mycobacterium

tuberculosis (ATCC 25177), *Mycobacterium avium* subsp. *avium* (ATCC 25291) and Hepatitis B virus (HBV). *S. aureus*, *P. aeruginosa*, *S. mutans, C. albicans* and *B. subtilis* were obtained from Infection control laboratory, Department of Clinical Microbiology and Infectious Diseases, National Health Laboratory Service, Johannesburg and stored in semisolid agar as stock cultures. *M. tuberculosis* and *M. avium* subsp. *avium* were obtained from Mycobacteriology referral laboratory, National Health Laboratory Services, Braamfontein, Johannesburg and stored in aliquots at -70 ^{o}C as stock cultures. Hepatitis B virus was obtained from the immunology laboratory, National Health Laboratory Service, Braamfontein, Johannesburg and quantitated at the specialized molecular diagnostic unit at National Institute of Communicable Diseases, National Health Laboratory Service.

2.3 PREPARATION OF INOCULA

Each test organism was grown on the appropriate medium to obtain a primary culture for preparation of inoculum suspensions. *S. aureus* and *P. aeruginosa* were grown on Tryptone Soy Agar media and incubated at 37^{o}C for 24 hours. *S. mutans* was grown on Blood agar and incubated at 37^{o}C for 48 hours under carbon dioxide. *C. albicans* was grown on Sabouraud Dextrose Agar medium at 37^{o}C for 48 hours. *M. tuberculosis* and *M. avium* subsp. *avium* were grown in BACTEC mycobacteria growth indicator tube (MGIT) liquid medium. MGIT tube contains 7ml of modified Middlebrook 7H9 Broth base and is one of the commonly used liquid media for the cultivation of Mycobacteria. The MGIT tube contains a fluorescent compound embedded in silicone on the bottom of the tube. The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth. The initial concentration of dissolved oxygen quenches the emission from the compound

and little fluorescence can be detected. Later, actively growing and respiring micro-organisms consume the oxygen, which allows the compound to fluoresce.

BACTEC MGIT growth supplement is added to each MGIT tube to provide substances essential for the rapid growth of mycobacteria. Oleic acid is utilized by tubercle bacteria and plays an important role in the metabolism of mycobacteria. Albumin acts as a protective agent by binding free fatty acids which may be toxic to *mycobacterium* species, thereby enhancing their recovery. Dextrose is an energy source and catalase destroys toxic peroxides that may be present in the medium. Tubes are loaded into the BACTEC MGIT 960 system and are continuously incubated at 37^{o}C and monitored every 60 minutes for increasing fluorescence. An instrument positive tube, which contains approximately 10^{5} to 10^{6} colony forming units per millilitres (CFU/ml), was used as an inoculum. For each experiment, fresh cultures were used.

For *S. aureus, P. aeruginosa, S. mutans* and *C. albicans*, the resultant growth was harvested using a sterile wire loop and suspended in 20 ml sterile distilled water. The suspension was mixed by gentle shaking to obtain a homogenous organism suspension. The optical density of the inoculum was adjusted to 0.2 McFarland standards.

B. subtilis was grown on Tryptone Soy Agar with 2 mg/l manganese sulphate ($MnSO_4$) to enhance sporulation and the culture incubated at 37^{o}C for 7 days (Perez et al., 2005). The Schaefer and Fulton's method for staining spores (Jawetz et al., 1991) was used to confirm >90% spore production. The sporulating culture was suspended in 20ml of sterile distilled water and placed in a water-bath at 70^{o}C for 20 minutes, in order to kill the vegetative cells.

The optical density of the resultant spore suspension was adjusted to 0.2 McFarland standards (approximately 10^6 test organism per milliliter) and used as an inoculum.

2.4 TEST PROCEDURES FOR PERCENTAGE KILL (BACTERIA)

This test procedure applies to ***S. aureus, P. aeruginosa, S. mutans, C. albicans*** and ***B. subtilis***

Standard quantitative suspension test (percentage kill) or exposure test was done. In time kill experiment, 2ml of 48ppm and 24ppm chlorine dioxide solution (Aseptrol®) and chlorine releasing solution (Presept®) were inoculated with 20µl of inoculum containing approximately 10^6 test organism per millilitre and 10µl of skim milk. Skim milk was used to simulate organic material. Number of organisms in the inocula was determined for each experiment using serial dilution test. After inoculation, every 30 seconds for 5 minutes, 20µl of inoculated test compounds were removed, neutralized with a universal neutralizer (Perez et al., 2005) containing quarter strength Ringer's solution, 0.5% Tween 80 and 0.5% sodium thiosulphate, diluted and then spread onto appropriate medium to achieve the number of surviving organisms. Culture plates were incubated at 37°C for 24 or 48 hours depending on the type of organism. The colony count for each plate was determined and percentage kill was calculated using the inoculum count. The formula is given below:

$$\frac{\text{Challenged org. (Inoculum count)} - \text{(surviving org)}}{\text{Challenged org. (Inoculum count)}} \times 100 = \text{percentage (\%) kill}$$

Time taken to kill the challenged organisms was recorded against percentage kill. For each test organism, experiment was repeated 5 times using 48ppm, 24ppm chlorine dioxide solution (Aseptrol®) and sodium dichloroisocyanurate chlorine solution (Presept®).

2.5 TEST PROCEDURE FOR PERCENTAGE KILL (MYCOBACTERIA)

This test procedure applies to ***M. tuberculosis*** and ***M. avium* subsp. *avium.***

Quantitative suspension test described by Hernández et al., (2003) was used in this study. Cultures were grown in the BACTEC mycobacteria growth indicator tube (MGIT). Viable bacterial count was obtained using serial dilution technique. 20µl of this prepared suspension was added to 2ml of disinfectant, containing 10µl of skim milk. After inoculation, every 30 seconds for 5 minutes, 20µl of inoculated test compound was removed, neutralized with a universal neutralizer, and spread onto middlebrook 7H10 agar plate. Culture plates were incubated at 37^{o}C for 4 -6 weeks. The colony count for each plate was determined and percentage kill calculated using the inoculum count. Percentage kill was recorded for each contact time period.

Each experiment was repeated 5 times using 48ppm, 24ppm chlorine dioxide solution (Aseptrol®) and sodium dichloroisocyanurate chlorine solution (Presept®).

2.6 TEST PROCEDURE FOR PERCENTAGE KILL (Hepatitis B Virus)

COBAS® Ampliprep/COBAS® Taqman® HBV Test was performed to test the efficacy of test disinfectant against Hepatitis B virus. It is a nucleic acid amplification test for the

quantitation of Hepatitis B virus (HBV) DNA in human plasma. The test is based on two major processes: specimen preparation to isolate HBV DNA and simultaneous PCR amplification of target DNA and detection of cleaved dual-labelled oligonucleotides detection probe specific to the target. The test combines the COBAS® Ampliprep instrument, which performs fully automated extraction of HBV DNA and the COBAS® Taqman® 48 Analyzer, which performs fully automated real-time PCR amplification and detection, followed by interpretation of HBV DNA levels by means of Amplilink® software.

