# Chemical Disinfectant Resistance in

# Multiple Antibiotic Resistant and Susceptible Bacteria

By

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# Chemical Disinfectant Resistance in Multiple Antibiotic Resistant and Susceptible Bacteria

**BY**

**Rachel Suarez**

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University**

**of Manitoba in partial fulfillment of the requirements of the degree**

**of**

**Master of Science**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ATS | Artificial Test Soil |
| BA | Blood Agar |
| CFU | Colony forming units |
| CNA | Colistin/Naladixic Acid Agar |
| FBS | Fetal bovine serum |
| GISA | Glycopeptide intermediate resistant *Staphylococcus aureus* |
| MBC | Minimum bactericidal concentration |
| MIC | Minimum inhibitory concentration |
| MRSA | Methicillin resistant *Staphylococcus aureus* |
| MSSA | Methicillin sensitive *Staphylococcus aureus* |
| PBS | Phosphate-buffered saline |
| QAC | Quaternary ammonium compound |
| TSA | Trypticase soy agar |
| TSB | Trypticase soy broth |

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## ABSTRACT

Chemical disinfectants are used extensively in hospitals and other health care settings for a variety of hard-surface applications. The widespread use of chemical disinfectants has prompted some speculation that there may be a link between disinfectant resistance and antibiotic resistance. This type of link could have significant implications regarding nosocomial infections. The aim of the study was to evaluate whether such a link exists in bacterial strains that have been responsible for hospital outbreaks. We acquired several different bacterial species that were resistant to multiple antibiotics and had caused severe nosocomial outbreaks. These were compared to counterparts of the same species that were antibiotic susceptible strains. The comparison was performed using surface carrier as well as suspension testing. Because patient secretions may protect survival of organisms in the hospital setting, we incorporated an organic challenge in our test model.

The Surface Carrier Test allowed us to determine the efficacy of the disinfectant and the difference in survival rates of bacteria on a polyvinyl chloride lumen carrier. The carrier introduced a physical challenge because the organism was dried onto the surface in the presence of an organic challenge (ATS). This affects how readily the disinfectant reaches the bacteria. Quantitative measurements of the residual bacteria were then taken after disinfectant exposure. The Suspension Test allowed us to determine the efficacy of the disinfectant with no physical barriers (ie. surface vs. suspension). The suspension test was essentially a growth curve of the bacteria in the presence of varied disinfectant concentrations over a 24-hour time frame. Bacterial replication was detected using absorbance measurements. To correlate our

experimental data to actual in use issues, in-use data measurements were taken to establish realistic contact times and these contact times (1 min. and 10 min.) Were then used in the surface carrier test to re-evaluated disinfectant efficacy.

Results of the Surface Carrier Test showed that 0.5% sodium hypochlorite and 0.04% hydrogen peroxide were the most effective and least effective disinfectants, respectively. The Modified Surface Carrier Test revealed a close link between the reduced disinfectant contact time and an increased ability of the test bacteria to survive. The Suspension Test demonstrated that 3% hydrogen peroxide was the most effective for killing a wide range of bacterial strains, even at a 1:128 dilution.

Our data for surface carrier and suspension tests indicated that even in the presence of an organic challenge, the multiple antibiotic resistant pathogens were not any more resistant to disinfectant killing compared to susceptible strains of the same species.

## LITERATURE REVIEW

### 1. Introduction

### i) Historical Perspective

Although the scientific application of disinfectants and sterilants is limited to the past 150 years, empiric practices date back to ancient times of the Romans, Greeks and Jews (14). The first disinfectant, circa 800 B.C., to be reported was sulfur dioxide. It was described in Homer's *The Odyssey XXII* when Odysseus returned home after a long absence, killed his wife's suitors and disposed of the bodies. He burned the sulphur to produce sulphur dioxide to disinfect the area where the killings took place. Sulphur dioxide was used in this way during the Great Plagues of the middle ages and it is still used as a disinfectant to this day.

The eighteenth century ushered in the age of chemistry and a whole new set of chemical disinfectants emerged. In 1774, Swedish chemist, Scheele, discovered the disinfectant properties of chlorine, followed by the discovery of hypochlorites in 1789. Phenols were discovered in 1834 and used as a disinfectant a few years later. In 1858, English physician, Richardson discovered that hydrogen peroxide could eliminate foul odors and proposed its use as a disinfectant while the disinfectant use of quaternary ammonium compounds did not take place until 1935.

The 20th century has brought us many great advances in chemistry, particularly, organic chemistry and with that has brought us many new disinfectants that have become invaluable to us in healthcare settings, industry and right in our own homes.

**ii) Infection Control and Disease Transmission**

Approximately, 5-10% of the 35 million patients annually admitted to hospitals, acquire an infection while hospitalized (57, 135, 51). Infections in hospitalized patients can arise from an endogenous source meaning that the infection was caused by microorganisms already present in the patient before admission. Nosocomial infections can also be transmitted to and from patients and hospital staff. Hospital infections can also result from improperly processed medical devices. Due to the increasing numbers of nosocomial infections, concern over prevention has also increased. Appropriate measures for sterilization and disinfection are key factors in reducing the spread and incidences of hospital-acquired infections.

A biocide is a general term indicating a chemical agent that is capable of inactivating microorganisms. Within the range of biocides, there are three distinct groupings. Antibiotics refer to naturally occurring or synthetic organic substances which inhibit or destroy selective microorganisms. Antibiotics are usually active in low concentrations and administered to patients either intravenously or orally. Antiseptics are biocides that inhibit or destroy microorganisms in or on living tissue. Antiseptics are used in hand washing agents and in preoperative skin preparations. Disinfectants are quite similar to antiseptics but refer to their use on inanimate objects or surfaces (78). Sterilants are very similar to disinfectants as they are utilized on inanimate objects and surfaces but are different in that they are able to destroy all living organisms present. Disinfectants may also be sterilants when the disinfectant is used at higher concentrations and/or prolonged exposure times. Antiseptics, chemical disinfectants and sterilants are used at much higher concentrations than antibiotics.

A great number of disinfectants and sterilants are used extensively in hospitals and

other healthcare settings. They are an essential part of infection control practices and aid in the prevention of nosocomial infections (69*). The necessity for appropriate disinfection and sterilization procedures has been emphasized by numerous articles documenting infection after improper decontamination of medical devices.

A hospital outbreak of *Acinetobacter baumannii* was reported and associated with contaminated respiratory equipment and intravascular access devices (76). Between July 1987 and January 1998, five patients receiving long term hemodialysis developed systemic *Mycobacterium chelonae abscessuss* infections (63). Transmission was linked to contaminated water used in the dialyzers. Proper disinfection of the dialyzers could have prevented this outcome. In 1993, 12 out of 23 patients in a dialysis centre in Columbia, South America, tested positive for the human immunodeficiency virus (HIV) antibody (63). Dialyzers were reprocessed using 5% formaldehyde and were reused only on the same patient. Access needles on the other hand, were flushed with saline, soapy water and saline again and reprocessed by soaking the needles in a low-level disinfectant, 0.15% benzalkonium chloride. These needles were then used between different patients.

**iii) Medical Device Reprocessing**

Failures in disinfection and sterilization of medical devices may entail significant institutional costs, patient morbidity and even mortality. Proper cleaning, disinfection and/or sterilization of medical devices are important steps in reducing these.

Sterilization is referred to the complete elimination or destruction of all forms of microbial life, including bacterial endospores and is accomplished by either physical or chemical processes. Steam under pressure is the most common method of sterilization

utilized in the hospital setting. Many medical devices can not be reprocessed in this manner because of they are heat-labile and the high temperatures of these processes would compromise their integrity. It is therefore necessary to use methods that do not involve such high temperatures. One way to achieve this is to use low-temperature sterilization methods. The currently approved low-temperature sterilization methods include ethylene oxide, hydrogen peroxide gas plasma, chlorine dioxide, paracetic acid and ionizing radiation.

Disinfection describes the process whereby all microorganisms are eliminated with the exception of bacterial endospores. A few disinfectants which kill spores with prolonged exposure times of six to ten hours are chemical sterilants. At similar concentrations and shorter exposure times ($\leq$ 45 minutes) these same disinfectants may kill all microorganisms except fungal and bacterial spores. These are called high-level disinfectants. Intermediate-level disinfectants may be cidal for tubercle bacilli, vegetative bacteria, most fungi and most viruses but do not eliminate bacterial spores while low-level disinfectants may kill most vegetative bacteria, some fungi and some viruses (15).

Since all patient-care items are not equal in regards to their degree of risk of infection with use, Earle H. Spaulding devised an approach to disinfection and sterilization of these objects (122). He believed that the degree of disinfection or in other cases, sterilization, could be understood and applied if patient-care items were divided into three categories depending on their intended use. The three categories he described were critical, semi-critical and non-critical (Table 1).

Critical items are objects that pose a high risk of infection if it is contaminated with any microorganism including bacterial spores. Critical items that enter sterile body sites like

| | Exposure | Examples | Minimum Level of Disinfection |
|---|---|---|---|
| **Non-critical** | contact with intact skin but not mucous membranes | bedpans, blood pressure cuffs, crutches, bedrails etc. | low level |
| **Semi-critical skin** | contact with unintact and/or mucous membranes | respiratory and anesthesia equipment and endoscopes etc. | high level |
| **Critical** | contact with sterile tissue or vascular system | surgical instruments, cardiac and urinary catheters implants etc. | sterilization |

**Table 1: Spaulding's Classification**

The table represents the 3 categories; non-critical; semi-critical and critical, devised by Spaulding (122) to differentiate patient care items and the level of disinfection/sterilization that must be performed on them. Non-critical items require only low level disinfection while semi-critical items minimally require high level disinfection. Critical items require sterilization.

tissue or the vascular system such as surgical instruments, cardiac and urinary catheters, implants and needles must be purchased sterile or be sterilized by autoclaving. If the object is heat-labile, low-temperature sterilization methods are suitable.

Semi-critical items come into contact with mucous membranes or skin that is not intact. Items that are considered semi-critical have to be free of all microorganisms except bacterial spores. Respiratory therapy and anesthesia equipment, endoscopes and thermometers are included within this category. These items do not necessitate sterilization but must be high-level disinfected. Glutaraldehyde, hydrogen peroxide and chlorine-releasing agents are dependable high-level disinfectants if pre-cleaning of the object is performed (107).

Non-critical items are objects that come into contact with intact skin but not mucous membranes. The inherent property of intact skin of providing a barrier to foreign objects and substances means that non-critical items require only low-level disinfection. Items such as bedpans, blood pressure cuffs and crutches pose little risk of transmitting infectious agents to patients but secondary transmission could occur by contaminating hands of healthcare workers or medical equipment that comes into contact with the patients (105).

Although Spaulding's classifications seem clear cut, problems arise when dealing with the reprocessing of complicated, heat-labile medical equipment, and therefore, it is still difficult to determine which level of disinfection must be used. This is the case for flexible endoscopes. (104).

Endoscopes are widely used for the diagnosis and therapy of medical disorders. According to Spaulding's classifications, flexible endoscopes are semi-critical items because they only come into contact with mucous membranes of the patient and do not penetrate into

sterile body sites. As such, high level disinfection is the minimum disinfection needed. To prevent nosocomial infection, all flexible endoscopes must undergo thorough cleaning and high-level disinfection following each use but these narrow-lumened flexible devices present the most significant challenge to adequate cleaning and reprocessing (3). Reported cases of disease transmission from the use of contaminated flexible endoscopes have stemmed from inadequate cleaning before high-level disinfection/sterilization, contamination of endoscope reprocessors or overgrowth of microorganisms as a result of improper drying of the endoscope after reprocessing (22, 121).

The problem arising with a semi-critical classification is that even though the flexible endoscope itself does not come into contact with sterile body sites, the critical, sterile medical devices that are used for endoscopic procedures are passed through the long, narrow-lumened channels of the flexible endoscope. Any microorganisms that are present in the endoscope channels may be picked up by the accessory device and now have the potential of being introduced into sterile body sites. Effective sterilization of flexible endoscopes would eliminate this problem, but it has not been possible to reliably demonstrate sterilization for flexible endoscopes.

## 2. Disinfectants

### i) Classes of Chemical Agents used as Disinfectants

Chemical disinfectants and/or sterilants are categorized into many different classes depending, primarily on their chemical structure and mechanism of action. The disinfectant classes that are in common use today include aldehydes, including formaldehyde and glutaraldehyde; alcohols; halogen-releasing compounds such as sodium hypochlorite and

iodophors; peroxygens; silver compounds; phenols; and quaternary ammonium compounds.

Glutaraldehyde (1,5 pentanedial) is a powerful biocide that has found usage as a disinfectant, sterilant and fixative in electron microscopy. The first indications of its antimicrobial potential came from a survey of saturated dialdehydes by Pepper and Lieberman in 1962 in their search for an efficient substitute for formaldehyde. In the following year, Stonehill et al. (127) advocated that a suitably alkalinated solution of glutaraldehyde was rapidly sporicidal and toward the end of 1963, a glutaraldehyde formulation was marketed for use as a chemosterilant.

Glutaraldehyde displays a broad spectrum of activity and a rapid rate of kill against a majority of microorganisms. It is classified as a chemosterilant (19) as it is also effective against bacterial spores at a concentration $\geq 2\%$ after prolonged exposure (10 hours at room temperature 22°C.) It is classified as a high-level disinfectant at shorter exposure times of 20-45 minutes at room temperature.

The presence of free aldehyde groups is considered a prerequisite for good biocidal activity (44). Glutaraldehyde possesses two free aldehyde groups that react readily under suitable conditions (21, 56). The mechanism of action of glutaraldehyde involves a strong association with the outer layer of vegetative bacterial cells, specifically the amino groups of proteins. The reaction of the free aldehyde with a primary amine on a protein is followed by condensation of additional free glutaraldehyde and leads to the formation of a 1,3,4,5 substituted pyridinium salt. These cross linked proteins are evident by a new absorption peak at 265 nanometres. (144). However, the biocidal action of glutaraldehyde is not merely due to its sealing of the bacterial envelope alone. The inhibitory effect of glutaraldehyde on RNA,

DNA and protein synthesis is due to the inhibition of precursor uptake as a consequence of the glutaraldehyde/protein reaction in the outer structures of the cell (79) as well as deactivating bacterial surface enzymes (44).

Sodium hypochlorite (bleach) is a halogen-releasing agent capable of releasing free chlorine. The most important types of chlorine-releasing agents are chlorine dioxide, sodium dichlororisocyanurate and sodium hypochlorite. Sodium hypochlorite solutions are currently one of the most widely used agents for hard-surface disinfection in hospitals. The use of sodium hypochlorite is limited to non-metallic surfaces and objects as the corrosive effects of sodium hypochlorite on metals is quite detrimental. The disinfecting and deodorizing properties of chlorine were first recognized in the first half of the nineteenth century (16). In 1881, a German bacteriologist, Koch, demonstrated that under controlled laboratory conditions, that pure cultures of bacteria may be destroyed by the use of hypochlorites. Five years later, the American Public Health Association issued a favorable report on the use of hypochlorites as disinfectants (48). Sodium hypochlorite solutions are available in concentrations from 1%-15% but chlorine, in aqueous solutions, even in minute amounts, exhibits rapid bactericidal action. Although the actual mechanism of action is not known, many theories have been proposed. Baker (6), advanced a theory that chlorine destroys bacteria by combining with proteins of cell membranes forming N-chloro compounds which in turn interfere with cell metabolism, causing eventual death of the organism. Green et al. (45) postulated that because of the low chlorine level required for bactericidal action, chlorine must inhibit some key enzymatic reactions within the cell. In 1948, Knox et al.(68) confirmed that the bactericidal effect of hypochlorite is produced by the inhibition of certain enzyme

systems essential to life and that the mechanism here is the result of oxidative action of chlorine on the SH groups of vital enzymes or other enzymes sensitive to oxidation by chlorine.

Hydrogen peroxide is a peroxygen compound first reported in 1818 (17) and now serves particular functions of great value. It is currently used in disinfection, sterilization and antisepsis. Hydrogen peroxide solutions are commercially available in concentrations that range from 3%-90%. It can be easily destroyed by heat or the enzymes catalase and peroxidase to give the innocuous end products, oxygen and water. Hydrogen peroxide may be regarded as nature's own disinfectant. It is naturally present in milk and honey. It is also present in the phagocytic cells of mucous membranes in the mouth and it is a germicide that kills microorganisms that penetrate the outer defenses of the body and gain entrance to the bloodstream. It is produced in the phagocytic cells by the reduction of oxygen. The reaction of the superoxide radical with hydrogen peroxide produces a hydroxyl radical that is said to be the strongest oxidant known (41) Hydrogen peroxide is believed to kill bacteria by attacking essential cell components, including lipids, proteins and DNA. Hydrogen peroxide is active against vegetative bacteria, yeasts, fungi, viruses and bacterial spores. Anaerobes are even more susceptible to hydrogen peroxide because they do not produce the enzyme, catalase, that is known to break down hydrogen peroxide. Destruction of bacterial spores by hydrogen peroxide is greatly improved by both increasing the contact temperature or the concentration of hydrogen peroxide. Although its activity is affected by changes in pH, with greater activity on the acidic side, it is less affected than are many other disinfectants. Sporicidal activity is most effective at a pH of 3 and least effective at a pH of 9.

Phenolic (carbolic) compounds have long been used for their antiseptic and disinfectant properties. Since its adoption by Lister as a germicide, phenolic compounds have been the subject of study for many years. Phenolics are used as the active ingredient in hard-surface disinfectants, germicidal soaps and lotions, and antiseptics. The phenolic compounds used as hard-surface disinfectants are o-phenylphenol, o-benzyl-p-chlorophenol, and p-tert-amylphenol and are present in aqueous or aqueous/alcohol solutions of 400 ppm to 1300 ppm. Phenolic compounds are considered to be low to intermediate-level disinfectants and are appropriate for general disinfection of semi-critical and non-critical items. The biocide activity of phenolic compounds varies as a function of concentration, temperature, pH and other physical and chemical factors. Parasubstitutions to the phenolic ring of an alkyl chain, halogenation and nitration all contribute to increased antibacterial activity of the compound. The free hydroxyl group constitutes the reactive entity of the phenol molecule. Germicidal properties of the various phenolic compound formulations increase with the compound's molecular weight. Phenol and its derivatives exhibit several types of bactericidal action. At higher concentrations, these compounds act as a gross protoplasmic poison by penetrating and disrupting the cell wall and precipitating the cellular proteins (29, 30). However, at lower concentrations, phenolic compounds inactivate essential bacterial enzyme systems. In general, Gram-negative bacteria show a greater resistance to phenolics than do Gram-positives . The outer membrane of Gram-negatives provides an additional barrier to these biocides. Phenolic compounds are also highly effective tuberculocidal agents. Although the waxy cell wall of *Mycobacterium* has enabled it to be resistant to so many chemical disinfectants, the ability of phenolic compounds to dissolve this lipid material gives them their tuberculocidal

properties. Phenolic compounds are also fungicidal (2%) and fungistatic (lower concentrations) agents (132). Fungicidal mechanisms involve damage to the plasma membrane (97), resulting in leakage of intracellular constituents. Viruses are not heterogenous in their susceptibility to phenolic agents because of their varied lipophilic, hydrophilic and intermediate structures.

Although there were many stages in the historical development of quaternary ammonium compounds (QAC), two truly historical milestones were recognized. The first involves the work of Jacobs et al.(60) which examined the structure, preparation and antimicrobial properties of the QAC's and the second, the work of Jacobs and Heidelberger, (61), that reviewed various QAC formulations. In these publications, the antimicrobial activity was attributed to the structure of the compound.

The first generation QAC was benzalkonium chloride. Modifications of the first-generation quaternaries by substitution of the aromatic ring hydrogen with chlorine, methyl and ethyl groups resulted in second-generation QAC's. The third-generation QAC's, which contain a mixture of equal proportions of alkyldimethylbenzyl ammonium and alkyldimethylethylbenzyl ammonium chloride, are of the greatest commercial significance today. The third-generation QAC's or dual quats, offer improved biocidal activity, stronger detergency and a relatively lower level of toxicity (81).

Quaternary ammonium compounds that are used commercially are bacteriostatic, tuberculostatic, sporostatic and fungistatic at low concentrations of 0.5 to 5 ppm (52, 39, 116) and are bactericidal, fungicidal and virucidal against lipophilic viruses at medium concentrations of 10 to 50 ppm (70, 92, 67). QAC's are effective against both Gram-positive

and Gram-negative organisms but have greater activity against the Gram-positives. QAC's are not tuberculocidal or sporicidal or virucidal against hydrophilic viruses even at high concentrations (67, 120, 32). Because of these limitations, QAC's are considered low-level disinfectants and should be considered inappropriate for reprocessing of semi-critical and critical medical devices.

It's bactericidal effectiveness begins by adsorption of the QAC on the bacterial cell surface. The compound diffuses through the cell wall and binds to and disrupts the cytoplasmic membrane. This disruption leads to the release of potassium ions and other cytoplasmic constituents. Precipitation of the remaining cell constituents leads to the death of the cell.

**ii) Impact of Organic Soil**

In addition to the variable susceptibility of microorganisms to chemical germicides, the presence of organic and inorganic material can further complicate the killing efficacy of germicides. Residual blood, mucus, or feces on improperly cleaned medical devices may contribute to the failure of a given disinfectant. The reduction in disinfectant activity in the presence of organic matter is linked to complex physiochemical phenomena in which molecule reactivity such as oxidation and reduction, and adsorption mechanisms are involved. The organic soil may contain large or diverse microbial populations, may occlude microorganisms and prevent penetration of chemical disinfectants or the soil may even directly and rapidly inactivate certain germicides. Thorough cleaning of medical devices before they are disinfected or sterilized must be performed. Failure to do so may cause disinfection or sterilization procedures to fail because these organisms are protected by residual organic

matter.