The Quantitation of HBV DNA was performed using HBV Quantitation standard (QS). The QS compensates for the effects of inhibition and controls the preparation and amplification process. It was added through all the steps of specimen preparation, amplification and detection of dual labelled oligonucleotides detection probes. The Master Mix reagent contained primer pairs and probes specific for both HBV DNA and HBV QS DNA. The HBV DNA concentration in the test specimen was calculated by the COBAS® Taqman® analyzer by comparing the HBV QS signal for each control and specimen (Roche Molecular Systems).

2.6.1 Target selection

Selection of the target DNA sequence for HBV depends on identification of regions within the HBV genome that show maximum sequence conservation among the various HBV genotypes. Generic silica-based specimen preparation was used to capture the HBV DNA and HBV QS DNA and defined oligonucleotides are used as primers in amplification of the HBV DNA and HBV QS DNA. A target-specific and a QS-specific

dual-labelled oligonucleotides probe permitted independent identification of HBV amplicon. The appropriate selection of the primers and the dual-labelled oligonucleotides probe was critical to the ability of the test to amplify and detect the HBV genotypes. The COBAS® Taqman® HBV Test used three amplification primers for PCR.

2.6.2 Specimen preparation

The COBAS® Ampliprep/COBAS® Taqman® HBV Test utilized automated specimen preparation on the COBAS® Ampliprep instrument by a generic silica-based capture technique. The procedure processes 850µl of plasma. The HBV virus particles were lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that released nucleic acids and protected the released HBV DNA from DNases in plasma. Protease and a known number of HBV QS DNA molecules were introduced into each specimen along with the lysis reagent and magnetic glass particles. The mixture was incubated and the HBV DNA and HBV QS DNA are bound to the surface of the glass particles. Unbound substances were removed by washing the magnetic glass particles. The processed specimen, containing the magnetic glass particles, as well as released HBV DNA and HBV QS DNA, was added to the amplification mixture and transferred to the COBAS® Taqman® 48 Analyzer.

2.6.3 PCR Amplification

The PCR amplification reaction was performed with the thermostable recombinant enzyme *Thermus* specie DNA Polymerase (Z05). In the presence of manganese (Mn^{2+})

and under the appropriate buffer condition, Z05 has DNA activity. This allowed PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens were added to the amplification tubes (K-tubes) in which PCR amplification occurs. The thermal Cycler in the COBAS®Taqman® Analyser heats the reaction mixture to denature the double-stranded DNA and expose the specific primer target sequences on the HBV circular DNA genome and the HBV QS DNA. As the mixture cools, the primers anneal to the target DNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine triphosphates, Z05 polymerase extends the annealed primers along the target template to produce a double-stranded DNA molecule, termed an amplicon. The COBAS®Taqman® Analyser automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA.

2.6.4 Detection of PCR products

The use of dual-labelled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HBV and HBV QS-specific oligonucleotides probes with a reporter dye and a quencher. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher due to Förster-type energy transfer effects.

Once the reporter and quencher dyes are released and separated, quenching no longer occurs and the fluorescent activity of the reporter dye is increased. The amplification of HBV DNA and HBV QS DNA are measured independently at different wavelengths.

These fluorescent readings are sent by the instrument to the AMPLILINK® software and stored in a database. The COBAS® Ampliprep/ COBAS® Taqman® HBV Test is standardized against WHO international standard for Hepatitis B virus DNA for Nucleic Acid Technology (NAT) assays testing (NIBSC 97/746) and titer results are reported in international units (IU/Ml).

2.6.5 HBV DNA Quantification

The test was performed using COBAS® Ampliprep/COBAS® Taqman® HBV test kit (described as above), according to the manufacturer's instructions. HBV CS1 (HBV magnetic glass particles reagent cassette) was placed onto a reagent rack and put into position A of the COBAS® Ampliprep instrument. HBV CS2 (HBV lysis reagent cassette), HBV CS3 (HBV multi-reagent cassette) and HBV CS4 (HBV test-specific reagent cassette) were placed into a separate reagent rack and loaded into rack position B, C, D or E. Sample processing units (SPUs) were placed in the racks and loaded onto appropriate rack position. Full K-tube and K-tip racks were also loaded in their positions. One millilitre of controls (negative, low positive and high positive) and 960µl of HBV-negative human EDTA-plasma were manually transferred into barcode-labelled sample input tubes (S-tubes). 20µl of universal neutralizer was added to each S-tube containing HBV-negative human EDTA-plasma. 20µl of positive HBV, with known viral load (1.0 X 10^5 iu/ml), was added to 2ml of disinfectant. Every 30 seconds for 5 minutes, 20µl of the

mixture was removed and added to the S-tubes and then placed into sample racks and loaded in the COBAS® Ampliprep instrument. Using the COBAS® Ampliprep instrument connected to the COBAS® Taqman® via docking station, transfer of extracted material for amplification and detection occurred automatically. After completion of the COBAS® Taqman® analyzer, the result reports were printed. The COBAS® Taqman® analyzer automatically determined the HBV DNA concentration for the specimens and controls and expressed them in IU/ml.

Skim milk was not added in these tests because plasma represented organic material. The test was performed 5 times using 48ppm and 24ppm Aseptrol® solution and Presept® solutions.

2.7 SHELF-LIFE STUDY PROCEDURE

According to manufacturer's recommendation, concentrations of Aseptrol® (48ppm) and Presept® (10 000ppm) were prepared. Time-kill study using above organisms were performed on the same day at contact time periods of 30 and 60 seconds and thereafter every fifth day. After 20 days, the test was performed everyday. Same experiment was performed until the prepared disinfectant solutions became ineffective. The last effective day for each organism was recorded. Only the qualitative analysis was done.
This test was not performed for *B. subtilis* spores and Hepatitis B virus. The test was performed once for the rest of the test organisms.

2.8 STATISTICAL ANALYSIS

Percentage kill study was performed 5 times for each test organism and each test concentration of the two disinfectants. Descriptive statistical analysis was performed. Mean and standard deviations were recorded.

CHAPTER 3: RESULTS

3.1 EFFECT OF ASEPTROL® AND PRESEPT® ON TEST ORGANISMS

The results are presented in table form (Tables 3.1 – 3.9). Each table contains results of one test microorganism, two concentrations of Aseptrol® and one concentration of Presept®. Each concentration was tested 5 times, therefore 5 results are depicted. The inoculum count for each test is given and the percentage kill is calculated and described. Mean of percentage kill for each concentration is also calculated.