Many organic soil formulations have been developed to simulate various real-life soils found on medical devices and have been utilized in studies that require an organic soil. Hucker Soil contains a mixture of food products such as peanut butter, butter, flour, lard, dehydrated egg yolk and evaporated milk (62). It is intended to simulate fecal material. Edinburgh soil has been developed to simulate the soil found on anaesthetic equipment and contains egg yolk, sheep's blood and hog mucin (62). Other test soils simulate material found on surgical instruments (Swedish soil) (62), bedpans (British Standard soil) (62) and anaesthetic equipment (Birmingham soil) (62). The choice of soil used is dependent upon the context of the investigation.

Organic loads of soils differ widely, but because they are often proteins, a number of tests employ yeast extract or animal/human serum. Because of the known inhibitory effect of residual patient material on disinfectant/sterilant efficacy, the United States Food and Drug Administration (FDA) requires pre-market evaluations of such biocides in the presence of a "worst-case" organic and inorganic soil load (62). The soil used should simulate the major components found in patient-used medical devices. Alfa et al. (3) determined the levels of several components of the soil found in flexible endoscopes immediately after patient use. The levels of carbohydrate, protein, salts, hemoglobin and endotoxin were measured. The worse-case soiling levels or the maximum levels of each component were used to develop an artificial test soil (ATS) for simulated-use evaluations. This is the first test soil whose formulation was based on data from patient-used endoscopes.

**iii) Other Factors Affecting Disinfectant Efficacy**

As expected, with all other variables equal, the longer the disinfectant is exposed to the object that is being disinfected, the greater its effectiveness. The problem arises when long exposure times are just not realistically practical. Medical device turn-over may affect the exposure time that the medical device receives. If a medical device is used frequently, prolonged exposure or even minimum exposure to the disinfectant and/or sterilant may not be achieved.

Generally, the higher the concentration of a disinfectant, the greater its effectiveness and the shorter the exposure time required for effective microbial killing. It would be ideal to be able to use all disinfectants at high concentrations in order to reduce exposure and reprocessing time but this also may not be realistically practical. The increase in disinfection concentration almost always entails an increase in cost of the disinfectant. The increase in cost to the institution may just not be permissable. Some disinfectants, such as the high-level disinfectants, are appropriately used only at strong concentrations. Some intermediate-level disinfectants may become useful sporicides when the concentration is increased significantly. This is true for hydrogen peroxide and chlorine-base agents like sodium hypochlorite but is not true for all intermediate-level disinfectants, especially phenolics. Biocide activity is represented by the value "n". Biocides with high "n" values like alcohols and phenolic compounds, are affected markedly by changes in concentration and dilution whereas those with a low "n" values such as oxidizing agents and QAC's are influenced to a lesser extent (9). This is important when dealing with the idea of a "use dilution". The "use dilution" is the actual dilution of the disinfectant when in-use. The "use-dilution" may be the same as the

manufacturer's recommended dilution but in most cases, the "use-dilution" is less than the manufacturer's recommended dilution. This occurs when disinfectants are used more than once. When the disinfectant is used more than once and not changed between different disinfecting procedures, dilution of the disinfectant may occur if extraneous liquid is introduced. The "n" values of disinfectants are important when considering "use-dilution" because any dilution of the original disinfectant may affect its efficacy. Depending on the "n" values of the disinfectant, an increase in dilution may or may not severely affect the disinfectant's killing ability.

The easiest surface to disinfect or sterilize is one that is smooth, non-porous, and easily cleanable. Crevices, joints, pores in surfaces and narrow lumened devices constitute barriers to cleaning and subsequent penetration of the liquid germicide. Prolonged exposure to the disinfectant may be necessary with these objects. Flexible endoscopes can not be subjected routinely to long contact times in chemical germicides without the risk of eventually degrading the components of the device.

Penetration of the disinfectant itself, regardless of the device surface it is applied to, is also an issue. Glutaraldehyde is a strong fixative that efficiently cross-links bacterial surface proteins. This inherent property of glutaraldehyde may contribute to the inability of the biocide to penetrate its target. Upon contact with glutaraldehyde, bacteria present in the outer most portion of the soil layer will be destroyed. This cross-linked outer layer of proteins may act as a barrier so that the glutaraldehyde can not reach the remaining bacteria in the deeper portion of the soil. While a reduction in the numbers of bacteria would be observed, sequestering of bacteria in deeper layers of the soil may lead to sporadic failure of

glutaraldehyde killing.

### 3. Antibiotic Resistance

#### i) Mechanisms of Antibiotic Resistance

It is estimated that 80,000 deaths per year in the United States are related to hospital-acquired or nosocomial infections (76). Many of these infections are caused by bacteria that are resistant to one or more antimicrobial agents. The development of bacterial antibiotic resistance is a major world-wide problem complicating the use of chemotherapeutic agents and the control of infectious diseases (91).

The mechanisms of antibiotic resistance can generally be classified into four basic types. These include the ability of the organism to inactivate or modify the antibiotic, alter the target site of the antibiotic, use of an alternate pathway (bypass) and decreased uptake of the antibiotic.

A drug inactivation mechanism of resistance is the most widely recognized and was the first mechanism to be characterized. It was discovered in penicillin, an antibiotic that inhibits bacterial cell wall synthesis. It was observed that enzymes produced by the bacteria could destroy penicillin and were later named penicillinases. Beta-lactamase is the general term describing these enzymes that inactivate all Beta-lactam antibiotics. All Beta-lactamases destroy the Beta-lactam ring of the antibiotic.

Genes encoding Beta-lactamases can reside on the bacterial chromosome or on plasmids. Plasmid-mediated resistance can be passed to distantly related bacterial species by conjugation. In contrast, the expression of chromosomally mediated Beta-lactamases is usually not constitutive but can be induced or derepressed by exposure to Beta-lactam

antibiotics (109) The common Beta-lactamases, TEM 1 and 2 found in all Gram-negative and some Gram-positive bacteria confer resistance to broad spectrum penicillins such as ampicillin. In response to an increase of resistance to broad spectrum penicillins, antibiotics such as cefotaxime and ceftazidime were introduced which were resistant to the action of Beta-lactamases. Rapid mutations in the Beta-lactamase gave greater access to the "extended spectrum" cephalosporins giving rise to the extended spectrum Beta-lactamases (ESBL's). ESBL's are becoming increasingly common.

Antibiotic resistance through an alteration in the antibiotic target site is also a very common mechanism. Methicillin resistance in *Staphylococcus aureus* is mediated through this mechanism. Penicillin-binding proteins (PBP) are responsible for synthesis of major bacterial cell wall structural components. In methicillin resistant strains, the PBP is altered (PCP2') and has a much lower affinity for methicillin than the unaltered form (PBP2)(49). The expression of PCP2' can either be induced by methicillin or be constitutive.

While the antibiotic susceptible target can remain in the bacterial cell, some resistant bacteria have acquired an alternative target. This alternate target site is resistant to inhibition by the antibiotic. The bacteria are viable in the presence of the antibiotic which "bypasses" the normal target route. This has been observed in vancomycin resistance in *Enterococcus*. Vancomycin is a glycopeptide antibiotic that inhibits bacterial cell wall formation by the inhibition of peptidoglycan synthesis. It binds to the free carboxyl end of the D-alanyl-D-alanine of the elongating peptidoglycan chain and inhibits the progression of synthesis. In vancomycin resistant strains of *Enterococcus faecium* and *Enterococcus faecalis*, an alternative cell wall precursor is made that contains a D-alanine-D-lactate chain end.

Vancomycin is unable to bind to this site and peptidoglycan synthesis can continue.

By preventing an antibiotic from entering the bacterial cell or by pumping the antibiotic out of the cell before it acts on the target, bacteria can be protected from the actions of the antibiotic and become resistant. Beta-lactam antibiotics against Gram-negative bacteria gain access to the cell by water filled hollow membrane proteins known as porins. Different antibiotics may enter through different porins therefore a lack of a specific bacterial porin may confer resistance for that antibiotic. Imipenem resistance in *Pseudomonas aeruginosa* is caused by the lack of the D2 porin. This type of mechanism is also seen in low level resistance to fluoroquinolones and aminoglycosides. Increased efflux of antibiotics is a well recognized mechanism for resistance to a wide range of antibiotics. This wide range resistance is contributed by the *mar* and *norA* genes which are widely distributed in the *Enterobacteriaceae* (31).

**ii) Antibiotic Resistant Bacteria**

*Acinetobacter* species are non-fermentative, aerobic Gram-negative coccobacilli. They are widely distributed in nature, in the hospital environment and may be found as human commensals on the skin and other body sites (20). Infections from these organisms are opportunistic in nature and develop in patients compromised by invasive diagnostic or therapeutic procedures (128). Infection presents as pneumonia, central venous catheter related infection, skin and wound sepsis, septicemia and urinary tract infections (20).
There are 3 groups within the species but *Acinetobacter baumannii,* formerly named as *Acinetobacter calcoaceticus* var. *anitratus* (20) is recognized as the species associated with the majority of human infections. This organism has several properties which are a concern.

These include a demonstrated propensity to develop resistance to multiple antibiotics and its relative resistance to environmental exposure (142). *Acinetobacter baumannii* has been detected in patient hospital pillows (141), ventilatory surfaces, hospital gloves, patients' mattresses (117) and hospital bed rails (26). This environmental resistance increases when *Acinetobacter baumannii* is dried in the presence of organic material or menstrua. The overall susceptibility of *Acinetobacter* isolates to cephalosporins, penicillins and aminoglycosides has steadily decreased over the last decade (35, 43, 73, 115, 138, 128, 134). Additionally, outbreak strains of *A. baumannii* were recently reported to be considerably more antimicrobial-resistant than non-outbreak strains (43, 73). *A. baumannii* has become an important nosocomial pathogen (94, 140, 26, 10, 131, 126, 117, 23). Many outbreaks of *A. baumannii* have been associated with respiratory equipment, intravascular access devices and contaminated hands of hospital care givers.

*Alcaligenes xylosoxidans*, formerly *Achromobacter xylosoxidans* is a Gram-negative, non-fermenting, aerobic, motile bacillus. It is widely distributed in the environment and has been isolated from the aqueous environments that come into contact with infected patients (137). Along with *A. baumannii*, *A. xylosoxidans* is a nosocomial pathogen responsible for multiple outbreaks due to its resistance to environmental drying.

Many of the antimicrobial agents introduced during the 1970's and 1980's such as fluoroquinolones and later generation cephalosporins, exhibited less activity against Gram-positive organisms than Gram-negative bacteria. This coincided with the emergence and spread of novel types of resistance among various species of Gram-positive bacteria such as penicillin resistance in pneumococci and glycopeptide resistance in enterococci.

*Staphylococcus aureus* is a Gram-positive, coccoid organism that causes pneumonia and infections of the bloodstream, skin, soft tissues and bone (55). This pathogen frequently causes community-acquired infections and is the most common cause of nosocomial infections. Soon after the introduction of benzylpenicillin into clinical use in the 1940's, it became clear that some strains of *S. aureus* were resistant due to the production of beta-lactamase. Under the selective pressure of penicillin usage, the prevalence of penicillin-resistant strains increased, particularly in hospitals. In the 1960's, upon the introduction of a penicillin-derivative, methicillin, rapid emergence of methicillin resistant *Staphylococcus aureus* (MRSA) followed (75).

Strains of MRSA are also commonly resistant to various other antimicrobial agents including erythromycin, aminoglycosides, tetracycline, fusidic acid, rifampicin or ciprofloxacin. In MRSA, the mechanism of resistance does not involve inactivation of the antibiotic but expression of an altered penicillin-binding protein, $PBP_2'$. The beta-lactam antibiotics can not recognize this altered protein and therefore can not interfere with cell wall synthesis. This is a mode of antibiotic resistance in which the antibiotic target site is altered.

MRSA strains are frequently only susceptible to glycopeptide antibiotics such as vancomycin. Gram-negative bacteria are resistant to glycopeptides, because the large glycopeptide molecule can not penetrate the outer membrane. The emergence of reduced vancomycin susceptibility in *S. aureus* has heightened the fears of a totally antibiotic-resistant strain. In May 1996, the first isolate of *Staphylococcus aureus* with a reduced susceptibility to vancomycin was observed (54). The minimum inhibitory concentration (MIC) for this organism was 8 ug/ml indicating it had intermediate-level resistance to vancomycin (55).

Therefore, the strains were called glycopeptide intermediate-resistant *S. aureus* (GISA). Although *van* genes are the only specific genes that confer resistance to vancomycin in other organisms like enterococci, the GISA strains were shown not to have these genes. The mechanism of resistance in GISA appears to be due to an increased rate of cell wall synthesis. As a result, more cell wall material, peptidoglycan, is produced. This thickened cell wall has been demonstrated by electron microscopy (56). Vancomycin's targets are the D-alanyl-D-alanyl residues present in the peptidoglycan of the microbial cell wall. The large, glycopeptide molecule acts by sterically impeding trans-glycosylation which is needed to extend the peptidoglycan backbone. In addition, vancomycin prevents transpeptidase from cross-linking the growing peptidoglycan chain. Since there is an overproduction of peptidoglycan in GISA strains, more D-alanyl-D-alanyl residues are available for vancomycin. These "extra targets" aid in consuming and sequestering vancomycin molecules thereby protecting deeper portions of peptidoglycan (56).

The genus *Enterococcus* are Gram-positive, aerobic organisms. They colonize the bowels of healthy humans and are found in counts of up to $10^7$ cfu / gram of stool. *E. faecalis* is more common than *E. faecium* and the other sixteen species are rarely found in humans. Enterococci frequently causes infections in association with other virulent organisms however, enterococci can cause more invasive infection and are sometimes responsible for cholecystitis, chloangitis, peritonitis, septicemia, endocarditis, meningitis and wound infections (86, 83).

Vancomycin resistance in *Enterococcus* can be attributed to different genes. The *vanA* gene is usually plasmid borne but is now known to be encoded on a transposon that may

pass to the chromosome. Resistance by the *vanA* gene results in resistance to vancomycin and teicoplanin. Most outbreaks of VRE have involved the *VanA* phenotype. In these cases the resistance plasmid often appears in both *E. faecium* and *E. faecalis* and readily transfers to multiple different strains of these species during the outbreak (40).

*VanB* resistance is usually chromosomal and is occasionally transferable from chromosome to chromosome on a transposon. This type of resistance renders the bacterium vancomycin resistant but teicoplanin susceptible. Several outbreaks of organisms with the *VanB* phenotype have also been reported, more often with single strains. As with the *VanA* phenotype, *VanB* is most commonly seen in *E. faecium* and *E. faecalis* (40).

The constitutive low-level vancomycin resistance seen in some strains of *Enterococcus gallinarum* is called *VanC*. A *VanD* phenotype has been reported in a single strain of *E. faecium* (40). This organism has constitutive resistance to vancomycin (MIC = 64mg/L) and to low levels of teicoplanin (MIC = 4mg/L). Because *vanA* and *vanB* are so readily transferred, these types of resistance pose a more significant infection control risk compared to low-level resistance due to *vanC*, which is much less likelier to be transferred.

**iii) Bacterial Cross Resistance to Antibiotics and Disinfectants/Sterilants**

Resistance to disinfectants or sterilants has been less extensively studied compared to antibiotic resistance. As with antibiotics, resistance to disinfectants and sterilants may be acquired but is more commonly intrinsic. Intrinsic resistance to such biocides has been reported for Gram-negative bacteria (95), bacterial spores (18), mycobacteria (98) and under certain conditions, staphylococci (78). Acquired, plasmid-mediated resistance is most widely associated with mercury compounds and other metallic salts (99). In recent years, acquired

resistance to certain types of biocides has been observed, notably in staphylococci (78).

Intrinsic mechanisms of microbial resistance to biocides (IRB) most often involve the outer layer of the organism. The composition of the outer layer may act as a permeability barrier in which there is a reduced penetration of the biocide (96, 98, 78). Alternatively, but less commonly, constitutively synthesized enzymes may bring about the degradation of the biocide (59, 88).

For Gram-positive bacteria, IRB may be attributed to the plasticity of the bacterial cell envelope (78). Growth rate and any growth limiting factors will affect the thickness and degree of cross-linking of peptidoglycan and will likely modify the microorganism's susceptibility to disinfectants. In nature, *S. aureus* may exhibit a mucoid, slime layer. Non-mucoid strains are killed more rapidly than mucoid strains by some biocides. Therefore, the slime layer plays a protective role either by acting as a barrier or absorbing the biocide molecules.

For Gram-negative bacteria, IRB is due to the outer membrane of the Gram-negative cell wall. This membrane acts as a barrier that limits the entry of chemically, distinct antibacterial agents (78). It has also been proposed that the inner, cytoplasmic membrane can also provide intrinsic resistance but little evidence has been shown (78).

*Mycobacterium* are well known to possess resistance to many disinfectants. The most likely explanation of its IRB is attributed to the highly complex cell wall. The mycobacterial cell wall is a highly hydrophobic structure with a mycoylarabinogalactan-peptidoglycan skeleton (78), complex lipids, lipopolysaccharides and proteins. This unique structure presents itself as a waxy, outer coat that is an effective barrier to the entry of biocides.

Plasmid-mediated resistance in Gram-negative and Gram-positive bacteria has been well studied (99). Increased biocide MIC's have been observed in *S. aureus* strains. These strains possess a plasmid carrying the genes that encode for resistance to gentamicin (72). Plasmid-mediated resistance to biocides has also been observed in Gram-negative organisms. Plasmid-encoded changes in outer membrane proteins are associated with decreased susceptibility to formaldehyde in *Escherichia coli* and *Serratia marcescens* (66). Generally, however, it appears that intrinsic resistance in Gram-negative bacteria is of greater significance than plasmid-mediated resistance.

Considering the intrinsic properties of antibiotic resistance and the transfer of genes of multiple antibiotic resistance from one bacterium to another, it has been proposed that multiple antibiotic resistant organisms are more likely to be disinfectant or sterilant resistant than organisms that are susceptible to multiple antibiotics. Many studies have been performed to test this theory. Suller and Russell (129) investigated biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. The test disinfectants included chlorhexidine, several quaternary ammonium compounds, and triclosan among others. Although these strains showed low-level resistance to the test disinfectants, they remained relatively sensitive to the biocides compared to their susceptible counterparts. Antibiotic resistant bacteria were also investigated by Barry et al. (8) against chlorhexidine and povidone iodine. They determined that antibiotic resistance does not affect the activity of chlorhexidine and povidone iodine as antiseptic agents. Many more studies have also come to the conclusion that antibiotic resistant bacteria do not show an increased resistance to disinfectants compared to multiple antibiotic susceptible strains (4, 5).

## 4. Models for Studying Disinfectant Efficacy

### i) Introduction

Since the beginning of the twentieth century, a large number of scientific publications have been devoted to developing methods for testing disinfectants. Koch in 1881 introduced the first method of testing chemical disinfectants by impregnating the surface of silk threads with *Bacillus* spores and then exposing the inoculated threads to the test disinfectant for varying lengths of time. The threads were washed and then used to inoculate nutrient agar plates. Today, the approved surface carrier method involves the use of disposable glass penicylinders (111). Recently, in Belgium, investigators developed a new test which is a quantitative, standardized and reproducible suspension test (14). Both surface and suspension tests, although modified from their progenitors, have been the two most utilized methods in testing disinfectant efficacy.

### ii) Critical Model Parameters

No matter which test method is used to determine disinfectant efficacy, certain test conditions must be well defined. It is important to include strains from the main bacterial groups which include bacilli, cocci, Gram-positive, Gram-negative and mycobacterial strains. Culture medium, whether it is broth or agar should contain all the elements required for abundant growth of each strain. It has been suggested that test bacteria should be tested when they are at the exponential phase of growth and that two or three subcultures should be performed in the same medium before use. The inoculum should be prepared in either peptonic water, broth or buffer as preparation in distilled water may have a bactericidal effect. The size of inoculum is an essential parameter of both the surface carrier and the suspension

tests. For high level disinfection, a minimum 6 $\log_{10}$ reduction is required and therefore when testing high-level disinfectants, the inoculum should contain at least $10^6$ cfu.

Temperature of the assay is also important. The higher the activity of a disinfectant, the greater its sensitivity to changes in temperature. Generally the reaction temperature is 25°C or room temperature (22°C) unless specified otherwise by the disinfectant manufacturer.

The activity of a disinfectant is proportional to its concentration and action time. Some disinfectants, when diluted, must be exposed for prolonged measures to retain the same activity of an undiluted solution while certain disinfectants have rapid lethal action and tests must be carried out rapidly. Suspension methods usually have exposure times of 5 to10 minutes while surface carrier tests have contact times of 5 to 360 minutes.

Neutralization of disinfectant is very important in both the surface carrier and suspension tests. Dilution alone is normally sufficient to suppress the antimicrobial residual activity of the test disinfectants. Quaternary ammonium compounds may have a bacteriostatic activity against certain organisms even when highly diluted. Neutralizing agents usually contain phospholipids such as lecithin or nonionic surfactants such as polysorbates.

Washing the sample is also very important. Washing procedures imply mechanical separation of microbial cells and disinfectant containing suspension. This can be done by centrifugation, membrane filtration or by mechanical removal of the disinfectant solution from the carrier.

**iii) Suspension Test Method**

Suspension tests estimate the in-vitro bactericidal activity of the disinfectant under a totally fluid environment. Contact between the bacteria and disinfectant is complete meaning

there are no added mechanical barriers provided by the investigator. This method gives an assessment of the direct chemical killing ability of the disinfectant.