3.1.1 Effect of test disinfectants on *S. aureus*

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^6 CFU of *S. aureus* within 30 seconds (Table 3.1). The results are reproducible because the test was repeated 5 times and the results did not differ.

3.1.2 Effect of test disinfectants on *P. aeruginosa*

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^6 CFU of *P. aeruginosa* within 30 seconds (Table 3.2). The results are reproducible because the test was repeated 5 times and the results did not differ.

3.1.3 Effect of test disinfectants on *S. mutans*

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^6 CFU of *S. mutans* within 30 seconds (Table 3.3). The results are reproducible because the test was repeated 5 times and the results did not differ.

3.1.4 Effect of test disinfectants on *C. albicans*

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^4 CFU of *C. albicans* within 30 seconds (Table 3.4). The results are reproducible because the test was repeated 5 times and the results did not differ.

3.1.5 Effect of test disinfectants on *B. subtilis* spores

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^5 to 10^6 CFU of *B. subtilis* spores within 2 and 2.5 minutes (Table 3.5). In this table, after the exposure to disinfectants, number of surviving organisms is also given because the percentage kill was not 99.99 in every test. Aseptrol® performed slightly better than Presept®. The survival of B. *subtilis* spores varied in each test, therefore mean and standard deviations were calculated. The results also showed that at ineffective contact time periods, five test results were different and therefore standard deviations are high. However, as the contact time period increased, the test concentration became effective and consistent results were obtained showing low standard deviations.

3.1.6 Effect of test disinfectants on *M. tuberculosis* and *M. avium subsp. avium*

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^5 CFU of *M. tuberculosis* and *M. avium subsp. avium* within 30 seconds (Tables 3.6 and 3.7). The results are reproducible because the test was repeated 5 times and the results did not differ.

3.1.7 Effect of test disinfectants on Hepatitis B virus

Aseptrol® at concentrations 48ppm and 24ppm and Presept® at 10 000ppm killed approximately 10^5 particles of Hepatitis B virus within 30 seconds (Table 3.8). The results are reproducible because the test was repeated 5 times and the results did not differ.

The Summary of above results is shown in Table 3.9, where it is clear that both disinfectants are bactericidal, mycobactericidal, fungicidal and viricidal at 30 seconds exposure. They are sporicidal at 2 – 2.5 minutes contact time period.

3.2 ANTIMICROBIAL SHELF-LIFE OF ASEPTROL® AND PRESEPT®

This test was not performed for Hepatitis B virus and *B. subtilis* spores. Although the manufacturers recommend the disinfectant solutions should be prepared daily, the results showed that if Aseptrol® solution is prepared and stored in a screw cap bottles, it retains bactericidal effect for at least 27 days with contact time period of 30 seconds (Table 3.10). On the other hand, Presept® was found to be effective even until 37 days. The tests were not performed after 37 days (Table 3.10).

Table 3.1 The effect of Aseptrol® and Presept® on *S. aureus* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.8 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	4.7 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.2 The effect of Aseptrol® and Presept® on *P. aeruginosa* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.1 X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.3 The effect of Aseptrol® and Presept® on *S. mutans* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.4X 10^6	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.4 The effect of Aseptrol® and Presept® on *C. albicans* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	6.0 X 10^4	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.5 The effect of Aseptrol® and Presept® on *B. subtilis* spores at various contact time period.

Disinfect-ant	**Tests**	**No. of challenged organisms Cfu/ml**	**½ min**		**1 min**		**1½ min**		**2 min**		**2½ min**	
			No. surviving org cfu/ml	**% kill**	No. surviving org cfu/ml	**% kill**	No. surviving org cfu/ml	**% kill**	No. surviving org cfu/ml	**% kill**	No. surviving org cfu/ml	**% kill**
Aseptrol® 48 ppm	1	3.4×10^6	2.6×10^6	23.53	3.0×10^4	99.12	4.8×10^3	99.86	0	99.99	0	99.99
	2	3.5×10^5	1.8×10^5	48.57	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99	0	99.99
	3	3.5×10^5	1.3×10^5	62.86	3.5×10^4	90	5.0×10^3	98.57	0	99.99	0	99.99
	4	3.5×10^5	1.1×10^5	68.57	3.0×10^4	91.43	5.0×10^3	98.57	0	99.99	0	99.99
	5	3.5×10^5	1.2×10^5	65.71	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99	0	99.99
	Mean ±SD			**53.85± 18.61**		**95.54± 4.44**		**98.83± 0.58**		**99.99± 0**		**99.99± 0**
Aseptrol® 24 ppm	1	3.4×10^6	1.4×10^6	58.8	4.9×10^4	98.56	4.9×10^4	98.56	0	99.99	0	99.99
	2	3.5×10^5	1.4×10^5	60	1.3×10^5	62.86	5.0×10^3	98.57	0	99.99	0	99.99
	3	3.5×10^5	1.0×10^5	71.43	1.0×10^4	97.14	5.0×10^3	98.57	0	99.99	0	99.99
	4	3.5×10^5	1.5×10^5	57.14	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99	0	99.99
	5	3.5×10^5	2.0×10^5	42.86	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99	0	99.99
	Mean ±SD			**58.05± 10.18**		**91.14± 15.82**		**98.57± 0**		**99.99± 0**		**99.99± 0**
Presept® 10 000 ppm	1	3.4×10^6	2.4×10^5	92.94	1.0×10^4	99.70	4.8×10^3	99.86	5.0×10^3	99.85	0	99.99
	2	3.5×10^5	1.0×10^5	71.43	4.9×10^4	86	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99
	3	3.5×10^5	2.7×10^5	22.86	1.2×10^5	65.71	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99
	4	3.5×10^5	1.6×10^5	54.29	9.0×10^4	74.29	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99
	5	3.5×10^5	1.0×10^5	71.43	2.5×10^4	92.86	5.0×10^3	98.57	5.0×10^3	98.57	0	99.99
	Mean ±SD			**62.59± 26.10**		**83.71± 13.76**		**98.83± 0.58**		**98.83± 0.57**		**99.99± 0**

Table 3.6 The effect of Aseptrol® and Presept® on *M. tuberculosis* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	5.0×10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.7 The effect of Aseptrol® and Presept® on *M. avium* subsp. *avium* at various contact time period.

Disinfectant	Tests	No. of challenged organisms Cfu/ml	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol 24 ppm	1	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept 10 000 ppm	1	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	0.4 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.8 The effect of Aseptrol® and Presept® on Hepatitis B virus at various contact time period.