A fixed volume of disinfectant solution is mixed with the bacterial suspension in the presence of absence of an organic load. After a defined contact time, the sample is neutralized to eliminate any effect of the disinfectant, post exposure. The microbicidal capacity is assessed by filtering the bacterial/disinfectant/neutralizing solution and by either subculturing all or part of the sample into nutrient broth, which only indicates whether all bacteria have been destroyed or not or subculturing part of the sample onto nutrient agar to determine the number of residual, surviving bacteria. The former method is only qualitative in nature while the latter is quantitative as it gives valuable information on the disinfectant and its killing rate. The quantitative method is most commonly used to assess disinfectants, whereas the qualitative method of broth inoculation is more commonly used to assess sterilization.

**iv) Surface Carrier Test Method**

Surface carrier test methods attempt to reproduce in-use disinfection conditions in which the disinfectant is applied to a surface contaminated with microorganisms (eg. environmental surface or medical device). Different surface carriers have been proposed and used. The Hard Surface Carrier Test (HSCT) uses glass penicylinders, while others use glass vials (111) or polyvinyl chloride tubing segments (3 ). The choice of surface carrier material and shape is also important. The test carrier surface should mimic the particular in-use surface that the disinfectant will be applied to. The shape and physical structure of the test carrier may also affect the evaluation of disinfectant efficacy. Disinfectant penetration or

access is not hindered by flat, smooth, non-porous carriers while test carriers that have a small diameter lumen, an uneven surface or pores create a more stringent challenge.

Inoculated carriers are dried for specific times and either dropped into specified volumes of test disinfectant or disinfectant is applied directly to the dried inoculum on the carrier. It is exposed to the test disinfectant for a specified interval. The washing procedure is performed followed by the neutralizing procedure. The following step is identical to the suspension test method in which as sample is either qualitatively or quantitatively assessed for microbicidal activity of the disinfectant.

Controls are very important when testing with the surface carrier method. Since the surface carrier is being exposed to fluid effects at several stages, controls must be included to ensure adequate interpretation of the results. If the test organism is washed off by the fluid exposure but is not killed by the disinfectant, erroneous conclusions may be made. The "fluid effect" may vary depending on the bacterial strain, disinfectant exposure, washing methods and neutralizing agent. Many investigations utilizing the surface carrier test method fail to use this type of control and the efficacy of disinfectant was assessed from the residual bacteria level and the initial inoculum level. The most accurate measurement of disinfectant efficacy would be to compare the residual bacterial, post disinfectant exposure, to the recoverable bioburden level determined by using a fluid control. Not accounting for these liquid manipulations would suggest a false level of disinfectant efficacy because the reduction of viable bacteria on the carrier due to the liquid exposure to the disinfectant, washing and neutralizing would be wrongly attributed to the disinfectant.

## 5. In-use Disinfection/Cleaning Practices

It is well-known that cleaning is an essential step in the reprocessing of medical devices and infection control. If devices and surfaces are not properly cleaned, subsequent disinfection and/or sterilization can not be guaranteed. The cleaning process should reduce the natural bioburden on the object and remove organic/inorganic contaminants. Rutala et al (105), found that after a standard cleaning procedure on surgical instruments, 72% contained less than $10^1$ cfu. In a study of rigid, lumened medical devices, Chan-Myers et al. (27) found that bioburden on both the inner and outer surface of the lumen was relatively low ($10^1$ to $10^4$ cfu/device). After the instruments were cleaned, 83% had bioburden of less than $10^2$ cfu/device. The importance in cleaning to remove organic/inorganic material was highlighted in several studies (62). These studies suggested that the presence of organic/inorganic material on medical devices, prior to disinfection or sterilization would detrimentally affect the ability to disinfect or sterilize. Jacobs et al (62) suggest that this is due to the formation of crystals from the organic/inorganic material. These crystals occlude any microorganisms and thereby "protect" them against the action of the disinfectant or sterilization procedure.

After proper cleaning, medical devices undergo either disinfection or sterilization depending on its use.

## 6. Issues Regarding Disinfectant Resistance

In consideration of the current literature regarding disinfectant resistance in antibiotic resistant bacteria, there are some issues that have not been fully elucidated. The range of organisms tested in previous studies have not been exhaustive. Previous studies have focused on specific bacteria like methicillin resistant *Staphylococcus aureus* and vancomycin resistant

on specific bacteria like methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus* (129) or Mycobacteria (125). This study involves a full range of test bacteria encompassing each important bacterial group including Gram-negative and positive bacteria, cocci and bacilli and Mycobacteria.

No data has been published to date to determine possible disinfectant resistance in organisms that exhibit non-specific mechanisms of antibiotic resistance such as permeability limitations and non-specific efflux pumps. Permeability is an important factor in GISA strains because of the hyperproduction of cell wall material and in Mycobacteria because of the waxy, glycolipid cell wall. Non-specific efflux pumps such as the *mar* pump are found in all enterobacteria and may play a role in disinfectant resistance. This study includes two GISA strains, a novel glutaraldehyde-resistant *Mycobacterium chelonae* and several strains of *Enterococcus faecium*.

Previous studies have included highly antibiotic resistant bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* as test bacteria. We have also chosen to do so but have increased our criteria to include these organisms and others that have been implicated in hospital outbreaks. This choice of test bacteria serves to encompass all important bacterial groups while it explores the variety in resistance mechanisms and it's possible association with disinfectant resistance.

It is known that organic/inorganic material present on medical devices complicates the process of disinfection and/or sterilization. Most of the previous studies have taken this fact into consideration and have included an organic soil in their test protocols. The research published to date however, has not addressed the effect of an organic soil that has

experimentally been developed to contain the levels of blood, protein, bilirubin and salts found in patient-used flexible endoscopes. The goal of this study was to use a test soil that simulates the soil found in patient-used flexible endoscopes, not merely any organic substance, to determine whether this organic challenge has an effect on disinfectant efficacy against the test bacteria.

Reuse of chemical germicides for disinfection of heat sensitive medical devices is routine in health-care facilities. Dilution of the disinfectant is also known to occur during reuse. Although manufacturers often recommend product reuse for only a specific period, this is not always adhered to and may contribute to inadvertent dilution of the disinfectant through multiple use. Previous studies almost always focused on specific disinfectant concentrations. The range of disinfectant concentrations was not great. This study took into consideration the fact that disinfectants used in real-life may inadvertently be diluted through use. This dilution of the disinfectant may eventually create an environment in which bacteria are able to survive. The use of the suspension test method protocol and the use of a range of concentrations of disinfectants addresses the issue that multiple antibiotic resistant bacteria may overcome and survive the effects of sub-optimal concentrations of disinfectant.

In addition to specific disinfection concentrations, the previous studies also focused on specific disinfectant exposure times. The investigators picked certain time points in the disinfectant to test the survivability of the particular test strain. In this study, we propose the idea that multiple antibiotic resistant bacteria may be able to tolerate the effects of the disinfectant over prolonged exposure times. To test this idea, the suspension test protocol was created to include the observation of the test bacteria in the disinfectant over a period

of 24 hours.

**7. Experimental Hypothesis of Current Project**

The research published to date indicate that antibiotic resistant bacteria do not exhibit increased resistance to disinfectants than antibiotic susceptible bacteria. Our study investigates the issues that have not been dealt with in the previous studies such as disinfectant resistance in the GISA and other multiple antibiotic resistant strains. Previous studies have not addressed the issue of penetration of the disinfectant into the bacterial cell. The use of *Mycobacterium* and other organisms expressing non-specific mechanisms of antibiotic resistance attempts to determine the effect of penetration. Our study also evaluates the effects of disinfectant exposure time/concentration and the ability of multiple antibiotic resistant bacteria to outgrow in the test disinfectant.

It is our hypothesis that the multiple antibiotic resistant test bacteria that have shown increased resistance to antibiotics or have been implicated in hospital outbreaks, will be more resistant to the test disinfectants when compared to the multiple antibiotic susceptible test bacteria under the specific test conditions we have chosen.

The objective was to utilize both the surface carrier and suspension methods. The surface carrier method will allow for the comparison of resistance to disinfectants of the multiple resistant and susceptible test bacteria that are in the presence of an organic soil (ATS), which is a soil that has been formulated to simulate the worst case soil levels measured on patient-used flexible endoscopes (3). The bacterial/ATS suspension are inoculated onto a small diameter lumened surface. Because the suspension method will be observed over 24 hours, this method will allow us to determine the resistance of the test

bacteria to decreasing concentrations of the test disinfectant and to observe over a specific period of time whether there is a difference in outgrowth abilities of the resistant strains compared to the susceptible ones.

## MATERIALS AND METHODS

### 1. Test Bacteria

Bacterial strains were obtained from various research groups around the world. The choice of isolate was based on our hypothesis of wanting to test multiple antibiotic resistant isolates that have been involved in hospital outbreaks. We chose these isolates as environmental survival may have contributed to their spread with the hospitals. All bacterial strains have been approved for specified use by the provider and proper permits were obtained from Health Canada and the Canadian Food Inspection Agency.

*Acinetobacter baumannii*$^{R1}$ (76) is a clinical strain obtained from a medical intensive care unit in Birmingham, Alabama, U.S.A. It is a multiple antibiotic resistant strain resistant to amikacin, gentamicin, tobramycin, third-generation cephalosporins, ciprofloxacin, ampicillin/sulbactam, ticarcillin/clavulanate and piperacillin. This strain is only susceptible to imipenem.

*Acinetobacter baumannii*$^{R2}$ (94) is a gentamicin-resistant outbreak strain obtained from a medical intensive care unit in Perth, Australia.

*Acinetobacter baumannii*$^{S1}$ is a clinical, multiple antibiotic sensitive strain obtained from the St. Boniface General Hospital, Winnipeg, Canada.

*Alcaligenes xylosoxidans*$^{R1}$ (137) is an outbreak strain originating from a pediatric burns unit in Paris, France. The susceptibility pattern of this strain shows that it is susceptible to ticarcillin, piperacillin, ceftazidime, moxalactam, imipenem and cotrimoxazole and resistant to cefotaxime, cefepime, cefpirome, aztreonam, colistin, ciprofloxacin, gentamicin, tobramycin, netilmicin and amikacin. This strain is also highly resistant to the antiseptic,

| Bacterial Strain | Source | Type | Study Designation |
|---|---|---|---|
| *Acinetobacter baumannii* | U.S.A. (76) | MR, O | *Acinetobacter baumannii* $^{R1}$<br>*A. baumannii* $^{R1}$ |
| *Acinetobacter baumannii* | Australia (94) | MR, O | *Acinetobacter baumannii* $^{R2}$<br>*A. baumannii* $^{R2}$ |
| *Acinetobacter baumannii* | Canada | MS, C | *Acinetobacter baumannii* $^{S1}$<br>*A. baumannii* $^{S1}$ |
| *Alcaligenes xylosoxidans* | France (137) | MR, O | *Alcaligenes xylosoxidans* $^{R1}$<br>*A. xylosoxidans* $^{R1}$ |
| *Alcaligenes xylosoxidans* | Canada | MS, C | *Alcaligenes xylosoxidans* $^{S1}$<br>*A. xylosoxidans* $^{S1}$ |

**Table 2: Gram Negative Test Bacteria**

The table represents all of the Gram negative bacterial strains tested and where each strain originated. The type of organism (MR= multiple antibiotic resistant; MS= multiple antibiotic susceptible; O = hospital outbreak strain and C = clinical strain) was also indicated. Each bacterial strain was also given a study designation to differentiate the multiple test strains.

chlorhexidine with a minimum bactericidal concentration of greater than 2000 mg/L.

*Alcaligenes xylosoxidans*$^{S1}$ strain is a clinical, multiple antibiotic sensitive strain obtained from the St. Boniface General Hospital, Winnipeg, Canada. A summary of the Gram-negative test bacteria can be found in Table 2.

*Enterococcus faecium*$^{R1}$ and *Enterococcus faecium*$^{R2}$ are outbreak strains obtained from Regina and Saskatoon, Canada respectively and provided by Dr. Michael Mulvey of the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada. Both strains are resistant to vancomycin, ampicillin, ciprofloxacin, gentamicin, penicillin, streptomycin and tetracycline.

*Enterococcus faecium*$^{R3}$ strain is a clinical strain obtained from the St. Boniface General Hospital, Winnipeg, Canada. It is also resistant to all the antibiotics mentioned for the 2 other resistant *E. faecium* strains

*Enterococcus faecium* ATCC 35667 was obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. This strain is a multiple antibiotic sensitive strain. A summary of the *Enterococcus faecium* test strains can be found in Table 3.

*Staphylococcus aureus*$^{R2}$ (55) is a homogeneous resistant, vancomycin intermediate resistant (VISA) strain resistant to vancomycin and methicillin. *S. aureus*$^{R1}$ (54) is a strain that exhibits heterogeneous resistance to vancomycin. It is capable of generating *S.aureus*$^{R2}$-like cells by vancomycin selection with a frequency of 1 in 1,000,000 cells. Both test strains were obtained from a patient in Tokyo, Japan. Both strains exhibit an MIC of 8ug/ml of vancomycin.

| Bacterial Strain | Source | Type | Study Designation |
|---|---|---|---|
| *Enterococcus faecium* | Canada | MR, O | *Enterococcus faecium* R1<br>*E. faecium* R1 |
| *Enterococcus faecium* | Canada | MR, O | *Enterococcus faecium* R2<br>*E. faecium* R2 |
| *Enterococcus faecium* | Canada | MR, C | *Enterococcus faecium* R3<br>*E. faecium* R3 |
| *Enterococcus faecium* | ATCC | MS | *Enterococcus faecium* S1<br>*E. faecium* S1 |

**Table 3: *Enterococcus faecium* Test Bacteria**

The table represents all of the *Enterococcus faecium* bacterial strains tested and where each strain originated. The type of organism (MR= multiple antibiotic resistant; MS= multiple antibiotic susceptible; O = hospital outbreak strain; C= clinical strain) was also indicated. Each bacterial strain was also given a study designation to differentiate the multiple test strains.

| Bacterial Strain | Source | Type | Study Organization |
|---|---|---|---|
| *Staphylococcus aureus* | Japan (55) | MR | *Staphylococcus aureus* R1<br>*S. aureus* R1 |
| *Staphylococcus aureus* | Japan (55) | MR | *Staphylococcus aureus* R2<br>*S. aureus* R2 |
| *Staphylococcus aureus* | Canada | MR, O | *Staphylococcus aureus* R3<br>*S. aureus* R3 |
| *Staphylococcus aureus* | Canada | MR, O | *Staphylococcus aureus* R4<br>*S. aureus* R4 |
| *Staphylococcus aureus* | Canada | MR, O | *Staphylococcus aureus* R5<br>*S. aureus* R5 |
| *Staphylococcus aureus* | ATCC | MS | *Staphylococcus aureus* S1<br>*S. aureus* S1 |

**Table 4: *Staphylococcus aureus* Test Bacteria**

The table represents all of the Staphylococcus aureus bacterial strains tested and where each strain originated. The type of organism (MR= multiple antibiotic resistant; MS= multiple antibiotic susceptible; O = hospital outbreak strain) was also indicated. Each bacterial strain was also given a study designation to differentiate the multiple test strains.

| Bacterial Strain | Source | Type | Study Designation |
|---|---|---|---|
| *Mycobacterium chelonae* | United Kingdom (46) | MR | *Mycobacterium chelonae* $^{R1}$<br>*M. chelonae* $^{R1}$ |
| *Mycobacterium chelonae* | ATCC | MS | *Mycobacterium chelonae* $^{R2}$<br>*M. chelonae* $^{R2}$ |

**Table 5: *Mycobacterium chelonae* Test Bacteria**

The table represents all of the *Mycobacterium chelonae* bacterial strains tested and where each strain originated. The type of organism (MR= multiple antibiotic resistant; MS= multiple antibiotic susceptible) was also indicated. Each bacterial strain was also given a study designation to differentiate the multiple test strains.

*Staphylococcus aureus*[R3], *Staphylococcus aureus*[R4] and *Staphylococcus aureus*[R5] are methicillin resistant strains obtained from an outbreak in Toronto, Canada; Kingston, Canada and Toronto, Canada respectively. All three strains were provided by Dr. Michael Mulvey at the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada.

*Staphylococcus aureus* ATCC 25923 was obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. This strain is a multiple antibiotic resistant strain. The *Staphylococcus aureus* test strains are summarized in Table 4.

*Mycobacterium chelonae*[R1] (46) is glutaraldehyde resistant strain obtained from endoscope washer disinfectors in Birmingham, United Kingdom.

*Mycobacterium chelonae* [S1] obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. is a glutaraldehyde sensitive strain. The summary list for the *M. chelonae* strains is listed in Table 5.

Upon receiving each culture, it was sub-cultured on trypticase soy agar (TSA) (Becton Dickinson, Cockeysvillle, Maryland, U.S.A.). *M. chelonae* strains were sub-cultured onto TSA supplemented with 5% whole, sheep blood (BA)(Oxoid, Toronto, Canada) to check for purity and stocked down in skim milk aliquots and stored at -70 °C until needed.

**2. Test Disinfectants**

The following disinfectants were utilized in both the surface carrier and suspension tests (Table 6): Glutaraldehyde (Metricide™, Sybron Canada, Ontario, Canada) at a stock concentration of 2% (w/v), sodium hypochlorite (Javex™, Colgate-Palmolive, Toronto, Canada) with a stock solution of 5% (v/v), hydrogen peroxide (Hydrox™, Virox

| Disinfectant | Disinfectant Usage<br>Low Level<br>(non-critical medical devices, environmental surfaces) | <br>High Level<br>(semi-critical and critical medical devices) |
|---|---|---|
| Glutaraldehyde (2% w/v) | | <--------------------------> |
| Sodium Hypoclorite (0.5% v/v) | <-------------------------------- | ---------------------------------><br>* |
| Hydrogen Peroxide (3% v/v stabilized) | <-------------------------------- | ---------------------------------><br>** |
| Phenol | <--------------------------------> | |
| Quaternary Ammonium Compound | <--------------------------------> | |

**Table 6: Disinfection Uses and Levels for Test Disinfectants**

The table represents the United States FDA approved uses for the disinfectants used in this study. Two percent (w/v) glutaraldehyde is the only test disinfectant approved for high level disinfection of semi-critical and critical items. Sodium hypochlorite (0.5% w/w) is a low level disinfectant but can be utilized as a high level (*) disinfectant on rubber surfaces and plastic tubing. Three percent hydrogen peroxide (v/v) is categorized as a low level disinfectant but when the concentration is increased to 7% (**), hydrogen peroxide is approved as a high level disinfectant. Both phenolic and quaternary ammonium compounds are low level disinfectants.

Technologies, Mississauga, Canada ) at a stock solution of 3% (v/v), phenolic compound (LpHse, Calgon-Vestal, Mississauga, Canada) and a quaternary ammonium compound (Coverage 256, Calgon-Vestal, Mississauga, Canada) both used as a 1:250 dilution in sterile, distilled water as per manufacturer's recommendations.

**3. Surface Carrier Test**

**i) Bacterial Preparation and Inoculation**

All test bacteria were evaluated in the Surface Carrier Test. Both *M. chelonae* strains were sub-cultured from the -70 °C stock onto BA plates and incubated 48 hours at 30°C. This resulting culture was again passaged two additional times in the same manner. The remaining test strains were sub-cultured onto TSA plates from the -70 °C stock and incubated at 37 °C overnight. The resulting culture was again passaged two additional times in the same manner to ensure an optimal growth and metabolic status (87). *S. aureus*$^{R2}$ and *S. aureus*$^{R1}$ were additionally sub-cultured onto CNA supplemented with 8 ug/ml of vancomycin after the three passages on TSA. The additional growth on CNA with vancomycin plates was to ensure the isolate was expressing increased cell wall synthesis which is thought to be the basis of its vancomycin-resistance (55).

For all the test bacteria, excluding the *M. chelonae*$^{S1}$, a bacterial suspension with turbidity equivalent to a 0.5 McFarland turbidity standard was prepared in TSB to give a suspension of approximately $10^8$ cfu/ml. A suspension of approximately $10^8$ cfu/ml of *M. chelonae*$^{S1}$ strain was prepared by swabbing 5 BA plates that were incubated for 48 hours at 30 °C. The bacteria were suspended into 2 ml of TSB, vortexed for 30 seconds and sonicated for 3 seconds, two times. The suspension was allowed to settle for 2 minutes to

sediment bacterial clumps and 1ml was removed and transferred into a 1 ½ ml microcentrifuge tube and centrifuged at 7,000 rpm for 10 minutes at 4 °C. The supernatant was removed and the pellet was resuspended in 1 ml of TSB.

One millilitre of each test suspension containing approximately $10^8$ cfu's/ml was transferred into a separate microcentrifuge tube and centrifuged at 14,000 rpm for 5 minutes at 4 °C. The supernatant was removed and the pellet was resuspended in 1 ml of artificial test soil (ATS). To determine initial bacterial inoculum counts of the artificial test soil/bacterial suspension, serial 1:10 dilutions in TSB were performed, 100 uls. of the $10^{-3}$ to $10^{-6}$ dilutions were spread over the surface of BA plates for *Mycobacterium* strains and incubated at 30 °C for 48 hours. For the remaining test bacteria, TSA plates were used and incubated at 37 °C overnight.

Polyvinyl chloride tubing (Nalgene, Rochester, New York, U.S.A.) with an internal diameter of 1/8 inch, was cut into 2 cm. lengths and were wrapped using autoclaveable peel pouches (Steriking™, Wipak Medical, Nastola, Finland) and steam sterilized on a fifteen minute liquid sterilization cycle (121 °C, 15 psi, 15 minutes). The lumen carriers were chosen to mimic the plastic surfaces frequently encountered in medical devices. The 1/8 inch diameter of the lumen is similar to that of the biopsy channel of flexible endoscopes. The lumen carriers were left in the pouches for at least 1 week as this ensured that they were well dried before use. Ten microlitres of the artificial test soil/bacterial suspension were inoculated into each lumen carrier that was then dried by holding them in a sterile petri dish at room temperature overnight.

### ii) Artificial Test Soil

The artificial test soil (ATS) used is a proprietary formulation that provides protein, carbohydrate, endotoxin, hemoglobin and a salt concentration similar to the worst-case levels reported by Alfa et al. (3). A new batch of artificial test soil was prepared before each day an assay is run.

### iii) Neutralizing Agent

To eliminate any residual effects of the test disinfectant against the bacteria after experimental exposure was complete, all carriers were rinsed, then a neutralizing agent was utilized. The agent that was chosen for this assay was 10% FBS made up in sterile, distilled water.

### iv) Protocol

The entire assay was performed in a Class II B3 safety cabinet. Inoculated lumen carriers were placed into 3 ml of the test disinfectant in 12 ml plastic, snap-cap test tubes (Simport, Quebec, Canada) using sterile, medical hemastats. Tubes were inspected to ensure that the lumen was positioned at the bottom of the tube and that no air bubbles were present within the lumen of the carrier. Total disinfectant contact must be achieved. The lumen carriers were allowed to sit in the disinfectant for the predetermined exposure time; twenty minutes for glutaraldehyde, sodium hypochlorite and hydrogen peroxide; ten minutes for phenolic and quaternary ammonium compounds. These exposure times were chosen as per manufacturers' recommendations.

After exposure, the test disinfectant was removed with a sterile Pasteur pipette connected to a vacumn line. Care was taken not to touch the inner lumen of the carrier and

to use a gentle vacumn so as not to remove any of the bacteria from the lumen carrier. Three millilitres of sterile, 0.01M phosphate-buffered saline (PBS) with a pH of 7.5 was added carefully to the tube to rinse the lumen carrier. The PBS was removed by the Pasteur pipette and vacumn. The PBS rinse step was repeated twice.

Upon completion of the last rinse, the lumen carrier was removed from the tube with sterile, medical hemastats and placed into a 4 ml, plastic, snap-cap test tube (Simport, Quebec, Canada) containing 2 ml of 10% FBS neutralizing agent. To release the bacteria from the lumen carrier, the tube was vortexed for 5 minutes and sonicated for 3 seconds. The sonication step was repeated one more time. Serial 1:10 dilutions in TSB supplemented with 10% FBS were performed and 100 uls. of the direct through to $10^{-4}$ dilution was spread onto TSA plates and incubated at 37 °C for 48 hours to determine the cfu's/ ml of residual, viable bacteria.

For *Mycobacterium* strains, the 1:10 serial dilutions were also made up in TSB and 100 uls. of the direct through to the $10^{-4}$ dilution were spread onto BA plates and incubated at 30 °C for between 72 and 96 hours. Incubation beyond 96 hours made counting bacterial colonies difficult for *M. chelonae*$^{S1}$ strain because individual colonies tended to grow together.

A recoverable bioburden control was done for every test bacterial strain to determine the residual viable bacteria on the carrier if the whole assay was performed in the absence of disinfectant. This recoverable bioburden (RB) count allows analysis of the fluid effect of the disinfectant experiments. The RB controls were inoculated and processed exactly the same as the test carrier except that PBS was substituted for disinfectant. As a negative

control, a sterilized lumen carrier was aseptically placed into 10 ml of TSB and incubated at 37 °C for 72 hours and checked for sterility.

**4. In-use Disinfection Evaluation**

**i) Questionnaire**

A questionnaire was compiled to get pertinent information about routine cleaning and/or disinfection of patient rooms at the St. Boniface General Hospital, Winnipeg, Canada. A copy of the questionnaire has been included in Appendix I.

**ii) Ward**

The Infection Control department of the St. Boniface hospital facilitated contact with the director of support services who oversees support services personnel. The director agreed to permit the author to observe support service personnel on three different wards at the hospital.

Two geriatric wards and 1 neonatal intensive care unit (NICU) were observed. Within 1 geriatric ward, three staff members were observed. In the other geriatric ward, two staff members and in the NICU, 1 staff member was observed. For each staff member, cleaning/disinfecting of two rooms each, was observed.

**iii) Protocol**

Before meeting with the support service staff, they were briefed about what would take place. The Director informed them that observation of cleaning and/or disinfection would take place in order to determine actual contact times of cleaning and disinfecting agents on hard surfaces cleaned by the support services staff and this information would only be used to adjust experimental disinfectant exposure times for the Surface Carrier Test.

Prior to the actual cleaning of the rooms, support services staff were asked what the type and concentration of cleaning and/or disinfecting agents that they used to clean the rooms. Objects in the room were recorded as either being present in the room or absent. It was also determined for each object in the room, whether routine cleaning and/or disinfecting was performed on the specific object. At the start of cleaning and/or disinfecting of an object, a pocket-sized timer was used to measure the contact time of the cleaning or disinfecting agent for that object. The end of exposure was determined to be the time when the cleaning and/or disinfecting agent had visibly evaporated from the object or when the object was wiped dry by the support service staff.

**5. Suspension Test**

**i) Bacterial Preparation and Inoculation**

For this part of the project, only the following strains were tested: *A. baumannii*$^{R1}$, *A. baumannii*$^{S1}$, *E. faecium*$^{R2}$, *E. faecium*$^{S1}$, *S. aureus*$^{R2}$, *S. aureus*$^{R3}$, *S. aureus*$^{S1}$, *M. chelonae*$^{R1}$ and *M. chelonae*$^{S1}$. All strains, except *Mycobacterium chelonae* were sub-cultured from the $^{-}$70°C stock onto TSA plates and incubated overnight at 37 °C. The resulting culture was passaged two additional times to ensure that the organism had an optimal growth and metabolic status (87). *S. aureus*$^{R2}$ was additionally sub-cultured onto colistin/naladixic acid (CNA) supplemented with 8 ug / ml of vancomycin (Difco Laboratories, Detroit, Michigan, U.S.A.). This was performed to stimulate the augmentation of cell wall synthesis (55).

Five to ten colonies of an overnight culture on TSA was used to inoculate 5 ml of trypticase soy broth (TSB) (Sigma, St. Louis, Missouri, U.S.A.) (87). The media was then

incubated for four to five hours at 37 °C with mild shaking to promote uniformity and optimization and growth. After incubation, the culture was adjusted to a turbidity equivalent to a 0.5 McFarland turbidity standard (87). Serial 1:10 dilutions in TSB were performed and 100 microlitres of each dilution were spread over the surface of a TSA plate and incubated at 37 degrees overnight to determine the viable, cfu of each strain. The 0.5 McFarland was also diluted 1:100 in TSB supplemented with 10% (v/v) FBS. Ten microlitres of this dilution are used to inoculate the test wells for the suspension test. The final concentration of bacteria in the wells would approximately be $10^5$ cfu/ml (87)

*Mycobacterium* strains were sub-cultured, from the -70 °C stock, onto BA plates. The plates were incubated at 30 °C for 48 hours. It was passaged in this way two additional times. From the 48 hour culture of *M. chelonae*$^{R1}$, a bacterial suspension with turbidity equivalent to a 0.5 McFarland turbidity standard was prepared in TSB.

Five, BA plates of *M. chelonae*$^{S1}$ that had been incubated for 48 hours, were swabbed and suspended in 2 ml of TSB making sure to break up the aggregates of bacteria. This strain exhibited a drier, flakier colony characteristic compared to the *M. chelonae*$^{R1}$ strain. The suspension was vortexed and sonicated for three seconds, two times. The suspension was then allowed to settle for 2 minutes and 1 ml was removed and transferred to a 1 ½ ml microcentrifuge tube and centrifuged at 7000 rpm for 10 minutes at 4 °C. The supernatant was removed and resuspended in 1 ml of TSB. The resuspended culture of *M. chelonae*$^{S1}$ and the 0.5 McFarland suspension of *M. chelonae*$^{R1}$ were serially diluted 1:10 in TSB, 100 ul of the $10^{-3}$ to $10^{-6}$ dilutions were plated onto BA plates (eg. spread plate method used to determine the viable bacterial count) and incubated at 30 °C for 48 hours to determine cfu

present. The resulting concentration of bacteria in the wells should be approximately $10^5$ cfu/ml. These suspensions were also diluted 1:100 in TSB + 10% FBS to use for inoculation of the wells. Ten microlitres were used to inoculate the test wells.

**ii) Assay Media**

The media used in the test wells was TSB(Sigma, St. Louis, Missouri, U.S.A.) prepared as manufacturer's recommendations, supplemented with 10% fetal bovine serum (Gibco, Grand Island, New York, U.S.A.). The FBS was added to provide an organic challenge. ATS-B could not be used for this purpose as it interfered with absorbance determinations.

**iii) Disinfectant Concentration and Exposure**

Glutaraldehyde was prepared according to the manufacturers recommendations and the stock solution was diluted 1:2 with sterile, distilled water to give the first test concentration of glutaraldehyde of 1%. The 5% stock solution of sodium hypochlorite was diluted 1:10 in sterile, distilled water to give the first test concentraion of 0.5% (v/v). Hydrogen peroxide was used directly from the stock solution giving a preliminary hydrogen peroxide concentration of 3% (v/v). Both phenolic and quaternary ammonium were diluted 1:250 with sterile, distilled water as per manufacturers' recommendations.

Growth of each test bacterium in the presence of the disinfectant, excluding *Mycobacterium*, was evaluated over 24 hours. Since *Mycobacterium chelonae* is considerably slower growing, growth in the presence of the test disinfectants was evaluated at 120 hours.

**iv) Positive Controls**

Two types of positive controls were prepared for each assay run. Both positive controls contained only TSB with 10% FBS media and the specific test bacteria. These controls did not contain any disinfectant within the wells. One positive control was placed on the same assay tray as the test, meaning that this positive control was not in direct contact with disinfectant but may be exposed to it, if vaporization or aerosolization of the disinfectant occurred. The second positive control was placed on a separate tray that contained no disinfectant. Both trays were subjected to identical assay conditions.

**v) Equipment**

The Bioscreen C System (Labsystems, Helsinki, Finland) was used to determine growth curves in 100 well assay trays. The growth curve reader was located at the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada. The run conditions chosen for this experiment were taken from the Standard Operational Procedure Manual of the Bioscreen C system and were as follows:

1) Single Wavelength

2) Wideband filter (gives an average of several wavelengths, which is a more robust reading than from a single wavelength)

3) Incubating Temperature of 30 °C for *M. chelonae* strains and 37 °C degrees for the remaining test bacteria

4) No preheating time was used

5) Kinetical Measurement

6) Measurement time of 120 hours for *M. chelonae* and 24 hours for the remaining

test bacteria

7) Interval between turbidity measurements was set at thirty minutes for *M. chelonae* and ten minutes for the remaining test bacteria

8) Medium shaking intensity

9) Continuous shaking regime

**vi) Protocol**

Two, 100 well trays were used for each test bacterial strain against each disinfectant. In one tray, 200 ul of TSB with 10% FBS were added each to three wells. This tray was the positive control with no disinfectants present.

In the other tray, 4 ( 1 blank and 3 replicates) series of 8 vertical wells were prepared with the first well in each series containing no media. Two hundred microlitres of TSB with 10% FBS were added to the following seven wells. Four hundred microlitres of the test disinfectant were then added to the first well of each series. By transferring 200 uls, serial 1:2 dilutions were then made to the following vertical wells in the series. The final 8 specific serial dilutions of each disinfectant are shown in Table 7.

In the same tray, 200 uls. of TSB with 10% FBS was added to three wells located the farthest away from the disinfectants. These wells were our positive controls in the presence of disinfectant. Each well, except the first series, was then inoculated with 10 uls. of the bacterial preparation with a multi-channel pipettor. The first series was used as a blank control for the disinfectant and media. Before inserting the trays into the Bioscreen C apparatus, the bottoms of the trays were wiped with Kimwipes (Kimberly-Clark, Mississauga, Canada) to eliminate any material that might interfere with absorbance readings.

| G | SH | HP | P | QA |
|---|---|---|---|---|
| 1% | 0.5% | 3% | 1:250 | 1:250 |
| 0.5% | 0.25% | 1.5% | 1:500 | 1:500 |
| 0.25% | 0.125% | 0.75% | 1:1000 | 1:1000 |
| 0.125% | 0.06% | 0.4% | 1:2000 | 1:2000 |
| 0.06% | 0.03% | 0.2% | 1:4000 | 1:4000 |
| 0.03% | 0.016% | 0.09% | 1:8000 | 1:8000 |
| 0.016% | 0.008% | 0.04% | 1:16000 | 1:16000 |
| 0.008% | 0.004% | 0.02% | 1:32000 | 1:32000 |

**Table 7: Test Disinfectant Concentrations and Dilutions for the Suspension Test Method**

Test disinfectant was added to the suspension test wells in a series of 8 serial dilutions. The table represents the range of 8 concentrations used for the test disinfectants (G=glutaraldehyde; SH=sodium hypochlorite; HP= hydrogen peroxide) or dilutions used for the phenolic disinfectant (P) and the quaternary ammonium compound (QAC) in the Suspension Test.

## RESULTS

### Section One: Surface Carrier Test

#### i) Experimental Controls

A critical step in assessing disinfectant efficacy is that of neutralization of any residual disinfectant. A disinfectant must be neutralized once the test exposure has terminated in order to prevent a "carry-over" effect where the disinfectant continues to kill organisms despite removal of disinfectant. There have been many neutralizing agents used based on the disinfectant and bacteria tested ( 125, 129, 108). The two neutralizing agents we tested were a phosphate-buffered solution supplemented with 5% Tween 80 (46) and a 10% FBS solution in sterile, distilled water.

The protocol used was identical to the Surface Carrier Test protocol described in the Materials and Methods section except that the sonication period was 5 seconds instead of 2. The test disinfectants included 2% glutaraldehyde, 0.5% sodium hypochlorite and 0.04% hydrogen peroxide and *Staphylococcus aureus* ATCC strain 25923, *Enterococcus faecalis* ATCC strain 2912 and *Escherichia coli* ATCC strain 25922 were the test bacteria. These test bacteria were chosen for their antibiotic susceptibility and would be expected to be the most sensitive to disinfectant killing. *E. coli* ATCC 25922 was chosen for evaluating the effect on Gram-negative organisms. Each bacterial species was tested with each of the disinfectants and each of the potential neutralizing agents. As a control for this preliminary test, the protocol was performed in the absence of a neutralizing agent. Table 8 shows the test bacteria against specific disinfectants with each neutralizing agent. When exposed to glutaraldehyde and sodium hypochlorite, no residual bacteria were detectable when

a)

| | Inoculum | NEUTRALIZING AGENT<br>PBS-Tween 80 | 10% FBS | None |
|---|---|---|---|---|
| *S. aureus* | $1.3 \times 10^7$<br>$\pm 0.3 \times 10^7$ | < ld | < ld | < ld |
| *E. faecalis* | $9.36 \times 10^6$<br>$\pm 0.3 \times 10^6$ | < ld | < ld | < ld |
| *E. coli* | $1.23 \times 10^7$<br>$\pm 0.1 \times 10^7$ | < ld | < ld | < ld |

b)

| | Inoculum | NEUTRALIZING AGENT<br>PBS-Tween 80 | 10% FBS | None |
|---|---|---|---|---|
| *S. aureus* | $1.3 \times 10^7$<br>$\pm 0.3 \times 10^7$ | < ld | < ld | < ld |
| *E. faecalis* | $9.63 \times 10^6$<br>$\pm 0.3 \times 10^6$ | < ld | < ld | < ld |
| *E. coli* | $1.23 \times 10^7$<br>$\pm 0.1 \times 10^7$ | < ld | < ld | < ld |

c)

| | Inoculum | NEUTRALIZING AGENT<br>PBS-Tween 80 | 10% FBS | None |
|---|---|---|---|---|
| *S. aureus* | $1.3 \times 10^7$<br>$\pm 0.3 \times 10^7$ | $2.42 \times 10^4$<br>$\pm 0.3 \times 10^4$ | $4.87 \times 10^5$<br>$\pm 0.8 \times 10^5$ | $1.06 \times 10^4$<br>$\pm 0.4 \times 10^4$ |
| *E. faecalis* | $9.63 \times 10^6$<br>$\pm 0.3 \times 10^6$ | $8.08 \times 10^4$<br>$\pm 0.7 \times 10^5$ | $3.16 \times 10^5$<br>$\pm 0.3 \times 10^6$ | $9.33 \times 10^4$<br>$\pm 0.4 \times 10^5$ |
| *E. coli* | $1.23 \times 10^7$<br>$\pm 0.1 \times 10^7$ | < ld | < ld | < ld |

**Table 8 : Neutralizing Agent Evaluation**

Inoculated, dried lumen carriers containing 10 uls. of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 2912 and *Escherichia coli* ATCC 25922 suspended in artificial test soil were exposed to a) 2% percent glutaraldehyde; b) 0.5% sodium hypochlorite or c) 0.04% hydrogen peroxide for 20 mins. The table represents the viable cfu's/carrier after neutralization with either PBS-Tween 80, 10% FBS or no neutralizing agent. The initial colony forming units inoculated onto each carrier (Inoculum) is also recorded. The results indicate the average of three replicates (± SD) and the limit of detection (ld) is 20 cfu's/carrier.

neutralized with PBS with Tween 80 or 10% FBS. So no evaluation could be made from these specific tests. The only residual bacteria detected was from the hydrogen peroxide experiments.

From this data, we determined that the loss of bacteria when no neutralizing agent was used was approximately 3-4 $\log s_{10}$. The use of PBS with Tween 80 shows this equivalent loss. When 10% FBS was used for neutralization, there was approximately a 2 $\log_{10}$ increase in bacterial survival. Based on this data, we concluded that 10% FBS was a more effective neutralizing agent and it was chosen to be the neutralizing agent for all subsequent surface carrier tests. In the literature, 10% FBS is widely used as a neutralizing agent because it can be used with a wide variety of disinfectants.

*E. coli* ATCC 25922 showed no residual bacteria in any of the neutralizing tests. Since the vortex and sonication of the bacteria were the most physical steps of the protocol, we questioned whether the absence of residual *E. coli* was due to these manipulations. We postulated that *E. coli* that survived and remained on the lumen carrier post-exposure to the disinfectant may have been killed by the sonication steps. Sonication uses ultrasonic frequency waves to remove and separate bacteria. Bacteria must be exposed to this ultrasonic frequency at very low doses as prolonged exposure creates heat that will kill the bacteria. If prolonged exposure must be used, smaller intervals of exposure and a cooling step should be performed.

The sonication was tested by inoculating *E. coli* suspended in ATS onto lumen carriers and dried. The inoculated, dried carrier was placed into 2 ml of 10% FBS and vortexed for 5 minutes and sonicated for either 5 seconds or 2 seconds, twice. The

recoverable bacteria after the 5 and 2 second exposures indicated that a 2 second exposure kills fewer bacteria than the five second exposure. The two second sonication exposure was then incorporated into the Surface Carrier Test protocol.

The Surface Carrier Test protocol involves many steps in which bacteria could be removed from the lumen carrier simply because of the fluid effect. Therefore, controls must be performed to ensure that the viable bacterial count on the carrier is accurate. Bacteria that have been removed from the lumen carrier by fluid exposure, but are not killed by the disinfectant, will be lost when the carrier is removed from the disinfectant and washed. This removable of viable bacteria by the fluid may skew the data and result in conclusions that the disinfectant's efficacy is better than it actually is.

We set up four fluid and procedural controls with the same bacteria used to evaluate the neutralizing agent (Fig. 1). These controls ensure that none of the protocol's fluid exposure steps influence the final, viable, bacterial count. Figure 1 shows a combination of the controls tested. Table 8, provides the data for viable bacteria after the entire protocol was performed using 3 ml of PBS instead of any disinfectant. The results show us that the procedure of Control 1 contributes the most to the loss of bacteria. Since the loss of bacteria is substantial, especially for *E. faecalis* and *E. coli*, this control must be run with every surface carrier test performed to allow evaluation of the fluid effect on the experiment. If the fluid effect is not accounted for, there may be over-interpretation of the disinfectant's efficacy.