Disinfectant	Tests	No. of challenged	Percentage kill (%) and contact time period (minutes)									
			½ min	1 min	1½ min	2 min	2½ min	3 min	3½ min	4 min	4½ min	5 min
Aseptrol® 48 ppm	1	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Aseptrol® 24 ppm	1	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**
Presept® 10 000 ppm	1	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	2	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	3	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	4	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	5	1.0 X 10^5	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99
	Mean		**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**	**99.99**

Table 3.9 Summary of Percentage kill of the test organisms by chlorine dioxide (Aseptrol®) and chlorine (Presept®) releasing disinfectants

Disinfectant	Test org.	Mean No. of challenged org. cfu/ml or iu/ml	Percentage kill (%) and contact time period Mean ± SD, n=5				
			½ min	1 min	1½ min	2 min	2½ min
Aseptrol® 48 ppm available chlorine	*S. aureus*	4.7×10^6	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00
	P. *aeruginosa*	1.1×10^6					
	S. mutans	1.4×10^6					
	C. albicans	6.0×10^4					
	M. tuberculosis	5.0×10^5					
	M. avium complex	4.0×10^4					
	Hepatitis B virus	1.0×10^5					
	***B. subtilis* spores**	6.8×10^6	**61.43±7.69**	**95.54±4.44**	**98.83±0.58**		
Aseptrol® 24 ppm available chlorine	*S. aureus*	4.7×10^6	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00
	P. aeruginosa	1.1×10^6					
	S. mutans	1.4×10^6					
	C. albicans	6.0×10^4					
	M. tuberculosis	5.0×10^5					
	M. avium complex	4.0×10^4					
	Hepatitis B virus	1.0×10^5					
	***B. subtilis* spores**	6.8×10^6	**58.05±10.18**	**91.43±15.18**	**98.57±0.00**		
Presept® 10 000 ppm available chlorine	*S. aureus*	4.7×10^6	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00	99.99±0.00
	P. aeruginosa	1.1×10^6					
	S. mutans	1.4×10^6					
	C. albicans	6.0×10^4					
	M. tuberculosis	5.0×10^5					
	M. avium complex	4.0×10^4					
	Hepatitis B virus	1.0×10^5					
	***B. subtilis* spores**	6.8×10^6	**63.19±25.49**	**83.18±13.02**	**98.83±0.58**	**98.57±0.00**	

Table 3.10 Antimicrobial shelf-life of Aseptrol® and Presept®

Organisms	**Disinfectant**	**Effectiveness (days)**	
		Time taken to kill	
		30 seconds	**60 seconds**
S. aureus	Aseptrol®	27	27
	Presept®	>37	>37
P. aeruginosa	Aseptrol®	35	>37
	Presept®	>37	>37
S. mutans	Aseptrol®	35	>37
	Presept®	>37	>37
C. albicans	Aseptrol®	30	>37
	Presept®	>37	>37
M. tuberculosis	Aseptrol®	>28	>28
	Presept®	>28	>28
M. avium subsp. *avium*	Aseptrol®	>28	>28
	Presept®	>28	>28

CHAPTER 4: DISCUSSION

Chlorinated disinfectants have been used for a long time in healthcare settings and the antimicrobial activity of chlorine is attributed to undissolved hypochlorous acid (HOCl). However, these compounds are relatively unstable and the activity is dependent on pH (Dychdala GR, 2001). Demand-release chlorine dioxide compounds were introduced because they retain chorine longer and so exert a more prolonged bactericidal effect and they are stable. One such formula, Presept®, containing sodium dichloroisocyanurate, was studied here. Some formulas are prepared with similar compound but corrosion inhibitors are added, which become activated at the time of solution preparation. In this study, such formula was Aseptrol® which releases chlorine dioxide. Low- level disinfectants are expected to be effective against most vegetative bacteria, some viruses and fungi but cannot be relied on to kill resistant micro-organisms e.g. M. *tuberculosis* or bacterial spores. Intermediate-level disinfectants kill M. *tuberculosis,* vegetative bacteria, most viruses and fungi but not necessarily bacterial spores. High-level disinfectants destroy all micro-organisms with the exception of high numbers of bacterial spores. Although many studies have shown chlorinated compounds to have good microbicidal activity, including activity against HBV and mycobacteria (Bond et al., 1983; Griffiths et al., 1999; Silverman et al., 1999; Weber et al., 1999), it has been considered intermediate level disinfectant purely because of the low sporicidal activity. In this study, the results showed that both disinfectants had bactericidal activity at 30 seconds contact time period.

S. aureus, P. aeruginosa, S. mutans and *C. albicans* are easily eliminated by chlorinated compounds and it was shown in this study but challenge is the mycobacteria, Hepatitis B and bacterial spores. This study showed that Aseptrol® and Presept® had not only

bactericidal but mycobactericidal, viricidal and sporicidal activity at half to 2 minutes contact time period. This suggests that it can be used as an intermediate level disinfectant, as well as a high-level disinfectant. Our results are comparable to the results obtained by Isomoto et al., (2006), where they found 30ppm chlorine dioxide to be mycobactericidal in 60 seconds and sporicidal in 1 to 5 minutes.

4.1 USE OF DISINFECTANTS AND STERILANTS IN SEMICRITICAL AREAS

The United States Environmental Protection Agency (EPA) regulates surface disinfectants and the tuberculocidal claim is an EPA benchmark for measuring germicidal potency. Among microorganisms, such as the vegetative bacteria, viruses and fungi, mycobacteria (including *M. tuberculosis*), have the highest intrinsic level of resistance to disinfectants. Therefore, any germicide with a tuberculocidal claim on the label must be capable of inactivating a broad spectrum of pathogens, including Hepatitis B virus, Hepatitis C virus and Human immune-deficiency virus (Cleveland et al., 2009). Results in this study have also shown that test disinfectants are mycobactericidal and proved to have broad spectrum activity.

Objects that enter sterile tissue or the vascular system such as surgical instruments, cardiac, urinary catheters etc must be sterile because microbial contamination could transmit diseases. Usually most items are purchased sterile or they are sterilized with steam or heat-sensitive ones are sterilised with ethylene oxide or hydrogen hydroxide gas plasma. However, if these methods are unsuitable, then chemical sterilants are required. These sterilants are high level disinfectants. Some chemicals such as glutaraldehyde, hydrogen peroxide and peracetic acid based formulas are used as sterilants. Generally

chlorinated compounds are not considered as sterilants due to their ineffectiveness and corrosive property. Bronchoscopes can transmit *M. tuberculosis* between patients (Larson et al., 2003). Inadequate cleaning and disinfection of the instruments and accessories are likely contributing factors (Ayliffe, 2000; Lisgaris, 2003; Nelson, 2003). The only practical means of disinfecting heat labile, flexible bronchoscopes between patients is immersion in a suitably efficacious and non-damaging disinfectant (Babb, 1993; Ayliffe, 2000). In this study, we studied two chlorine based disinfectants and the results showed that both disinfectants can be used as sterilants, regarded that the contact time period is more than two minutes. Aseptrol® is non-corrosive, therefore can be used for metal instruments as well. After disinfection of endoscopes using chlorine dioxide, Isomoto et al., (2006) observed no functional or cosmetic damage in the instruments or accessories. They suggested that the use of chlorine dioxide, together with thorough pre-cleaning, can offer effective, faster and less problematic endoscope disinfection.