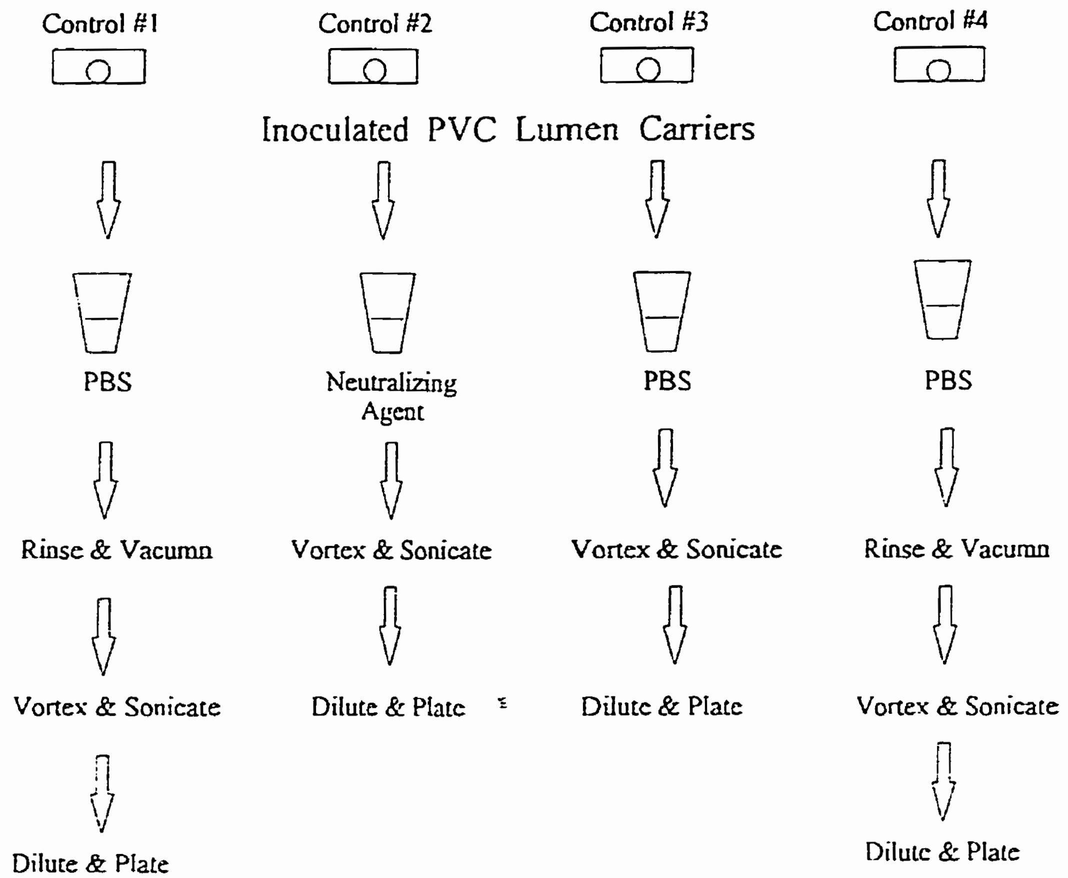



**Figure 1: Schematic Flow-Chart of the Four Surface Carrier Test Controls**

Four different test controls for the Surface Carrier Test were performed to determine which control is most likely to remove the most bacteria from the lumen carrier. In turn, we used this data to account for the fluid effect of the Surface Carrier Test protocol.

| | Inoculum | Control 1 | Control 2 | Control 3 | Control 4 |
|---|---|---|---|---|---|
| *S. aureus* | $1.3 \times 10^7$ $\pm 0.3 \times 10^7$ | $4.0 \times 10^6$ $\pm 0.9 \times 10^6$ | $8.9 \times 10^6$ $\pm 0.2 \times 10^7$ | $7.4 \times 10^6$ $\pm 0.3 \times 10^6$ | $1.7 \times 10^6$ $\pm 0.2 \times 10^5$ |
| *E. faecalis* | $9.6 \times 10^6$ $\pm 0.3 \times 10^6$ | $6.5 \times 10^4$ $\pm 0.8 \times 10^4$ | $1.9 \times 10^7$ $\pm 0.6 \times 10^6$ | $1.9 \times 10^7$ $\pm 0.3 \times 10^7$ | $9.3 \times 10^4$ $\pm 0.2 \times 10^5$ |
| *E. coli* | $1.2 \times 10^7$ $\pm 0.1 \times 10^7$ | < ld | $2.4 \times 10^2$ $\pm 0.3 \times 10^3$ | < ld | < ld |

**Table 9: Evaluation of the Fluid Effect on the Surface Carrier Test**

Lumen carriers were inoculated with 10 uls. of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 2912 and *Escherichia coli* ATCC 25922 suspended in artificial test soil and dried overnight. These inoculated, dried carriers were processed through four controls described in Figure #. The data represent the viable cfu's/carrier for the average of three replicates ($\pm$ SD). The number of cfu's inoculated onto each carrier (Inoculum) is also recorded. The limit of detection (ld) is 20 cfu's/carrier.

**ii) Assay**

**a) *Staphylococcus aureus***

Table 10 shows the results of the Surface Carrier Test of all *S. aureus* test strains with each of the test disinfectants. The minimum initial inoculum of $10^6$ cfu/carrier was achieved for all test strains. The minimum initial inoculum count is important as disinfectants must produce a 6 $\log_{10}$ kill to be considered a high level disinfectant. The recoverable bioburden (RB) values show only a slight fluid effect on the *S. aureus* strains. The decrease in counts is approximately 0.5 to 1 $\log_{10}$ in the controls. There was minimal loss of *S. aureus* strains on the carrier due to the protocol manipulations.

When the test strains were exposed to 2% glutaraldehyde, only *S. aureus*$^{R1}$, S.aureus$^{R4}$ and *S. aureus*$^{R5}$ strains showed detectable, viable bacteria. The residual bacteria were in very low amounts. This experiment was repeated twice with these strains against glutaraldehyde and interestingly, these strains showed variable results. The residual bacterial count for each strain was not consistent. In one experiment, one strain showed low-level survival and when repeated, it did not. This sporadic survival of bacteria may be attributed by the characteristics of glutaraldehyde.

No growth was observed in 0.5% sodium hypochlorite and the QAC in any of the test strains. Growth did occur in 3% hydrogen peroxide with *S. aureus*$^{R1}$, *S. aureus*$^{R4}$ and *S. aureus*$^{R5}$ strains. These are the same strains in which growth was observed in glutaraldehyde. *S. aureus*$^{R1}$, but not *S. aureus*$^{R2}$ showed growth in both 3% hydrogen peroxide and the phenolic compound. This was surprising as the *S. aureus*$^{R2}$ strain is a homogeneous resistant strain and *S. aureus*$^{R1}$ is a heterogeneous resistant strain.

Every test strain showed detectable, viable bacteria when exposed to 0.04% hydrogen peroxide. The residual bacteria ranged from 3 to 4 $\log_{10}$ with the *S. aureus*$^{S1}$ strain showing the lowest value of $2.6 \times 10^3$ and *S. aureus*$^{R2}$ showing the highest residual bacteria at $1.7 \times 10^4$ cfu.

Notable differences in strain resistance are found in 3% hydrogen peroxide. Three multiple resistant strains (*S. aureus*$^{R1}$, *S. aureus*$^{R4}$ and *S. aureus*$^{R5}$) showed residual bacteria while the multiple susceptible along with the remaining multiple resistant strains (*S. aureus*$^{R3}$ and *S.aureus*$^{R2}$) did not.

| | | | DISINFECTANTS TESTED (Concentration or Dilution) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Inoculum** | **RB** | **G (2%)** | **SH (0.5%)** | **HP (3%)** | **HP (0.04%)** | **P (1:250)** | **QAC (1:250)** |
| ***S.aureus*** R1 | $5.7 \times 10^6 \pm 0.5 \times 10^6$ | $2.4 \times 10^6 \pm 0.5 \times 10^6$ | $0.7 \times 10^1 \pm 9.4$ | < ld | $2.0 \times 10^1 \pm 0.1 \times 10^2$ | $4.2 \times 10^3 \pm 0.8 \times 10^3$ | $5.0 \times 10^2 \pm 0.7 \times 10^3$ | < ld |
| ***S.aureus*** R2 | $4.3 \times 10^6 \pm 0.8 \times 10^6$ | $9.0 \times 10^5 \pm 0.3 \times 10^6$ | < ld | < ld | < ld | $1.7 \times 10^4 \pm 0.2 \times 10^4$ | < ld | < ld |
| ***S.aureus*** R3 | $1.7 \times 10^7 \pm 0.3 \times 10^7$ | $2.9 \times 10^6 \pm 0.1 \times 10^7$ | < ld | < ld | < ld | $3.2 \times 10^3 \pm 0.9 \times 10^3$ | < ld | < ld |
| ***S.aureus*** R4 | $7.8 \times 10^6 \pm 0.1 \times 10^7$ | $2.0 \times 10^6 \pm 0.1 \times 10^5$ | $3.0 \times 101 \pm 0.4 \times 10^2$ | < ld | $0.7 \times 10^1 \pm 9.43$ | $6.8 \times 10^3 \pm 0.4 \times 10^4$ | < ld | < ld |
| ***S.aureus*** R5 | $8.9 \times 10^6 \pm 0.1 \times 10^5$ | $9.4 \times 10^5 \pm 0.1 \times 10^5$ | $2.0 \times 101 \pm 0.1 \times 10^2$ | < ld | $2.0 \times 101 \pm 0.2 \times 10^2$ | $9.3 \times 10^3 \pm 0.5 \times 10^4$ | < ld | < ld |
| ***S.aureus*** S1 | $5.6 \times 10^6 \pm 0.8 \times 10^6$ | $3.1 \times 10^6 \pm 0.2 \times 10^7$ | < ld | < ld | < ld | $2.6 \times 10^3 \pm 0.2 \times 10^4$ | < ld | < ld |

**Table 10: Surface Carrier Test of *Staphylococcus aureus* Strains**

Results show the viable cfu's/carrier of each *S. aureus* strain against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP), 0.04% hydrogen peroxide ( HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates (± SD). The limit of detection (ld) is 20 cfu's/carrier.

**b) *Enterococcus faecium***

The Surface Carrier Test results for *E. faecium* test strains are found in Table 11. The four test strains attained the minimal initial inoculum count of $10^6$ cfu/carrier. The control (RB) showed a decrease from the initial inoculum bacterial counts. A decrease of approximately 1.5 - 2 $\log_{10}$ of bacteria were observed. The three multiple resistant strains (*E. faecium*$^{R1}$, *E. faecium*$^{R2}$ and *E. faecium*$^{R3}$) showed more loss of bacteria in the control than the multiple susceptible *E. faecium*$^{S1}$ strain. The recoverable bioburden values were lower than those for the *S. aureus* strains even though the initial inoculum of *E. faecium* was higher. This suggests that the multiple antibiotic resistant *E. faecium* test strains adhere less to the lumen carrier than any of the *S. aureus* test strains. It is also interesting to note that both multiple antibiotic susceptible strains of *S. aureus* and *E. faecium* showed decreased removal from the lumen carrier compared to all of the multiple antibiotic resistant strains. None of the *E. faecium* strains showed viable bacteria, post-exposure to 2% glutaraldehyde, 0.5% sodium hypochlorite, 3% hydrogen peroxide or the quaternary ammonium compound. These disinfectants promote at least a 5 $\log_{10}$ kill in all test strains. These disinfectants showed more killing activity against the *E. faecium* than *S. aureus* strains.

The phenolic based disinfectant was effective against the multiple antibiotic resistant *E. faecium*$^{R3}$ strain and the susceptible *E. faecium*$^{S1}$ strain. The two other resistant strains, *E. faecium*$^{R2}$ and *E. faecium*$^{R1}$ showed survival in the phenolic disinfectant. The *E. faecium*$^{R1}$ strain showed survival of less than 10 cfu/carrier while *E. faecium*$^{R2}$ showed 2 $\log_{10}$ of survival.

Once again, as with the *S. aureus* strains, the 0.04% hydrogen peroxide was less

effective compared to 3% hydrogen peroxide. The 0.04% concentration of hydrogen peroxide only produced a 2.5 - 3 $\log_{10}$ kill. From the data in Tables 10 and 11, the *S. aureus* strains appeared more resistant to hydrogen peroxide killing compared to the *E. faecium* strains tested.

Notable resistance differences occur in the phenolic compound. *E. faecium*$^{R2}$ and *E. faecium*$^{R1}$ show slight resistance to the disinfectant while the other multiple antibiotic resistant strain, *E. faecium*$^{R3}$ and *E. faecium*$^{S1}$ do not.

| | | | DISINFECTANTS TESTED (Concentration or Dilution) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inoculum | RB | G (2%) | SH(0.5%) | HP(3%) | HP(0.04%) | P(1:250) | QAC(1:250) |
| *E. faecium*$^{R1}$ | $1.3 \times 10^7 \pm 0.2 \times 10^7$ | $6.3 \times 10^5 \pm 0.2 \times 10^6$ | < ld | < ld | < ld | $1.4 \times 10^3 \pm 0.2 \times 10^4$ | $0.7 \times 10^1 \pm 0.9 \times 10^1$ | < ld |
| *E. faecium*$^{R2}$ | $1.2 \times 10^7 \pm 0.2 \times 10^7$ | $4.2 \times 10^5 \pm 0.1 \times 10^5$ | < ld | < ld | < ld | $1.1 \times 10^3 \pm 0.1 \times 10^4$ | $9.1 \times 10^2 \pm 0.1 \times 10^4$ | < ld |
| *E. faecium*$^{R3}$ | $1.2 \times 10^7 \pm 0.3 \times 10^7$ | $2.3 \times 10^5 \pm 0.3 \times 10^6$ | < ld | < ld | < ld | $1.2 \times 10^3 \pm 0.1 \times 10^4$ | < ld | < ld |
| *E. faecium*$^{S1}$ | $8.3 \times 10^6 \pm 0.1 \times 10^7$ | $1.8 \times 10^5 \pm 0.1 \times 10^6$ | < ld | < ld | < ld | $5.8 \times 10^2 \pm 0.4 \times 10^3$ | < ld | < ld |

**Table 11: Surface Carrier Test of *Enterococcus faecium* Strains**

Results show the viable cfu's/carrier of each *E. faecium* strain against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP), 0.04% hydrogen peroxide (HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates(±SD). The limit of detection (ld) is 20 cfu's/carrier.

c) *Mycobacterium chelonae*

The Surface Carrier results for the *M. chelonae* strains are found in Table 12. The initial inoculum counts of both strains attained the 6 $\log_{10}$ minimum count. However, an initial suspension of $10^8$ cfu in ATS was difficult as homogeneous suspensions were difficult to achieve. The RB values indicate a fluid effect decrease from the initial inoculum counts of approximately 1.5 - 2 $\log s_{10}$. This is comparable to the loss observed in *E. faecium* strains.

No detectable, viable bacteria was observed when both *M. chelonae* strains were exposed to sodium hypochlorite and 3% hydrogen peroxide. In 2% glutaraldehyde, the *M. chelonae*$^{S1}$ strain showed no detectable, viable residual bacteria. The *M. chelonae*$^{R1}$ strain showed survival of approximately 2 $\log s_{10}$ of bacteria. This is not surprising considering the *M. chelonae*$^{R1}$ strain was discovered because of it's reduced susceptibility to glutaraldehyde. Residual bacteria were observed in 0.04% hydrogen peroxide, phenolic disinfectant and the quaternary ammonium compound.

| | Inoculum | RB | DISINFECTANTS TESTED (Concentration or Dilution) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | G(2%) | SH(0.5%) | HP(3%) | HP(0.04%) | P(1:250) | QAC(1:250) |
| *M. chelonae* R1 | $2.7 \times 10^6$ $\pm 0.1 \times 10^7$ | $3.3 \times 10^4$ $\pm 0.2 \times 10^5$ | $3.6 \times 10^2$ $\pm 0.1 \times 10^3$ | < ld | < ld | $1.8 \times 10^3$ $\pm 0.1 \times 10^4$ | $2.7 \times 10^4$ $\pm 0.1 \times 10^5$ | $1.8 \times 10^2$ $\pm 0.4 \times 10^2$ |
| *M. chelonae* S1 | $1.6 \times 10^6$ $\pm 0.8 \times 10^6$ | $2.8 \times 10^5$ $\pm 0.6 \times 10^5$ | < ld | < ld | $2.6 \times 10^1$ $\pm 0.4 \times 10^2$ | $2.5 \times 10^3$ $\pm 0.4 \times 10^3$ | $1.1 \times 10^5$ $\pm 0.1 \times 10^6$ | $1.9 \times 10^4$ $\pm 0.2 \times 10^5$ |

**Table 12: Surface Carrier Test of *Mycobacterium chelonae* Strains**

Results show the viable cfu's/carrier of each *M. chelonae* strain against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide ( HP), 0.04% hydrogen peroxide (HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants. The number of colony forming units inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates (± SD). The limit of detection (ld) is 20 cfu's/carrier.

**d) *Acinetobacter baumannii***

Table 13 shows the Surface Carrier Test results of *Acinetobacter baumannii*. The approximate initial inoculum is $10^6$ cfu/carrier. For all three test strains evaluated, the loss of the bacteria resulted in a recoverable bioburden of only 3 - 5 $\log s_{10}$ of bacteria. The fluid effect was most evident in the two multiple antibiotic resistant strains. The experiment was repeated to ensure reproducibility and the repeated experiment showed the same results. The loss of bacteria was not due to death during overnight drying as viable bacterial counts on dried carriers showed that counts were stable. *Acinetobacter baumannii* does not seem to adhere to the lumen carrier as well as the other test strains and when manipulated in the control procedures, there was substantial loss of bacteria due to the fluid effect. There were no viable bacteria detected, post-exposure to any of the disinfectants for the *A. baumannii*$^{R1}$ strain while low-level bacterial survival was observed when *A. baumannii*$^{R2}$ and *A. baumannii*$^{S1}$ exposed to 0.04% hydrogen peroxide.

| | Inoculum | RB | G(2%) | SH(0.5%) | HP(3%) | HP(0.04%) | P(1:250) | QAC(1:250) |
|---|---|---|---|---|---|---|---|---|
| | | | **DISINFECTANTS TESTED (Concentration or Dilution)** | | | | | |
| *A. baumannii* [R1] | $2.9 \times 10^6 \pm 0.4 \times 10^6$ | $1.3 \times 10^3 \pm 0.3 \times 10^4$ | < ld | < ld | < ld | < ld | < ld | < ld |
| *A. baumannii* [R2] | $1.1 \times 10^6 \pm 0.8 \times 10^5$ | $2.8 \times 10^3 \pm 0.5 \times 10^3$ | < ld | < ld | < ld | $0.1 \times 10^1 \pm 0.2 \times 10^2$ | < ld | < ld |
| *A. baumannii* [S1] | $1.9 \times 10^6 \pm 0.5 \times 10^5$ | $2.8 \times 10^5 \pm 0.3 \times 10^5$ | < ld | < ld | < ld | $0.1 \times 10^1 \pm 0.2 \times 10^2$ | < ld | < ld |

**Table 13: Surface Carrier Test of *Acinetobacter baumannii***

Results show the viable cfu's/carrier of each *A. baumannii* strain against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP), 0.04% hydrogen peroxide (HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates ($\pm$ SD). The limit of detection (ld) is 20 cfu's/carrier.

**e) *Alcaligenes xylosoxidans***

The Surface Carrier Test results are shown in Table 14. The results of *A. xylosoxidans* show distinct similarities to *A. baumannii* with respect to the recoverable bioburden values. The initial inoculum for both strains attained and exceeded $1 \times 10^6$ cfu/carrier. The difficulties arose when both strains were subjected to our procedural control. Again, substantial losses in bacterial numbers occurred due to the fluid effect. There were only 2 - 3 $\log_{10}$ of bacteria in the recoverable bioburden. Again, this experiment was repeated to test for the viability of these bacteria to overnight drying. The bacteria did survive the overnight drying process but do not adhere as well to the lumen carrier as *S. aureus, E. faecium* and *M. chelonae*. For both test strains, no viable bacteria were detected after exposure to any of the test disinfectants.

| | | | DISINFECTANTS TESTED (Concentration or Dilution) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Inoculum | RB | G(2%) | SH(0.5%) | HP(3%) | HP(0.04%) | P(1:250) | QAC(1:250) |
| *A. xylosoxidans* $_{RI}$ | $7.2 \times 10^6$ $\pm 0.2 \times 10^7$ | $7.9 \times 10^2$ $\pm 0.5 \times 10^2$ | < ld | < ld | < ld | < ld | < ld | < ld |
| *A. xylosoxidans* $_{SI}$ | $9.9 \times 10^6$ $\pm 0.3 \times 10^6$ | $1.2 \times 10^3$ $\pm 0.3 \times 10^3$ | < ld | < ld | < ld | < ld | < ld | < ld |

**Table 14: Surface Carrier Test of *Alcaligenes xylosoxidans***

Results show the viable cfu's/carrier of each *A. xylosoxidans* strain against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP), 0.04% hydrogen peroxide (HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates ($\pm$ SD). The limit of detection (ld) is 20 cfu's/carrier.

## Section Two: In-Use Disinfection Practices

The Surface Carrier Test procedure performed in this study uses a disinfectant exposure time based on the manufacturer's recommendations. The exposure times ranged from 10 - 20 minutes. The recommended exposure time for the disinfection of hospital surfaces is most often not achieved due to reasons of time (eg. many hospital rooms have to be disinfected/cleaned in a certain amount of time). This lack of time compromises the disinfectant's killing efficacy. The purpose of the in-use disinfection practices survey was to determine realistic exposure times of disinfectants used on hospital surfaces by hospital cleaning personnel. This data was used to modify the exposure time of the Surface Carrier Test method to clearly evaluate the efficacy of disinfectants at these reduced exposure times.

Observation of the cleaning of patient rooms has given much information on the cleaning practices of the test hospital. The main disinfectant used in all the rooms is a hydrogen peroxide-based disinfectant with a stock concentration of 3%. The stock solution is diluted to a 0.045% when used. Many of the rooms are cleaned when the opportunity arises. The cleaning personnel choose the room which is the most accessible at the time. The room may be empty or it may be occupied by a patient, staff or visitors. This makes room cleaning a non-standard process. If a patient room is unoccupied, cleaning procedures were standard but when the room was occupied, many alterations in the methods were observed. When the bathrooms were not in use, the cleaning personnel usually sprayed the sink and toilet with the disinfectant and let it soak until the rest of the room had been cleaned. If the bathroom was occupied, the bathroom was cleaned last and the objects in the bathroom would not be subjected to the disinfectant pre-soak as was observed for cleaning the

unoccupied bathrooms. This leads to great variations in exposure times for objects in the bathroom. Similarily, when the patients' beds were not occupied, the cleaning personnel may spray and pre-soak the bed but this was rarely performed when the patient was in the room. These factors greatly influence all disinfectant exposure times.

Most of the rooms observed were not for patients on isolation precautions. One room that was observed was occupied by a patient on MRSA isolation precautions. There was one dedicated cleaning personnel assigned to this particular room. Isolation precautions included the use of gowns, gloves and masks by cleaning personnel. The disinfectant used in this particular room was identical to the one used in other rooms except that the concentration was increased to 0.1%. All disinfectants and cleaning equipment were required to remain in that particular room so that possible contamination to other areas of the hospital was avoided. The disinfection exposure times observed in the hospital wards are shown in Table 15. The table only represents the objects that were routinely cleaned in each room. There were other patient care objects that were not routinely cleaned in every room and those have been excluded from the table. A complete list of objects that were observed to be cleaned has been included in the sample questionnaire found in the Appendix II. The data in the table were used to determine which exposure times would reflect the routine disinfectant exposure times found in the hospital setting. The most common, longest and shortest exposure times were taken from the in-use disinfection data and used in the Modified Surface Carrier Test against the *E. faecium*$^{R2}$ and *E. faecium*$^{S1}$ strains. According to Table 14, the most common and the longest exposure time was approximately 10 minutes. The most common and the shortest exposure time was approximately 1 minute, so the Modified Surface Carrier Test was

performed with all test disinfectants and the two *E. faecium* strains at an exposure time of 10 minutes and 1 minute.

**Exposure to Disinfectants (seconds)**

| | P. floor | Table | Sink 1 | Sink 2 | Toilet 1 | Toilet 2 | B. floor |
|---|---|---|---|---|---|---|---|
| Room 1 | 600 | 5 | 60 | 120 | 60 | 300 | 480 |
| Room 2 | 600 | 120 | 10 | 300 | 10 | 300 | 600 |
| Room 3 | 300 | 60 | 600 | 60 | 600 | 600 | 180 |
| Room 4 | 300 | 180 | 480 | 60 | 480 | 60 | 180 |
| Room 5 | 420 | 180 | 900 | 60 | 300 | 300 | 300 |
| Room 6 | 720 | 120 | 960 | 10 | 960 | 10 | 1020 |
| Room 7 | 600 | 60 | 180 | 180 | 300 | 300 | 480 |
| Room 8 | 600 | 60 | 240 | 240 | 60 | 60 | 480 |
| Room 9 | 360 | 60 | 60 | 60 | 60 | 60 | 300 |
| Room 10 | 420 | 30 | 300 | 60 | 60 | 60 | 480 |
| Room 11 | 300 | 300 | 300 | 300 | n/a | n/a | 300 |

**Table 15: In-Use Disinfection Practices**

Exposure times (seconds) are shown for each different hospital room observed. The objects represent a selection of objects that are routinely cleaned in each room. The patient's room floor (P.floor), Patient bedtable (Table), Bathroom sink fixtures (Sink 1), Bathroom sink basin (Sink 2), Toilet stand (Toilet 1), Toilet bowl (Toilet 2) and Bathroom Floor (B. floor) were all represented.

**Section Three: Modified Surface Carrier Test: In-Use Exposure Times**

**i) Ten Minute Exposure**

The 10 minute exposure Surface Carrier Test results are shown in Table 16. The table only shows the results of exposure to glutaraldehyde, sodium hypochlorite, 3% hydrogen peroxide and 0.04% hydrogen peroxide. The phenolic and QAC compounds were not included because the 10 minute exposure times for these disinfectants had already been performed and recorded in Table 11.

The initial inoculum counts exceed $1 \times 10^6$ cfu/carrier for both test strains evaluated. The RB data indicated there was not much fluid effect, as there was only a slight decrease in levels, approximately 1 $\log_{10}$, from the initial inoculum values. After a 10 minute exposure, no detectable, viable growth was observed in 2% glutaraldehyde, 0.5% sodium hypochlorite and 3% hydrogen peroxide. Residual bacteria were observed, in both strains, after 0.04% hydrogen peroxide exposure.

**ii) One Minute Exposure**

The 1 minute exposure (Table 17) showed that there was greater survival of *E. faecium* for all test disinfectants evaluated. The initial inoculum and recoverable bioburden values were comparable to the 1 minute exposure values. The fluid contact between 1 minute and 10 minutes did not alter the recoverable bioburden values. Two percent glutaraldehyde showed no detectable, viable growth for both *E. faecium* strains.

Upon exposure to 0.5% sodium hypochlorite, we observed residual bacteria in both strains but the amount of residual bacteria was quite different. *E. faecium*$^{R2}$ strain showed $1.2 \times 10^5$ cfu/carrier of residual bacteria, however, *E. faecium*$^{S1}$ shows a 4 $\log_{10}$ higher

| | Inoculum | RB | G(2%) | SH(0.5%) | HP(3%) | HP(0.04%) |
|---|---|---|---|---|---|---|
| | | | DISINFECTANTS TESTED (Concentration) | | | |
| *E. faecium* [R2] | $3.4 \times 10^6$ $\pm 0.5 \times 10^6$ | $7.8 \times 10^5$ $\pm 0.8 \times 10^6$ | < ld | < ld | < ld | $3.4 \times 10^5$ $\pm 0.5 \times 10^6$ |
| *E. faecium* [S1] | $3.0 \times 10^6$ $\pm 0.5 \times 10^6$ | $2.7 \times 10^5$ $\pm 0.3 \times 10^6$ | < ld | < ld | < ld | $2.5 \times 10^4$ $\pm 0.3 \times 10^5$ |

**Table 16: Modified Surface Carrier Test of *Enterococcus faecium* at 10 Minute Exposure to Test Disinfectants**

Results show the viable cfu's/carrier of two *E. faecium* strains against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP) and 0.04% hydrogen peroxide (HP) disinfectants for an exposure time period of 10 minute. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates (± SD). The limit of detection (ld) is 20 cfu's/carrier.

| | Inoculum | RB | DISINFECTANTS TESTED (Concentration or Dilution) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | G(2%) | SH(0.5%) | HP(3%) | HP(0.04%) | P(1:250) | QAC(1:250) |
| *E. faecium* R2 | $4.7 \times 10^6$ $\pm 0.2 \times 10^6$ | $2.6 \times 10^5$ $\pm 0.2 \times 10^6$ | < ld | $4.7 \times 10^1$ $\pm 0.7 \times 10^2$ | $1.7 \times 10^4$ $\pm 0.1 \times 10^5$ | $9.6 \times 10^4$ $\pm 0.3 \times 10^5$ | $3.9 \times 10^5$ $\pm 0.4 \times 10^6$ | < ld |
| *E. faecium* S1 | $5.4 \times 10^6$ $\pm 0.9 \times 10^6$ | $3.9 \times 10^5$ $\pm 0.2 \times 10^6$ | < ld | $1.2 \times 10^5$ $\pm 0.2 \times 10^6$ | $1.0 \times 10^4$ $\pm 0.5 \times 10^4$ | $8.1 \times 10^4$ $\pm 0.2 \times 10^5$ | $3.1 \times 10^5$ $\pm 0.4 \times 10^6$ | $1.3 \times 10^1$ $\pm 0.2 \times 10^1$ |

**Table 17: Modified Surface Carrier Test of *Enterococcus faecium* at 1 Minute Exposure to Test Disinfectants**

Results show the viable cfu's/carrier of two *E. faecium* strains against 2% glutaraldehyde (G), 0.5% sodium hypochlorite (SH), 3% hydrogen peroxide (HP), 0.04% hydrogen peroxide (HP), phenolic (P) and quaternary ammonium compound (QAC) disinfectants for an exposure time period of 1 minute. The number of cfu's inoculated onto each carrier (Inoculum) and recoverable bioburden control counts per carrier (RB) are also recorded. Values are averages of three replicates (± SD). The limit of detection (ld) is 20cfu's/carrier.

survival rate compared to the *E. faecium*$^{R2}$ strain. The resistance of *E. faecium*$^{S1}$ to sodium hypochlorite at this exposure time is interesting considering no residual growth was observed with any of the strains at the 20 and10 minute exposures.

In 3% hydrogen peroxide, we observed residual bacteria for both strains. This enforces the fact that recommended exposure times are very important because no bacterial growth was detected at the ten and twenty minute exposure times. Residual growth in 0.04% hydrogen peroxide after 1 minute exposure, are comparable to the 10 minute exposure and only slightly higher than the 20 minute exposure. Growth in the phenolic compound was slightly higher than observed in the 10 minute exposure. The residual bacteria observed in the QAC exposed bacteria increased slightly from the 10 minute exposure.

Regarding the values of residual bacteria of the two strains, the only disinfectant that showed significant differences was 0.5 % sodium hypochlorite. At this concentration of disinfectant, *E. faecium*$^{S1}$ strain seems to be more resistant than the multiple antibiotic resistant *E. faecium*$^{R2}$ strain

**Section Four: Suspension Test**

**i) Assay Standardization**

To assess the specific conditions in which the suspension test was to be performed, several preliminary tests were needed to determine which media would promote the growth of each test bacteria and the disinfectant concentrations that would be used.

Performing the suspension test in the presence of an organic load was an important factor because we wanted to simulate the hospital environment. Ideally, use of ATS for both the suspension and surface carrier tests would have been preferred. However, ATS interfered with absorbance measurements in the suspension test. Therefore, TSB supplemented with 10% fetal bovine serum was evaluated. The test bacteria were initially grown in this media and the growth assay was also performed using this media. The trypticase soy broth supplemented with 10% FBS could support the growth of the test bacteria and the FBS provided an organic load.

In the surface carrier test method, disinfectant concentration was chosen based on the manufacturers recommendations. In the suspension test method, multiple concentrations were being tested. Initial concentrations tested were 2% glutaraldehyde, 5% sodium hypochlorite, 3% hydrogen peroxide and 1:250 dilutions of the phenolic and QAC compounds. The starting disinfectant concentrations were then serially diluted, 1:2, for a series of 8 dilutions. The positive control growth curves, on the same tray as the test disinfectant, showed minimal growth for glutaraldehyde and sodium hypochlorite. This suggested that the presence of 2% glutaraldehyde and 5% sodium hypochlorite had detrimental effects on the growth of the positive control and therefore may have an effect on

the growth of test bacteria in the wells that contained lower concentrations of disinfectant. These detrimental effects could have been due to aerosolization of the disinfectant due to shaking during incubation or to natural vaporization of the disinfectant into adjacent wells. Lower starting concentrations of glutaraldehyde and sodium hypochlorite were tested to determine if what concentrations could be used without affecting the growth of bacteria in surrounding wells. Glutaraldehyde at 1% (w/v) and sodium hypochlorite at 0.5% (v/v) did not affect the growth of the internal positive control and therefore these starting concentrations were used in the suspension assay.

Due to the findings of the effect of possible disinfectant vaporization and aerosolization on the assay, two positive controls were set up for each disinfectant tray being tested. The first control was situated on the same tray as the disinfectant and the other control, on a separate tray that did not contain any disinfectant in any of the wells. If the positive control situated on the disinfectant assay tray showed decreased growth compared to the positive control situated on the separate tray, a repeat run was performed at lower disinfectant concentrations. The effect of the disinfectant was determined with each assay run. The positive control situated on the tray that contained disinfectant must show similar growth compared with the positive control on the separate tray otherwise, the test data could not be interpreted.

Since *Mycobacterium chelonae* is slow growing, the 24 hour observation period used with the other test bacteria would probably not be adequate. Preliminary tests using 24, 72 and 120 hours of monitoring were conducted. Upon 5 day (120 hours) observation, we were able to identify all three phases of growth. The growth curves generated indicated that a 5

day time frame produced optimal growth curves for *M. chelonae*. Therefore, all subsequent suspension test assays using *M. chelonae* were performed over a 5 day period.

There were a total of eight concentrations of each test disinfectant tested for each test strain. Because the area of interest was to determine where bacterial outgrowth occurred, only the highest and lowest concentrations of disinfectant and the highest concentration where bacterial growth occurred as well as the next dilution where no outgrowth occurred have been presented.

**ii) Assay**

**a) *Staphylococcus aureus***

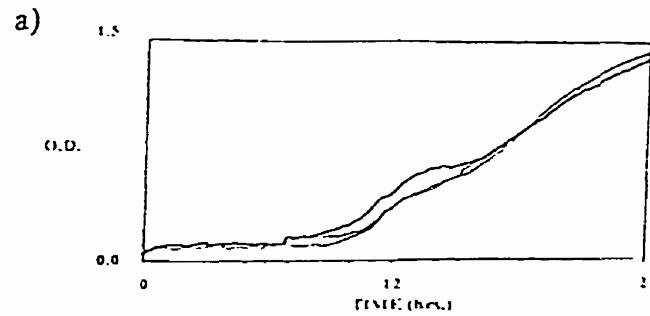
The three *S. aureus* test strains included two multi-resistant strains, *S. aureus*$^{R2}$ and *S. aureus*$^{R3}$, and 1 multi-sensitive (*S. aureus*$^{S1}$) strain. Figure 2, 4 and 6 show the positive growth controls of each strain when tested in conjunction with glutaraldehyde. All test wells were inoculated with approximately equal numbers of bacteria as shown by the inoculum cell counts but different maximum optical densities were achieved by the different test strains. Both *S. aureus*$^{S1}$ and *S. aureus*$^{R2}$ strains, showed a maximal optical density within the 24 hour observation of about 1.4 absorbance units (a.u.) while the *S. aureus*$^{R3}$ strain only showed a maximum optical density of 0.7 a.u. Each strain exhibited typical lag and logarithmic phases, but the stationary phase of growth was only observed in the *S. aureus* $^{R3}$ strain. Although different optical densities were observed between the different test strains, optical densities did not differ between the positive growth control which was situated in the disinfectant tray and the positive growth control which was on a separate tray, within the same test strain.

The $OD_{50}$ was chosen by us to denote the time in which the half of the maximum

optical density occurs. This gives us a value in which we can evaluate and compare the growth of various strains. The OD50 of *S. aureus*$^{R2}$ was approximately 19 hours, while the OD50 of the *S. aureus*$^{R3}$ strain was approximately 13.5 hours and 16.5 hours for the *S. aureus*$^{S1}$ strain. These values were consistent for both positive growth controls for each strain again indicating that the concentration of glutaraldehyde present did not have a vapor effect on surrounding wells in the tray. The lag phase of each strain was also quite similar. Lag phases for all strains were approximately 10-11 hours, post-inoculation.

The growth curves for the internal and external positive controls for each strain were identical with respect to maximum optical density, OD50 and duration of the lag phase therefore, the results from suspension testing in glutaraldehyde could be analyzed. Each test strain was exposed to identical concentrations of glutaraldehyde ranging from 1% to 0.008%. Figure 3, 5 and 7 show the growth curves of each bacterial test strain. *S. aureus*$^{R2}$ showed no detectable growth at 1% glutaraldehyde (Fig. 3) and the highest concentration of glutaraldehyde that this strain shows any outgrowth is at the lowest concentration of glutaraldehyde, 0.008%. No outgrowth was also detected in 1% glutaraldehyde with the *S. aureus*$^{R3}$ strain (Fig. 5). Growth did occur in 0.008% glutaraldehyde (Fig.5). The *S. aureus*$^{S1}$ strain also showed no growth in the 1% glutaraldehyde (Fig.7) but showed considerable growth at the 0.06% to 0.008 % glutaraldehyde suspension (Fig.7). In summary, outgrowth occurred in *S. aureus*$^{R2}$, *S. aureus*$^{R3}$ and *S. aureus*$^{S1}$ strains in 0.008%, 0.016% and 0.06% glutaraldehyde respectively

a)




b)

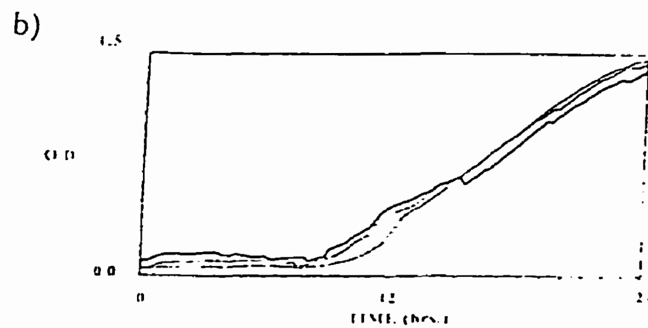



**Figure 2: Positive Control Growth Curve of *Staphylococcus aureus* $^{R2}$ Exposed to Glutaraldehyde**

Growth of *S. aureus* $^{R2}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

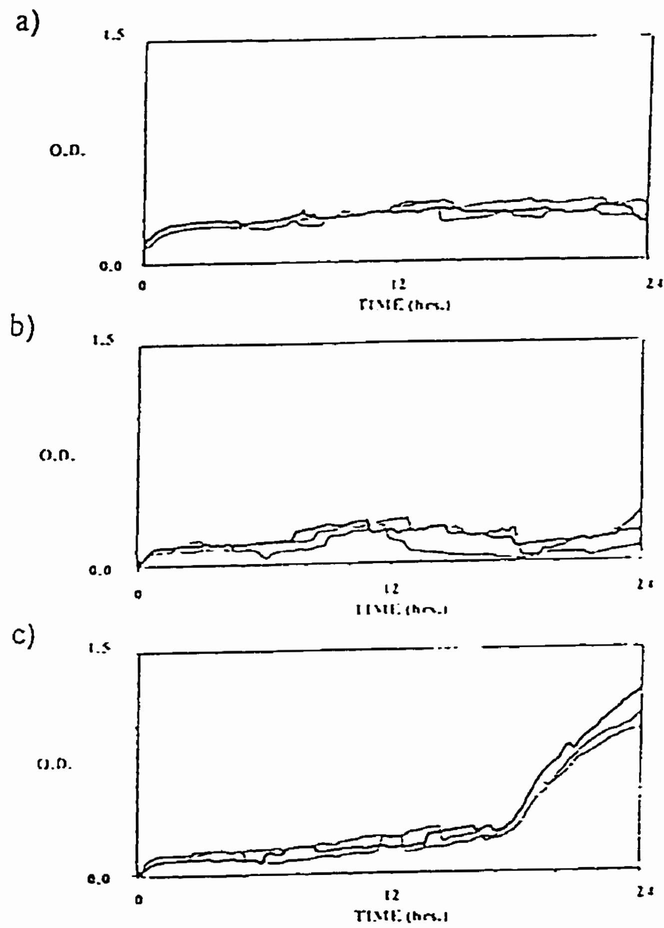
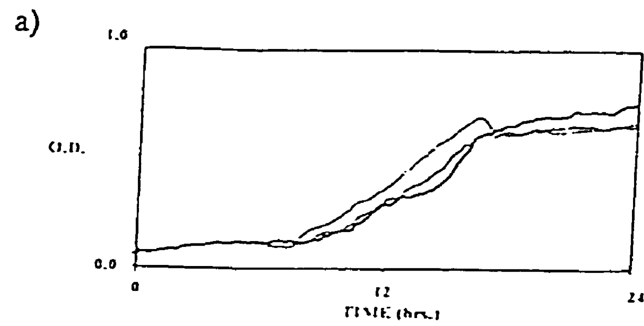



**Figure 3: Growth Curve of *Staphylococcus aureus*[R2] Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *S. aureus*[R2] was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.016 and c)0.08% glutaraldehyde concentrations.

a)




b)

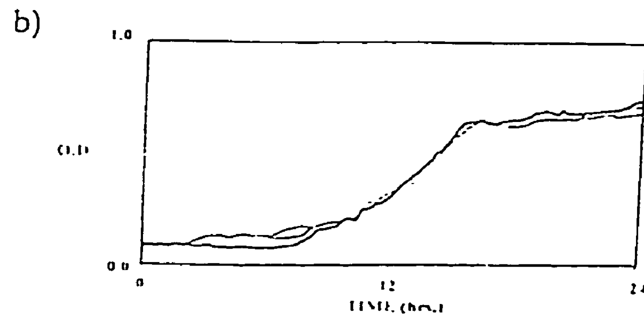



**Figure 4: Positive Control Growth Curve of *Staphylococcus aureus* $^{R3}$ Exposed to Glutaraldehyde**

Growth of *S. aureus* $^{R3}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