Disinfection is an important measure to prevent transmission of many infections by instruments. The CDC recommendation for high level disinfection of HBV-, HCV- , HIV- or mycobacteria-contaminated devices is appropriate and many studies have demonstrated the effectiveness of high-level disinfectants to inactive these and other pathogens that might contaminate semicritical devices (Bond et al., 1983; Hanson et al., 1990; Rutala et al., 1991; Sattar and springthorpe, 1991; Chanzy et al, 1999; Payan et al., 2004).

Haemodialysis systems include haemodialysis machines, water supply, water-treatment systems, and distribution systems. During haemodialysis, patients have acquired blood borne viruses and pathogenic bacteria (Alter et al., 2004) and therefore, cleaning and disinfection of this unit is important. EPA and Food and Drug Administration (FDA)

regulate disinfectants used to reprocess hemodialyzer, haemodialysis machine and water treatment systems. Hemodialyzer, haemodialysis systems are usually disinfected by chlorine-based disinfectants (500-600 ppm free chlorine). Aseptrol® and Presept® which were tested here can easily be used in this situation, particularly Aseptrol®, at much lower concentrations. Aseptrol® can be used for noncritical surfaces such as dialysis bed or chair, countertops and external surfaces of dialysis machines because it is noncorrosive to metals. In this study, surface/carrier disinfection testing was not done because it had been established by Rweyendela et al., (2009). In their study, impression materials were used instead of blocks and both disinfectants had good antimicrobial activity.

4.2 ROLE OF ORGANIC MATERIAL

All disinfectants are less effective in the presence of organic material (blood, saliva, body fluids), meaning, “you can't disinfect dirt”. Organic matter interferes with the action of disinfectants by: coating the pathogen and preventing contact with the disinfectant; forming chemical bonds with the disinfectants, thereby making it inactive against organisms; or reacting chemically with and neutralizing the disinfectant. Cleaning before the application of the disinfectant is essential. Here, tests were performed in the presence of organic material (skim milk and plasma), which suggests organic material did not compromise the efficacy of the disinfectants. However, some studies have shown that organic material can compromise the efficacy. Therefore Isomoto et al., (2006) has suggested that the use of chlorine dioxide together with precleaning in endoscope disinfection is still advantageous over glutaraldehyde due to its fast, effective and less problematic disinfection.

4.3 VIRICIDAL ACTIVITY

In this study, both disinfectants showed viricidal activity, therefore they can be used on surfaces that are contaminated with blood and blood spillages. For testing of HBV, HBV-negative human EDTA-plasma was used to make up the required volume and this organic material did not affect the microbicidal properties of chlorine and chlorine dioxide. This is contrary to the finding of Isomoto et al., (2006), who showed that organic material can compromise the efficacy of chlorinated compounds.

4.4 MYCOBACTERICIDAL ACTIVITY

Tuberculosis (TB) generally spreads through air but improperly decontaminated medical devices have also been implicated in its transmission. Environmental surfaces act as vehicles for mycobacteria. *M. tuberculosis* can survive for several days on inanimate surfaces (cited by Sattar et al., 1995). Non-tuberculous mycobacteria (NTM) are usually saprophytes but can be opportunistic and at times deadly pathogens. In the immunocompromised individuals, infections due to NTM have been observed to be an important cause of morbidity and mortality in the western countries (Wallace et al., 1990). Appropriate use of cleaners and disinfectants can minimize the incidence of health-care-associated infections and pseudo-outbreaks (Sehulster et al., 2003). Nosocomial infections and outbreaks caused by inadequate disinfection/sterilization of medical devices are well described (Larson et al., 2003). Pulmonary disease, lymphadenitis, skin, soft tissue, skeletal infections, catheter-related blood-stream infections in immunocompromised hosts and disseminated disease in persons with acquired immune-deficiency syndrome (AIDS) are clinical syndromes attributed to mycobacteria. *M. avium complex* infection results

from diverse and likely undetectable environmental and nosocomial exposures (Von Reyn et al., 2002). A study conducted by Griffiths et al., (1999) showed that chlorine releasing agents are effective against mycobacteria and blood borne viruses. In suspension tests, chlorine at 1100ppm was as effective as 10 000ppm sodium dichloroisocyanurate in achieving a Log 10 reduction >5 in *M. tuberculosis* and *M. avium-intracellulare*, tested under 1 minute (Griffiths et al., 1999). They also found that clinical isolates of *M. avium-intracellulare* was more resistant than *Mycobacterium tuberculosis* to Chlorine dioxide and sodium dichloroisocyanurate. However this study demonstrated 99.99% kill of M. *tuberculosis* and *M. avium subp. avium* by both disinfectants within 30 seconds. These results can categorise Aseptrol® and Presept® to be intermediate to high level disinfectants. Mycobactericidal disinfectants are also required for laboratory and respiratory equipment, the fixation of tissue and for removing culture and body fluid spills. These disinfectants could easily be used in those situations.

4.5 USE OF DISINFECTANTS IN DENTAL SETTINGS

Potential for transmission of infectious agents in dentistry have been stressed (Lewis et al., 1992, Lewis and Boe, 1992). In dental settings surfaces frequently touched by contaminated gloves, instruments, light handles become contaminated by saliva and blood and related organisms. Dental caries is one of the most prevalent diseases in humans; it causes irreversible damage to the grinding machinery involved in the intake of food and causes great distress. The change in the homeostasis of the oral cavity, with an overgrowth of *S. mutans* is recognized as the primary cause of the disease. In surgeries, due to the dental procedures, tremendous amount of blood contaminated salivary aerosols are created. These aerosols can be contaminated with caries causing *S. mutans*, oral