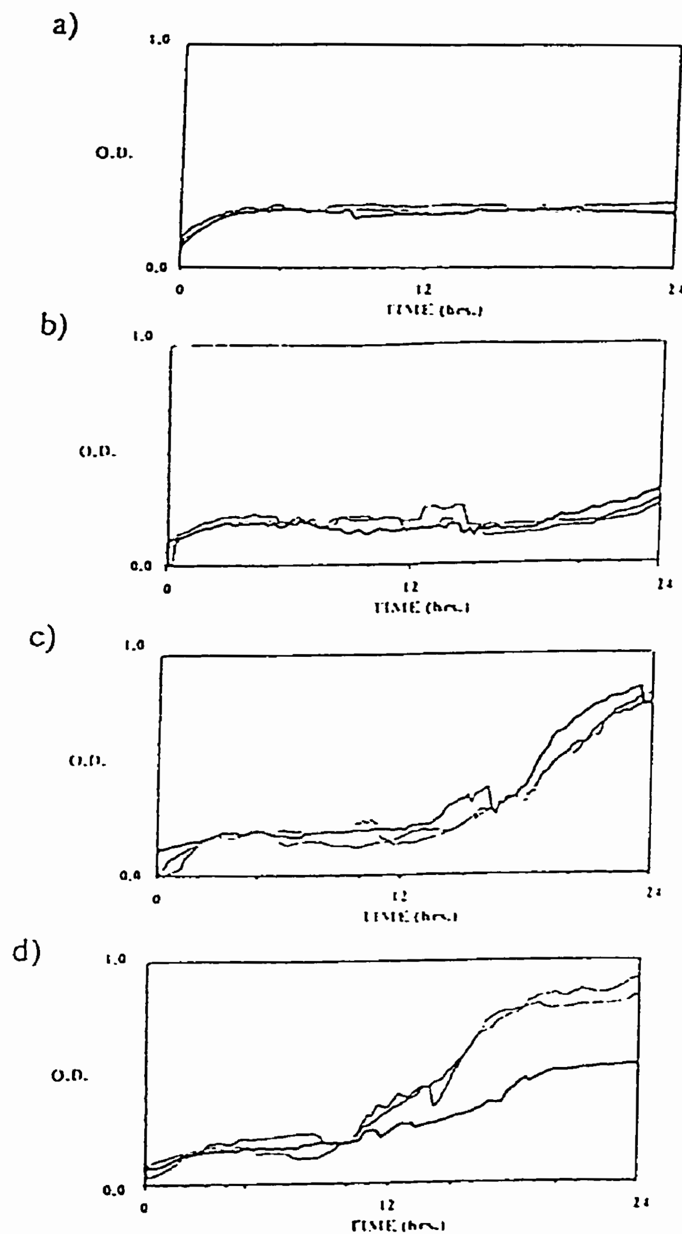
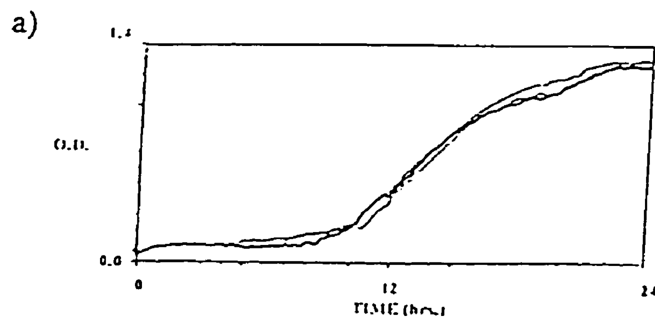



**Figure 5: Growth Curve of *Staphylococcus aureus* $^{R3}$ Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *S. aureus* $^{R3}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.03%; c)0.016% and d) 0.008% glutaraldehyde concentrations.

a)




b)

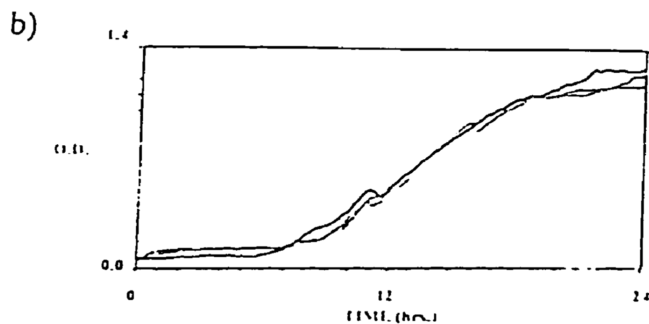



**Figure 6: Positive Control Growth Curve of *Staphylococcus aureus* [S1] Exposed to Glutaraldehyde**

Growth of *S. aureus* [S1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

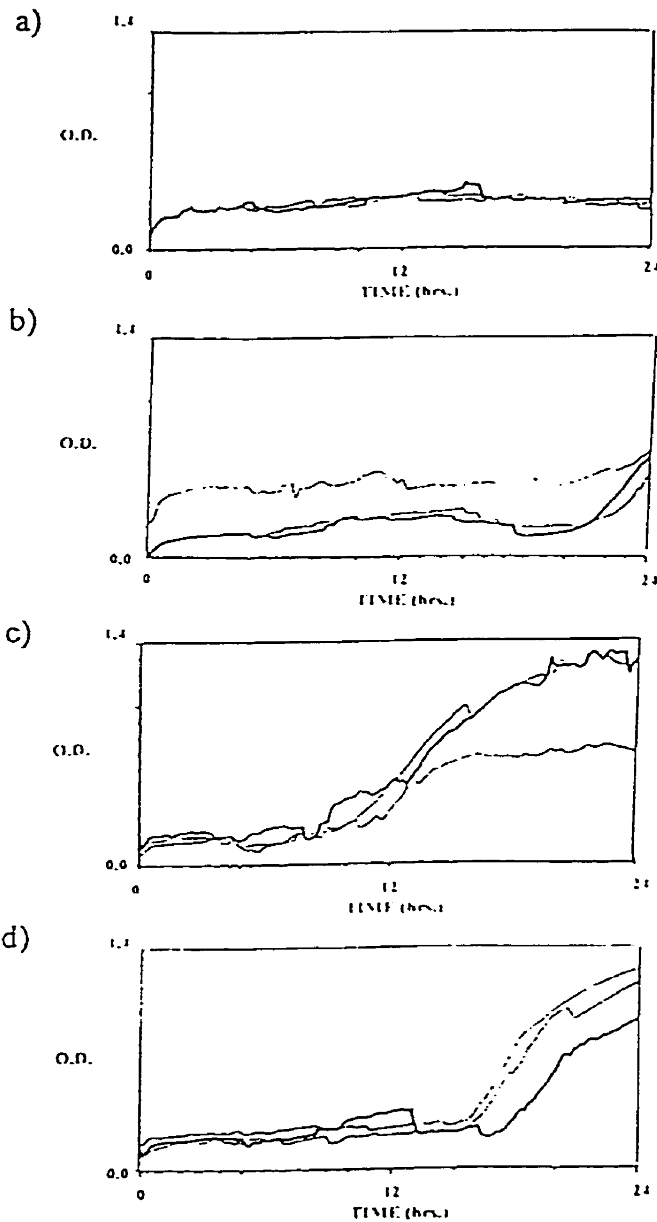



**Figure 7: Growth Curve of *Staphylococcus aureus*$^{S1}$ Exposed to Varied Concentrations of Glutaraldehyde**

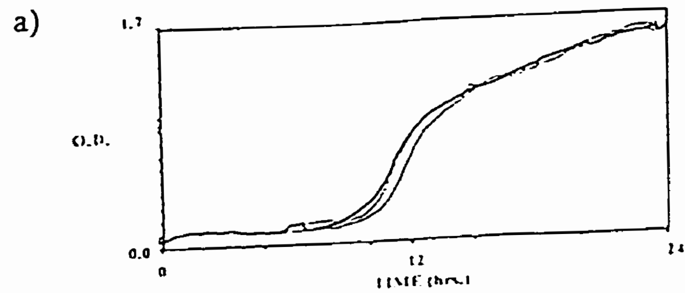
Growth of *S. aureus*$^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.125%; c)0.06% and d) 0.008% glutaraldehyde concentrations.

(Table 16). The duration of the lag phases of each test strain were increased upon exposure to glutaraldehyde indicating the increased time needed for the test bacteria to overcome the effects of the disinfectant.

The next disinfectant tested was sodium hypochlorite. The two positive controls performed for each test strain were quite similar to each other as well as compared to the other strains (Fig. 8, 10, and 12). The average maximum optical density for *S. aureus*$^{R2}$ was 1.6 a.u., for *S. aureus*$^{R3}$, it was 1.4 a.u. and for the *S. aureus*$^{S1}$ strain, the average maximum optical density was 1.3 a.u. All positive controls showed typical lag and logarithmic phases. The stationary phase was not observed during the 24 hour assay. The comparable positive control growth curves within each test strain allowed us to evaluate the sodium hypochlorite suspension results.

Sodium hypochlorite was tested with an initial concentration of 0.5%. The lowest concentration was 0.004%. All test strains showed no growth in 0.5% sodium hypochlorite while *S. aureus*$^{R2}$ first showed growth in 0.016%, *S. aureus*$^{R3}$ in 0.004% and the *S. aureus*$^{S1}$ strain in 0.06% sodium hypochlorite. Upon exposure to sodium hypochlorite and where growth was detectable, the lag phases of each test strain did not seem to differ compared to the positive control indicating that the test strains were able to overcome the effects of the disinfectant at that specific concentration.

a)




b)

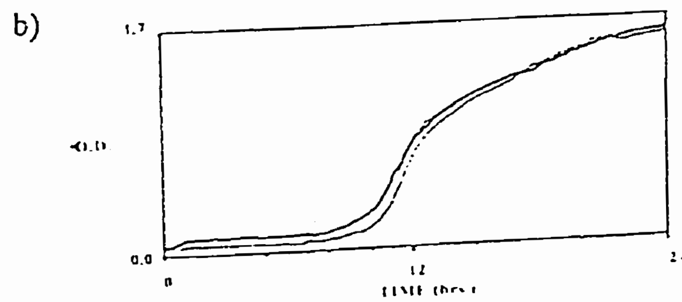



**Figure 8: Positive Control Growth Curve of *Staphylococcus aureus* $^{R2}$ Exposed to Sodium Hypochlorite**

Growth of *S. aureus* $^{R2}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

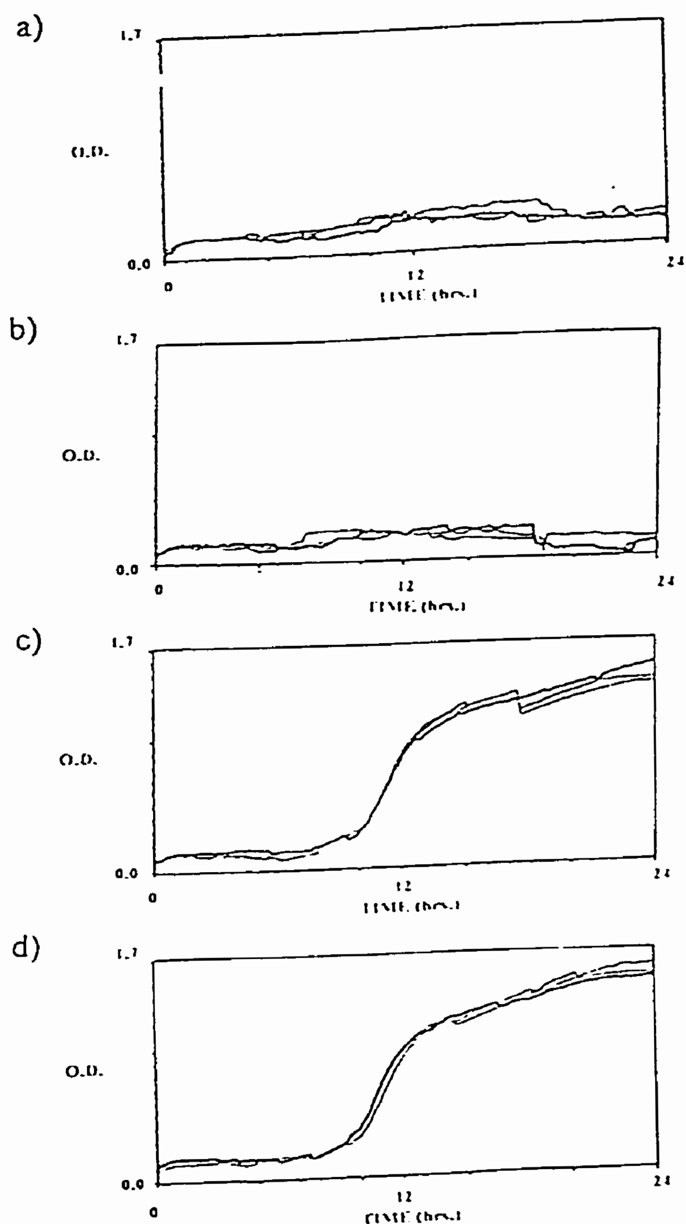



**Figure 9: Growth Curve of *Staphylococcus aureus* R2 Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *S. aureus* R2 was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.125%; c)0.06% and d) 0.004% sodium hypochlorite concentrations.

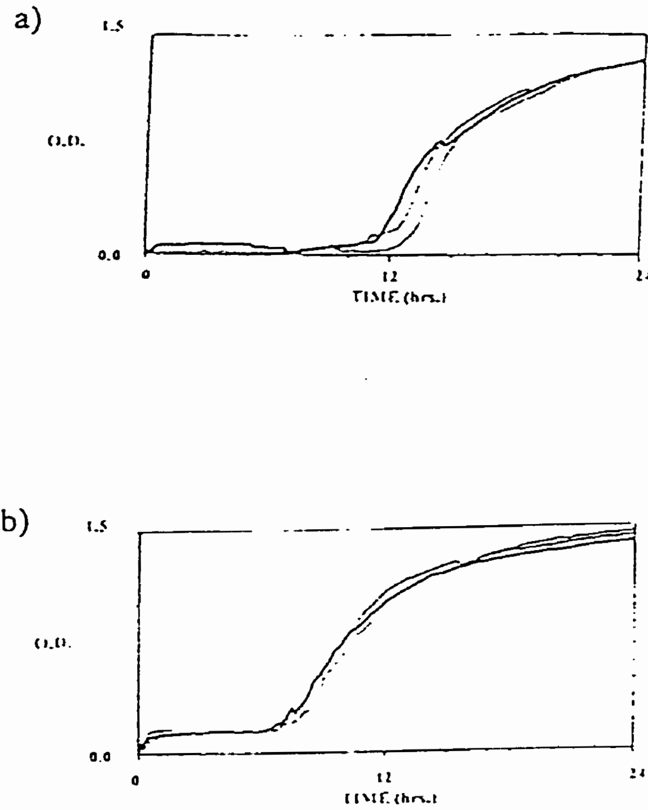



**Figure 10: Positive Control Growth Curve of *Staphylococcus aureus* [R3] Exposed to Sodium Hypochlorite**

Growth of *S.aureus* [R3] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

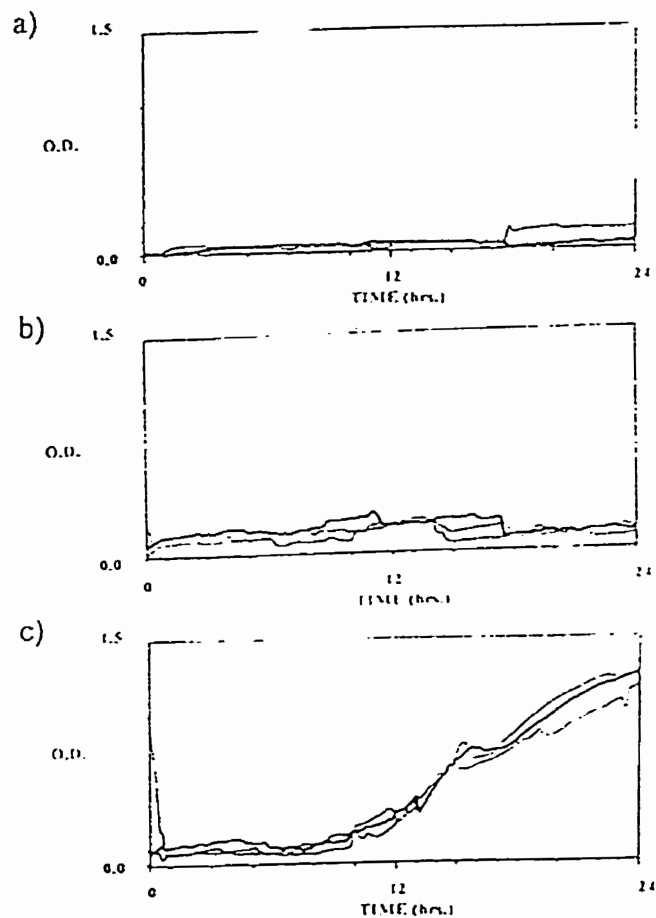



**Figure 11: Growth Curve of *Staphylococcus aureus* [R3] Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *S. aureus* [R3] was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.008% and c)0.004% sodium hypochlorite concentrations.

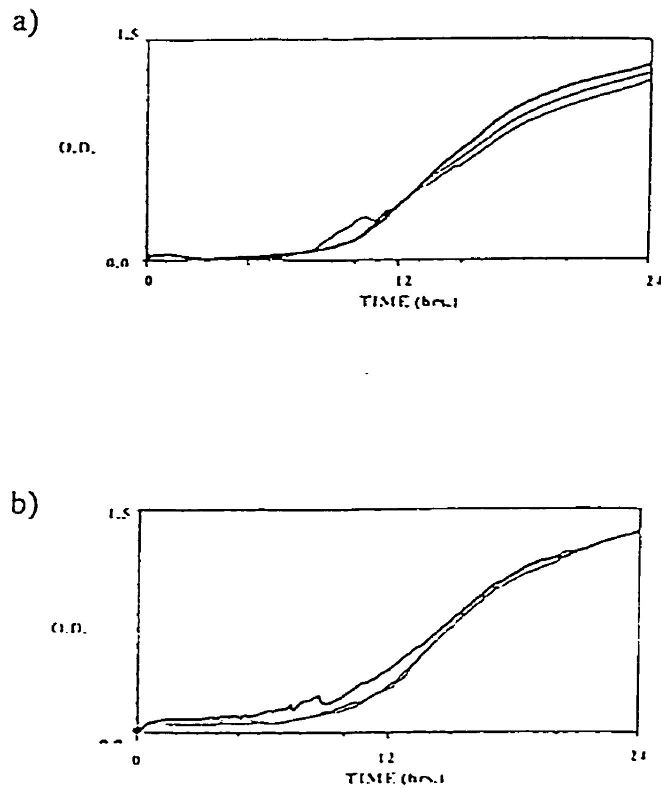



**Figure 12: Positive Control Growth Curve of *Staphylococcus aureus* S1 Exposed to Sodium Hypochlorite**

Growth of *S. aureus* S1 in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

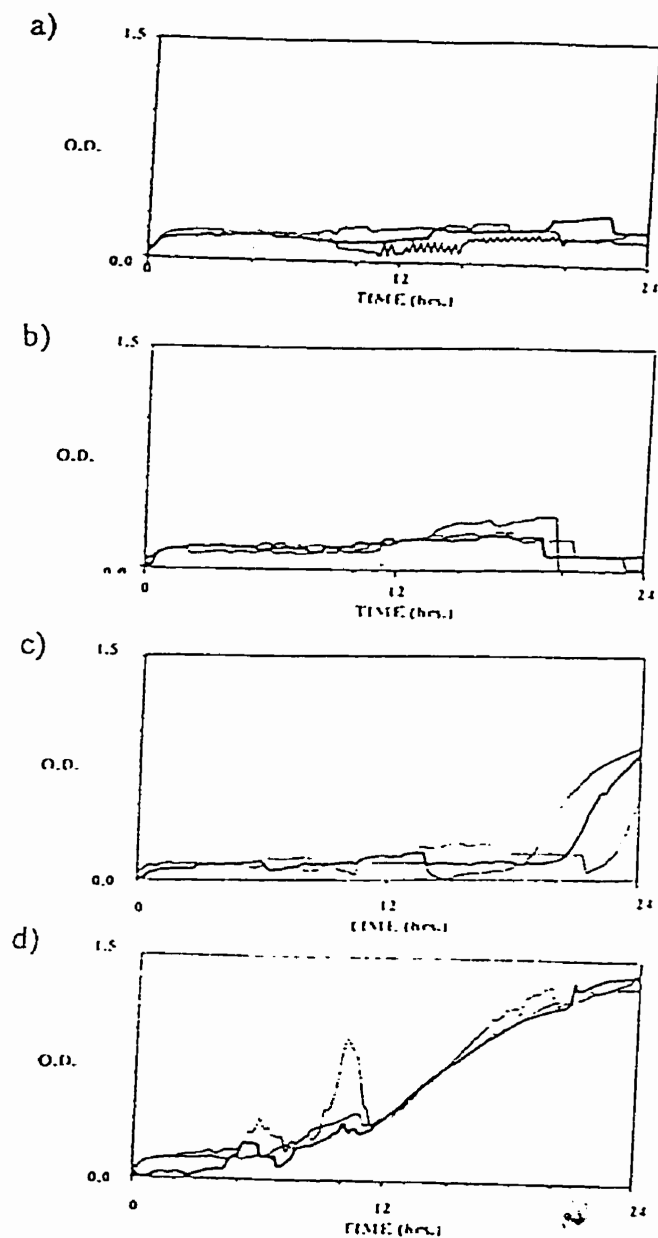



**Figure 13: Growth Curve of *Staphylococcus aureus* S1 Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *S. aureus* S1 was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.03%; c)0.016% and d) 0.004% sodium hypochlorite concentrations.

Positive growth controls for *S.aureus*$^{R3}$ and *S. aureus*$^{S1}$ when tested with hydrogen peroxide were similar to the positive growth curves observed with sodium hypochlorite. Maximum optical density for *S.aureus*$^{R3}$ averaged 1.2 a.u. and 1.3 a.u. for the *S. aureus*$^{S1}$ strain. Both positive controls were comparable. In the presence or absence of hydrogen peroxide, the growth curves for *S. aureus*$^{R2}$ showed minimal growth. Since the positive growth controls did not show any growth, we were not able to evaluate the corresponding suspension results for this strain. The positive controls must grow and the two positive controls must show similar growth patterns and OD values to consider the suspension test results.

Since the positive control growth curves for *S. aureus*$^{R2}$ exposed to hydrogen peroxide did not show growth, the experiment was repeated with the initial concentration of hydrogen peroxide of 0.75% instead of 3%. The decrease in initial concentration of the disinfectant resulted in positive controls that showed typical growth and maximum OD value of approximately 1.5. Upon exposure to hydrogen peroxide, no growth was detectable in any of the test strains for any of the concentrations tested.

Upon testing the phenolic compound, the positive control growth curves were similar to those when tested with hydrogen peroxide. *S. aureus*$^{R3}$ (Fig. 15) strain showed a maximum optical density of 0.9 while the *S. aureus*$^{S1}$ strain was 1.4 a.u. (Fig. 16). The positive controls for *S. aureus*$^{R2}$ showed no detectable growth. The experiment was repeated with *S. aureus*$^{R2}$ and an initial phenolic compound dilution of 1:500. The resulting positive control growth curves are shown in Fig. 14.