candidiasis causing *C. albicans*, *M. tuberculosis*, Hepatitis B and C viruses, HIV, prions, Influenza, Cytomegalovirus (CMV), Epstein Barr virus (EBV), Herpes simplex virus (HSV). These aerosols are inhaled and they contaminate surfaces. Some studies have shown that HIV positive patients' saliva contain HIV (Liuzzi et al., 1997)). The quantity of HIV in saliva depends on the blood viral load and the therapeutic status of patient (Liuzzi et al., 2000; Shepard et al., 2000). Therefore, sterilization and disinfection of any item that comes in contact with saliva is also important. Although the metal instruments are heat sterilized, some devices such as dental X-ray films, impression materials and prosthesis cannot be heat sterilized. An effective sterilant and surface disinfectant is a must in this situation. In this study, effect of two chlorinated disinfectants against three major dental setting contaminants such as *S. mutans, M. tuberculosis* and Hepatitis B were studied. The results showed that chlorine dioxide and NaDCC both killed all three organisms within 30 seconds. These results suggest that both these disinfectants can be used as sterilants and surface disinfectants in dental settings. Chlorine dioxide containing Aseptrol® particularly can be used on metal because it is noncorrosive. Use of Presept® in decontamination of dental X-ray films and use of Aseptrol® in decontamination of impression material have been studied (Coogan et al., 2004; Rweyndela et al., 2008), showing similar results to the ones shown in this study. Studies have shown the use of chlorine dioxide in plaque control (Nishikiori et al., 2008; Paraskevas et al., 2008) with no adverse effect on oral tissues and biofilm control in waterlines of dental units (Wirthlin and Marshall, 2001). Chlorine dioxide is a safe and clinically effective option in the management of chronic atrophic candidiasis. It can be used both as a topical antiseptic and for soaking dentures overnight after they have been removed from the mouth (Mohammad et al., 2004).

4.6 IMPLICATION OF SHELF-LIFE STUDY RESULTS

Although manufacturers of chlorinated compounds recommend daily preparation of solutions, our results showed that Presept®, as well as Aseptrol® solutions can be effective for greater than 37 and 27 days respectively. This result has financial implication because leftover disinfectant can be utilized. It can also save time in daily preparation of disinfectant solution. Similar results were found by Rutala et al., (1998). They also showed that brown screw cap bottles were ideal for storage of chlorinated disinfectants.

4.7 SAFETY OF CHLORINATED COMPOUNDS

The efficacy, 'user-friendliness and surface compatibility of a disinfectant should be established before use. Some disinfectants are irritant to the skin, eyes and respiratory tract and suitable personal protective equipments have to be worn. Due to concerns about the occupational safety of cleaning employees using sodium dichloroisocyanurate, Van Laer et al., (2008) conducted a small test to evaluate the concentration of chlorine in the air while cleaning employees disinfected a patient's room and they concluded that there is no occupational hazard for cleaning employees while performing decontamination procedure with a solution containing 4,500 ppm free chlorine made from sodium dichloroisocyanurate dehydrate tablets. Above study showed that it is safe to use chlorine-releasing disinfectants. A study has shown the safety of chlorine dioxide on gingival fibroblasts (Nishikiori et al., 2008). However, the safety of chlorine dioxide was not part of the scope of this study.

4.8 TECHNICAL DIFFICULTIES AND POSSIBLE SHORTCOMINGS

A

Shelf-life study for Hepatitis B Virus was not performed because the COBAS® Ampliprep/ COBAS® Taqman® HBV Test, which quantitates HBV viral DNA is very costly and the study funds were exhausted.

B

The growth incubation time for Mycobacteria ideally is 4-6 weeks. In shelf-life study some of the results were read after 4-5 weeks due to time constraints and due dates for completion of the study.

CHAPTER 5: CONCLUSION

In conclusion, this study has shown that Aseptrol®, a slow release chlorine dioxide formula at 48ppm and 24ppm and chlorine releasing sodium dichloroisocyanurate containing disinfectant, Presept® at 10 000ppm are bactericidal, viricidal, mycobactericidal at 30 seconds contact time period and at 2 to 3 minutes, sporicidal in the presence of organic material. Prepared disinfectants solutions of Aseptrol® and Presept®, if stored in screw cap bottles, can be effective for 27 and greater than 37 days respectively. Aseptrol® can be used on metal instruments because of its noncorrosive property.

Our preliminary results showed that both disinfectants have potentials to be used in medical and dental settings for intermediate to high-level disinfection and as sterilants. However, further relevant testing would be required.

CHAPTER 6: APPENDICES

APPENDIX A

Composition and preparation of media

1. **Blood Agar**

Oxoid Columbia agar base	100g
Demineralised water	2.5 L

These are well mixed, autoclaved at 121^{o}C for 30 minutes and then cooled at 50^{o}C. With aseptic precautions the following is added.

Sterile citrated horse blood (at room temperature) 100ml
pH is adjusted to 7.5 and aseptically distributed in sterile petri dishes.

2. **Tryptone Soy Agar**

Tryptone soy agar	40 g
Distilled water	1 L

The ingredients should be dissolved and autoclaved at 121^{o}C for 15 minutes.
pH 7.0
Aseptically distributed in sterile petri dishes

3. **Sabouraud Dextrose Agar**

Sabouraud agar	60 g
Distilled water	1 L

The ingredients should be dissolved and autoclaved at 121^{o}C for 15 minutes.
pH 7.0
Aseptically distributed in sterile petri dishes

4. **Middlebrook 7H10 Agar (BD 262710) with Middlebrook OADC Enrichment (BD 212240)**

Middlebrook 7H10	19g
Glycerol	5.0ml
OADC Enrichment	100ml
deionised water	900ml

5. **Neutralizing fluid**

Sodium chloride	2.25 g
Potassium chloride	0.105 g
Calcium chloride	0.12 g
Sodium bicarbonate	0.05 g
Distilled water	1.0 L
Sodium thiosulphate	5.0 g
Tween 80	5.0 ml

The ingredients should be dissolved and autoclaved at 121^{o}C for 15 minutes.
pH 7.0

6. **BACTEC Mycobacteria Growth Indicator Tube (MGIT)**
   MGIT contains 110µl of fluorescent indicator and 7 ml of broth. The indicator contains Tris 4,7-diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate in a silicone rubber base. The tubes are flushed with 10% CO_2 and capped with polypropylene caps.

   Approximate formula per litre of purified water:

Modified Middlebrook 7H9 Broth base	5.9g
Casein peptone	1.25g

   MGIT Growth supplement contains 15 ml Middlebrook OADC enrichment

   Approximate formula per litre of purified water:

Bovine albumin	50g
Catalase	0.03g
Dextrose	20g
Oleic acid	0.1g
Polyoxyethylene stearate (POES)	1.1g

APPENDIX B

Preparation of solutions for Hepatitis B Virus study
(COBAS® Ampliprep/COBAS® TaqMan® HBV Test)

1. **HBV CS1** (HBV magnetic glass particle reagent cassette)
   Magnetic glass particles
   Isopropanol 93%

2. **HBV CS2** (HBV lysis reagent cassette)
   Sodium citrate dehydrate
   Guanidine thiocyanate 42.5%
   Polydocanol < 14%
   Dithiothreitol 0.9%

3. **HBV CS3** HBV multi-reagent cassette containing:
   Pase (Proteinase solution)
   Tris buffer
   EDTA < 0.05%
   Calcium chloride
   Calcium acetate
   proteinase ≤ 7.8%
   Glycerol
   EB (Elution buffer)
   Tris-base buffer
   Methylparaben 0.2%

4. **HBV CS4** HBV test-specific reagent cassette containing:
   HBV QS (HBV quantitation standard)
   Tris-HCL buffer
   EDTA
   Poly Ra RNA (synthetic) <0.005%
   non-infectious, linearized, double-stranded plasmid DNA containing an insert with HBV primer binding sequence and a unique probe binding region < 0.001%
   sodium azide 0.05%

5. **HBV MIX** (HBV master mix)
   Tricine buffer
   Potassium acetate
   Potassium hydroxide
   sodium azide 0.09%
   Glycerol
   ATP, CTP, GTP, UTP <0.04%
   upstream and downstream HBV primers <0.003%
   Oligonucleotide aptamer <0.003%
   fluorescent-labelled oligonocleotide probes specific for HBV and the HBV QS standard <0.003%
   Z05 DNA polymerase (microbial) < 0.05%
   AmpErase (uracil-N-glycosylase) enzyme(microbial) < 0.1%

6. **HBV Mn^{2+}** (HBV Manganese solution)
   manganese acetate <0.5%
   Glacial acetic acid
   sodium azide 0.09%

7. **HBV H (+) C** (HBV high positive control)
   linearized, double stranded plasmid DNA containing HBVsequences
   Negative human plasma- non-reactive by tests to antibody to HBC, HIV- 1/2, HIV p24 <0.001%
   Antigen and HBsAG; HIV-1 RNA and HBV DNA not detectable by PCR methods.
   proclin® 300 preservative 0.1%

8. **HBV L (+) C** (HBV low positive control)
   linearized, double stranded plasmid DNA containing HBV sequences
   Negative human plasma- non-reactive by tests to antibody to HBC, HIV- 1/2, HIV p24 <0.001%
   Antigen and HBsAG; HIV-1 RNA and HBV DNA not detectable by PCR methods.
   proclin® 300 preservative 0.1%

9. **CTM (-) C (COBAS® TaqMan® negative control human plasma)**
   Negative human plasma- non-reactive by tests to antibody to HBC, HIV- 1/2, HIV p24
   Antigen and HBsAG; HIV-1 RNA and HBV DNA not detectable by PCR methods.
   proclin® 300 preservative 0.1%

10. **PG WR** (COBAS® Ampliprep/COBAS® TaqMan® wash reagent)
    Sodium citrate dehydrate
    N-Methylisothiazolone-HCL <0.1%

APPENDIX C

Result printout from AMPLILINK® for HBV

Figure C1 Negative control result printout from AMPLILINK® for HBV

NICD **Specialized Molecular Diagnostics**

AMPLILINK® 3.2.2 Report: Control Result Detail - NC

Order

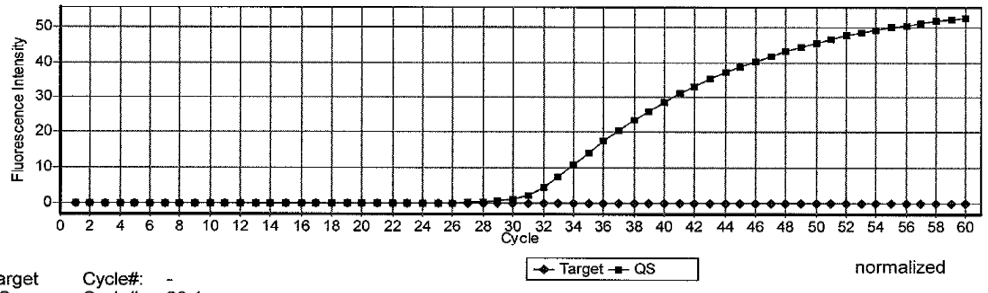
Lot Number: M0512300000
Order Date/Time 10/28/2009 2:17:02 PM
Sample ID

Test	Result	Flags	Date/Time
HBMCAP96	Target Not Detected		10/28/2009 5:26:09 PM

Workflow

Process Steps	Name	System ID	Position	Date/Time
AmpliPrep Preparation	HBMCAP96	392232	004 - 01	10/28/2009 3:01:20 PM
TaqMan Amplification	HBMCAP96	391415	TCB 004 - 12	10/28/2009 5:26:02 PM
TaqMan Detection	HBMCAP96	391415	004 - 01	10/28/2009 5:26:09 PM

Measurement Details



Target Cycle#: -
QS Cycle#: 29.1

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	M0512300000	234	5/31/2010	A: -0.0053, B: 0.3350, C: 3.7500

Detail Flags

TM71: _S_RFITOOLOW

Acceptance Information

Accepted by MARK Accepted Date/Time: 10/29/2009 11:01:33 AM

1 / 1 **Mark Goosen** **10/29/2009**
AMPLILINK® 3.2.2 (Build 0801)

Figure C2 Low positive control result printout from AMPLILINK® for HBV

NICD **Specialized Molecular Diagnostics**

AMPLILINK® 3.2.2 Report: Control Result Detail - LPC

Order

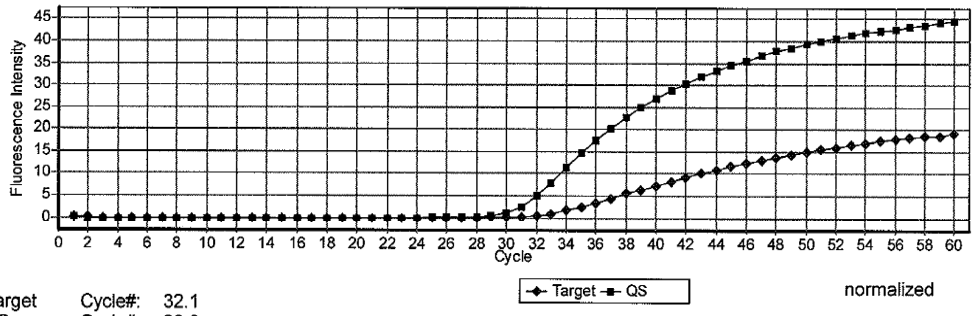
Lot Number: M0512300000
Order Date/Time 10/28/2009 2:17:02 PM
Sample ID
LPC Low: 1.60E+2 IU/mL
LPC High: 1.60E+3 IU/mL

Test	Result	Flags	Date/Time
HBMCAP96	4.62E+2 IU/mL		10/28/2009 5:26:09 PM

Workflow

Process Steps	Name	System ID	Position	Date/Time
AmpliPrep Preparation	HBMCAP96	392232	004 - 02	10/28/2009 3:04:56 PM
TaqMan Amplification	HBMCAP96	391415	TCB 004 - 13	10/28/2009 5:26:02 PM
TaqMan Detection	HBMCAP96	391415	004 - 02	10/28/2009 5:26:09 PM

Measurement Details



normalized

Target Cycle#: 32.1
QS Cycle#: 29.0

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	M0512300000	234	5/31/2010	A: -0.0053, B: 0.3350, C: 3.7500

Acceptance Information

Accepted by MARK

Accepted Date/Time: 10/29/2009 11:01:33 AM

1 / 1 **Mark Goosen** **10/29/2009**
AMPLILINK® 3.2.2 (Build 0801)

Figure C3 High positive control result printout from AMPLILINK® for HBV

NICD

Specialized Molecular Diagnostics

AMPLILINK® 3.2.2 Report: Control Result Detail - HPC

Order

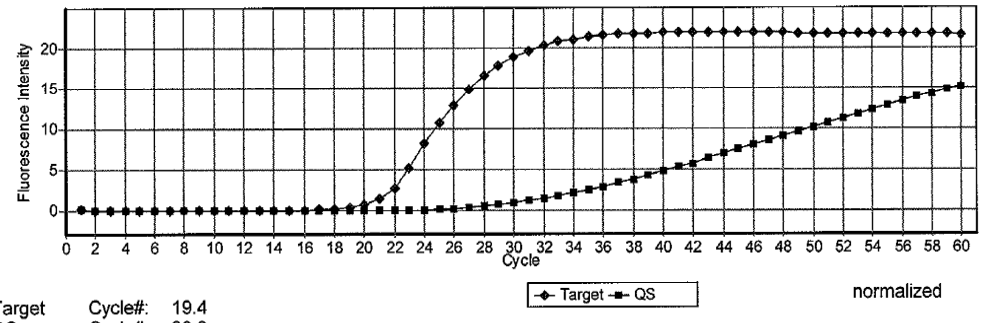
Lot Number: M0512300000
Order Date/Time 10/28/2009 2:17:02 PM
Sample ID
HPC Low: 4.80E+5 IU/mL
HPC High: 4.80E+6 IU/mL

Test	Result	Flags	Date/Time
HBMCAP96	2.05E+6 IU/mL		10/28/2009 5:26:09 PM

Workflow

Process Steps	Name	System ID	Position	Date/Time
AmpliPrep Preparation	HBMCAP96	392232	004 - 03	10/28/2009 3:08:32 PM
TaqMan Amplification	HBMCAP96	391415	TCB 004 - 08	10/28/2009 5:26:02 PM
TaqMan Detection	HBMCAP96	391415	004 - 03	10/28/2009 5:26:09 PM

Measurement Details



Target Cycle#: 19.4
QS Cycle#: 28.3

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	M0512300000	234	5/31/2010	A: -0.0053, B: 0.3350, C: 3.7500

Acceptance Information

Accepted by MARK

Accepted Date/Time: 10/29/2009 11:01:33 AM

1 / 1

Mark Goosen

10/29/2009

AMPLILINK® 3.2.2 (Build 0801)

Figure C4 Sample of negative result printout from AMPLILINK® for HBV

NICD — **Specialized Molecular Diagnostics**

AMPLILINK® 3.2.2 Report: Sample Result Detail

Order

Order Number: PC5
Order Date/Time: 10/28/2009 10:20:42 AM
Sample ID: PC5
Doctor:
Hospital:
OrderCom2:

Patient

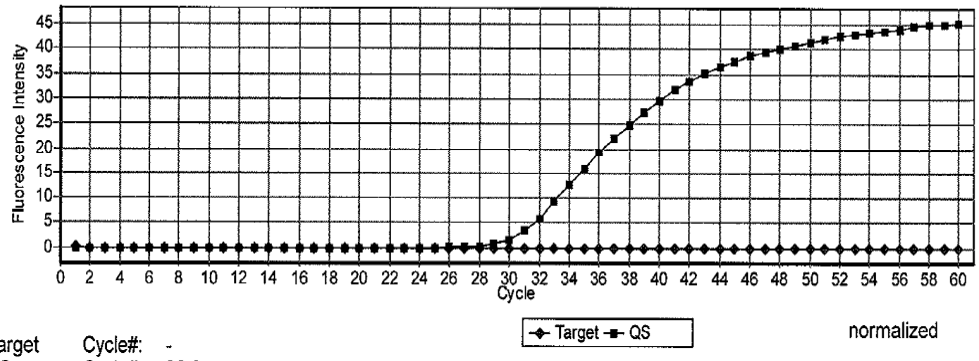
Patient ID:
Patient Name:
Date of Birth:
Sex:
PatientCom1:
PatientCom2:

Test	Result	Flags	Date/Time
HBMCAP96	Target Not Detected		10/28/2009 4:26:03 PM

Workflow

Process Steps	Name	System ID	Position	Date/Time
AmpliPrep Preparation	HBMCAP96	392232	006 - 18	10/28/2009 2:12:54 PM
TaqMan Amplification	HBMCAP96	391415	TCA 006 - 10	10/28/2009 4:25:53 PM
TaqMan Detection	HBMCAP96	391415	006 - 18	10/28/2009 4:26:03 PM

Measurement Details



normalized

Target Cycle#: -
QS Cycle#: 28.3

Reagent Kits

Reagent Kit	Lot Number	Cassette Serial Nr.	Expiration Date	Calibration
PCR Kit	M0512300000	234	5/31/2010	A: -0.0053, B: 0.3350, C: 3.7500

Detail Flags

TM71: _S_RFITOOLOW

Acceptance Information

Accepted by MONALISA — Accepted Date/Time: 10/28/2009 4:40:46 PM

1 / 1 — **Mark Goosen** — **10/29/2009**

AMPLILINK® 3.2.2 (Build 0801)

Figure C5 Ethical clearance 1

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical))
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-080226-1

26/02/2008

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigators: **Dr M Patel, Ms J Ebonwu Student No 341929**

Project title: **Antimicrobial effect of slow release chlorine dioxide disinfectant.**

Reason: The research for this project is fully laboratory using stock bacterial cultures and commercial Duck hepatitis B virus. There is no research on human participants.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2008 -02- 26
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Research Office, Senate House, Wits

Figure C6 Ethical clearance 2

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

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14 July 2010

Dr M Patel
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Dear Dr Patel

RE: Amendments on Ref W-CJ-080226-1

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved the use of "Human Hepatitis B Virus" on the abovementioned waiver number as detailed in your letter dated 09 July 2010.

Thank you for keeping us informed and updated.

Yours sincerely,

Anisa Keshav
Secretary
Human Research Ethics Committee (Medical)

CHAPTER 7: REFERENCES

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