Upon exposure to the phenolic compound, each test strain did not show growth until

the 1:4000 dilution (Figs. 17, 19 and 21). Maximal optical densities at these concentrations were similar to the positive controls even though the lag phase durations and OD50 were increased when exposed to the disinfectant.

The positive control growth curves for the quaternary ammonium compound showed similar results. *S. aureus*$^{R3}$ and *S. aureus*$^{S1}$ strains showed typical growth curves. The positive control that was situated on the tray containing the QAC showed a growth curve consistent with the positive control growth curve on a separate tray. The *S. aureus*$^{R2}$ strain, again, did not show typical growth when in contact with QAC. The QAC was diluted to 1:500 and this dilution was the new initial dilution when the experiment was repeated. The resulting curves showed typical growth.

When exposed to QAC, *S. aureus*$^{R2}$ and *S. aureus*$^{S1}$ strain showed no growth in any of the dilutions tested while *S. aureus*$^{R3}$ showed 1 replicate out of 3 exhibiting outgrowth at the 1:64,000 dilution.

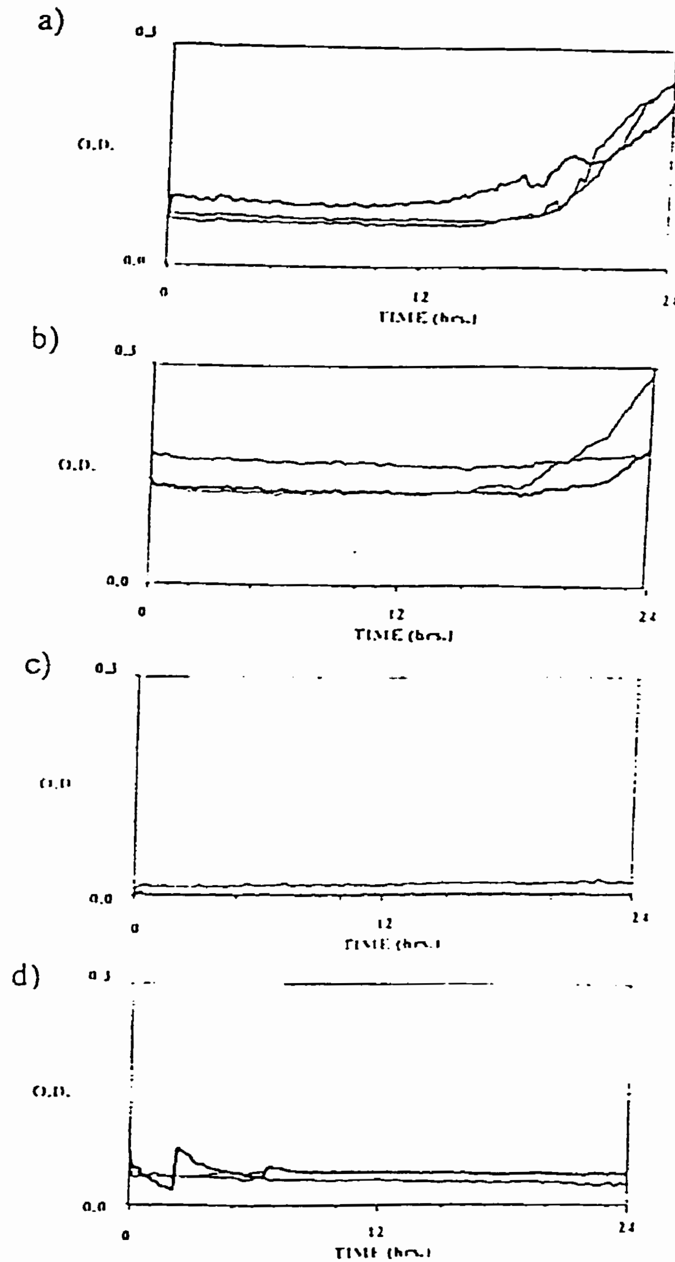



**Figure 14: Growth Curve of *Staphylococcus aureus* $^{R2}$ Exposed to Hydrogen Peroxide**

Growth of *S. aureus* $^{R2}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray withouthydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 1.5%; d) 0.01% hydrogen peroxide.

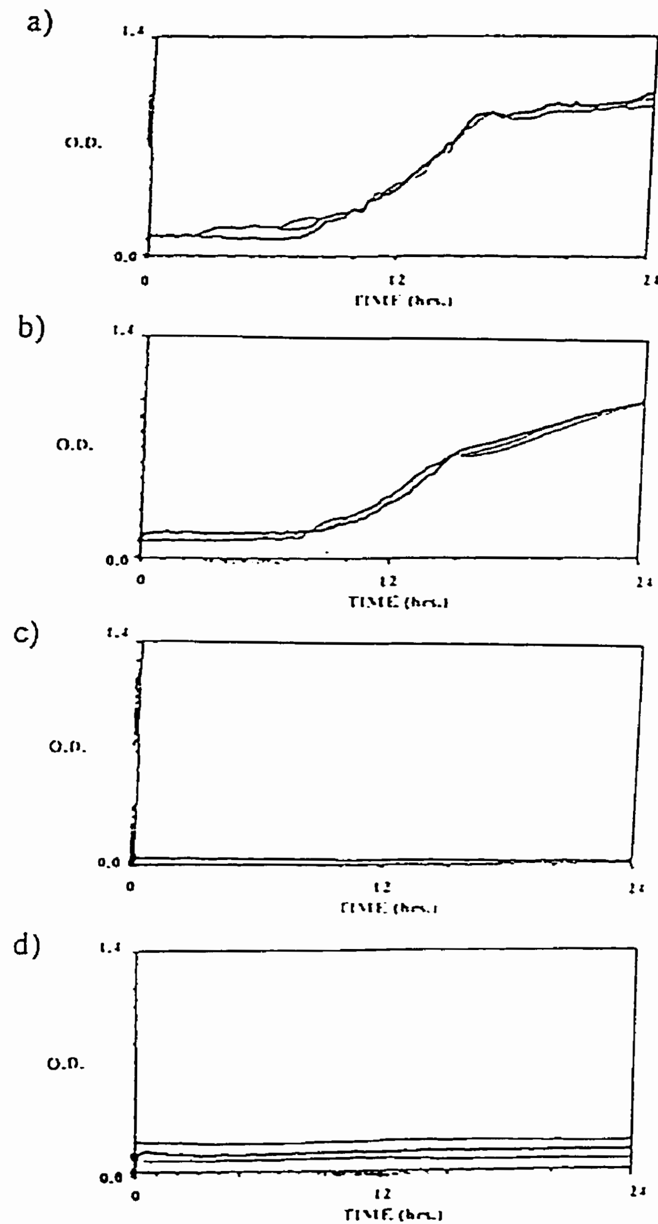



**Figure 15: Growth Curve of *Staphylococcus aureus* $^{R3}$ Exposed to Hydrogen Peroxide**

Growth of *S. aureus* $^{R3}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

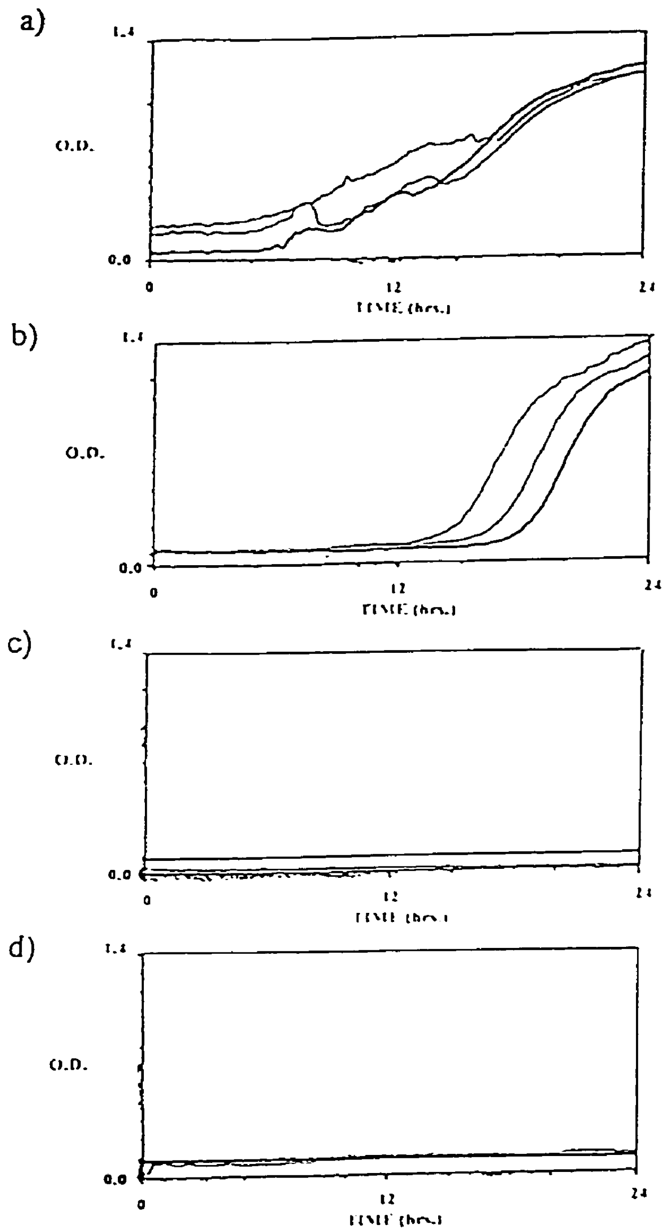



**Figure 16: Growth Curve of *Staphylococcus aureus* $^{S1}$ Exposed to Hydrogen Peroxide**

Growth of *S. aureus* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

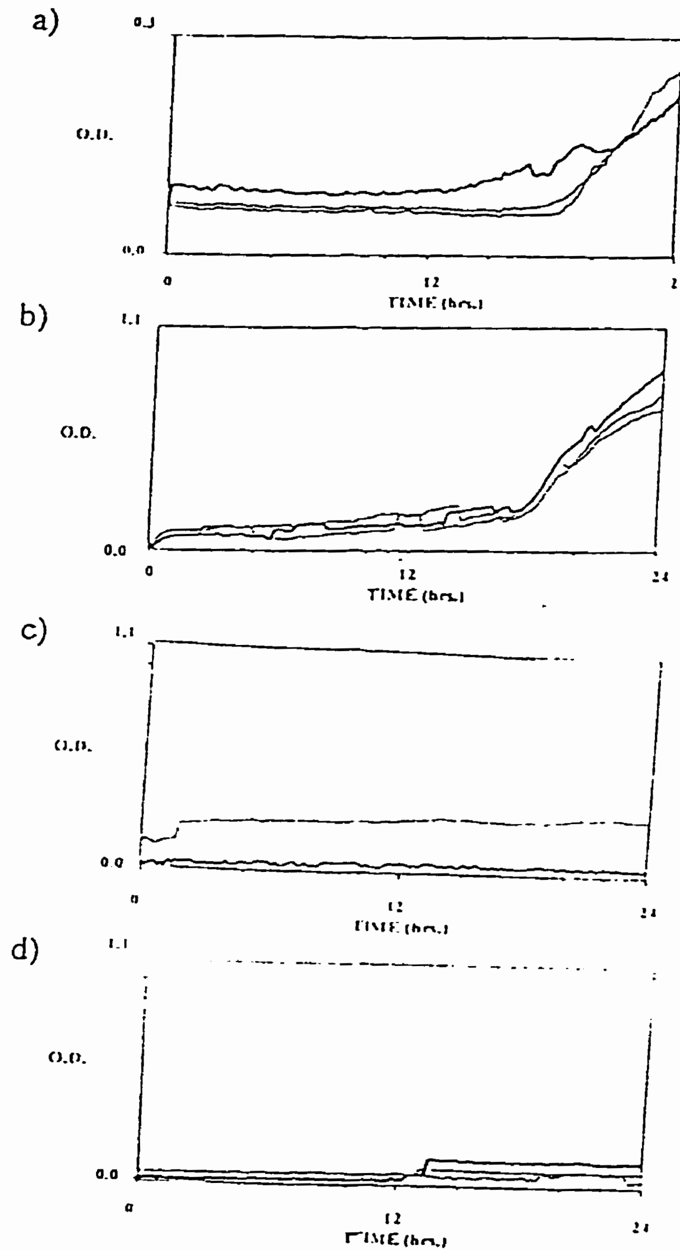



**Figure 17: Growth Curve of *Staphylococcus aureus* R2 Exposed to Phenolic Disinfectant**

Growth of *S. aureus* R2 was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without phenolic disinfectant; b) the same tray that contained various dilutions of phenolic disinfectant. Growth was also monitored in c) 1:500; d) 1:64000 dilutions of phenolic disinfectant.

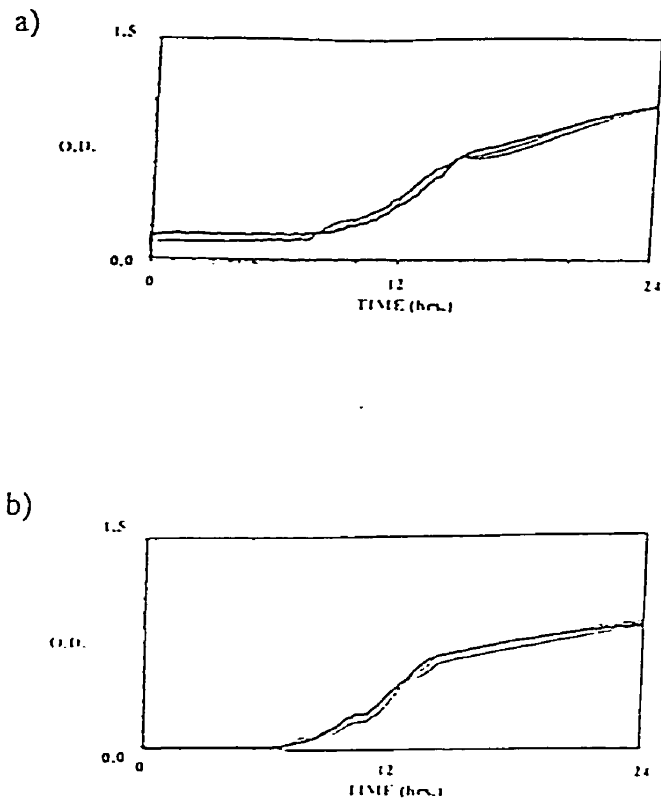



**Figure 18: Positive Control Growth Curve of *Staphylococcus aureus* $^{R3}$ Exposed to Phenolic Disinfectant**

Growth of *S. aureus* $^{R3}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

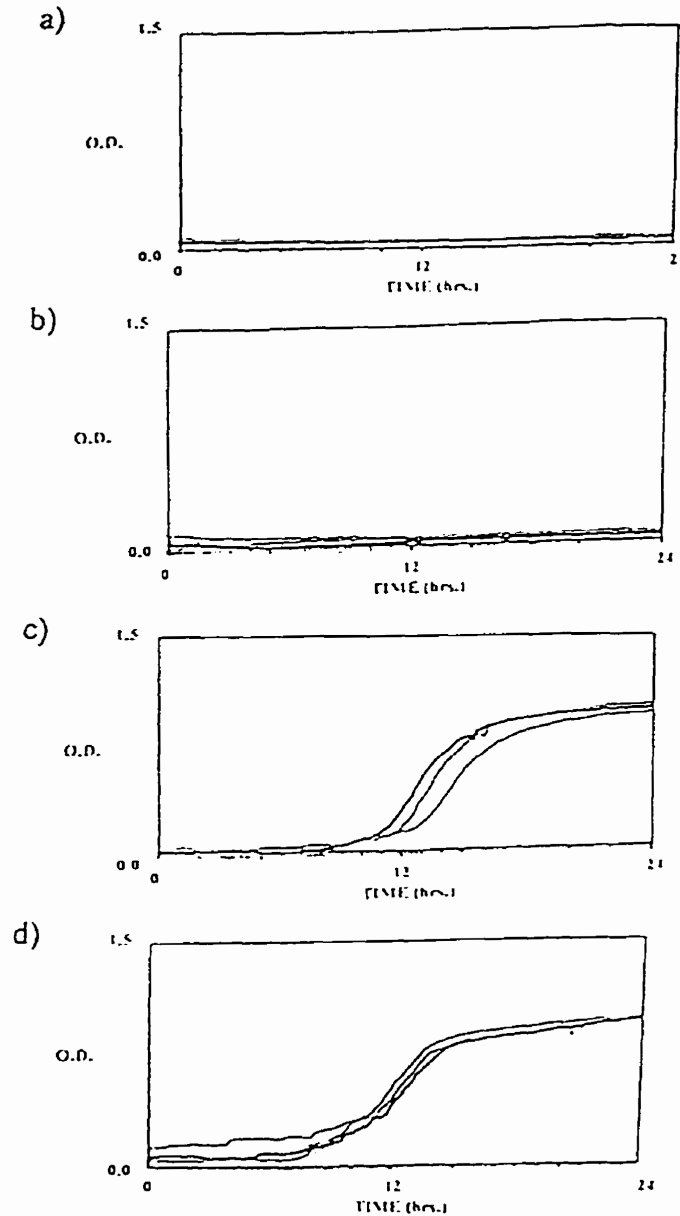
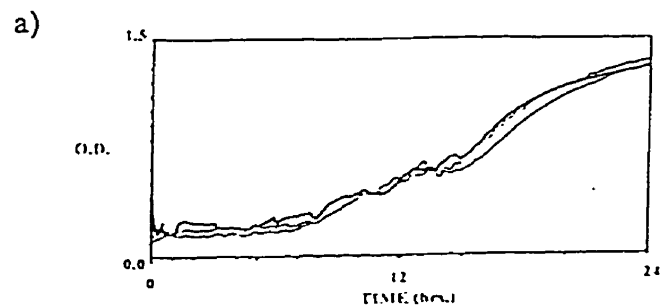



**Figure 19: Growth Curve of *Staphylococcus aureus* R3 Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *S. aureus* R3 was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:2000; c)1:4000 and d) 1:32000 dilution of phenolic disinfectant

a)




b)

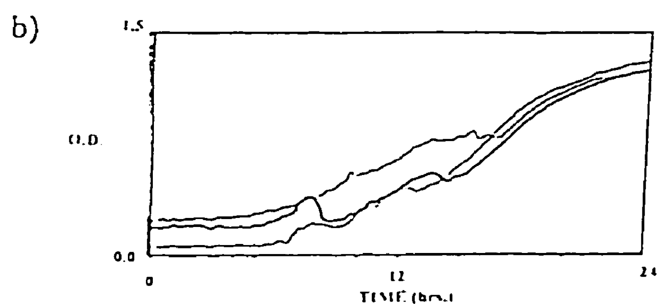



**Figure 20: Positive Control Growth Curve of *Staphylococcus aureus* $^{S1}$ Exposed to Phenolic Disinfectant**

Growth of *S. aureus* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

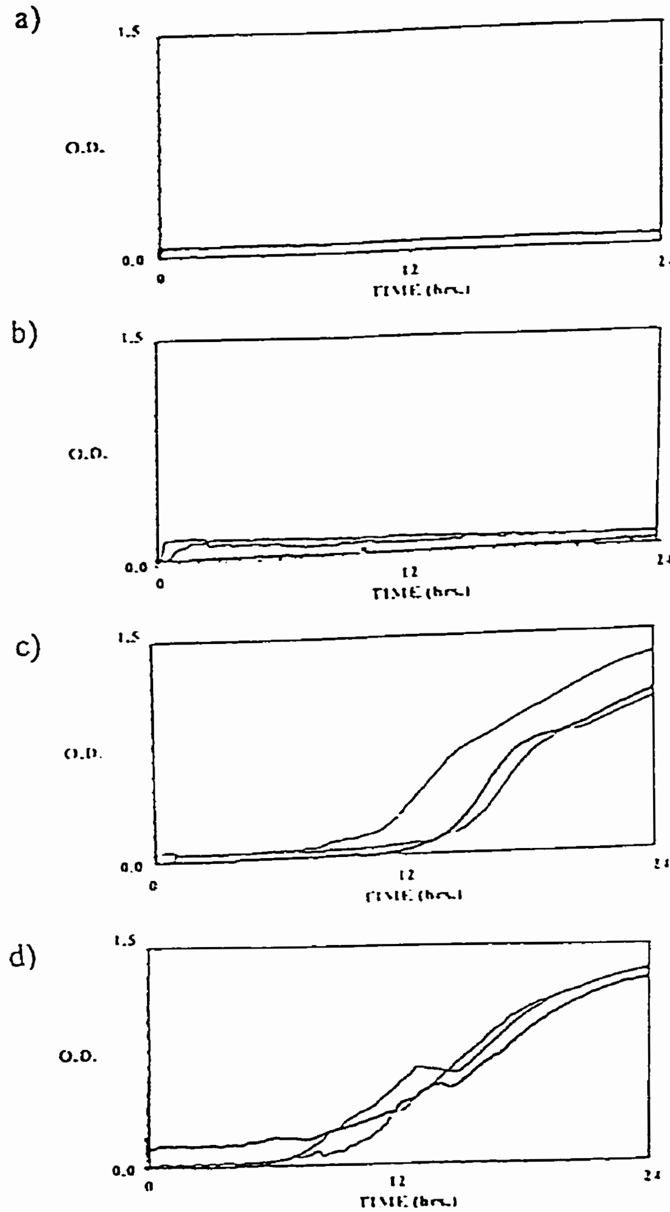



**Figure 21: Growth Curve of *Staphylococcus aureus* S1 Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *S. aureus* S1 was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:2000; c)1:4000 and d) 1:32000 dilution of phenolic disinfectant

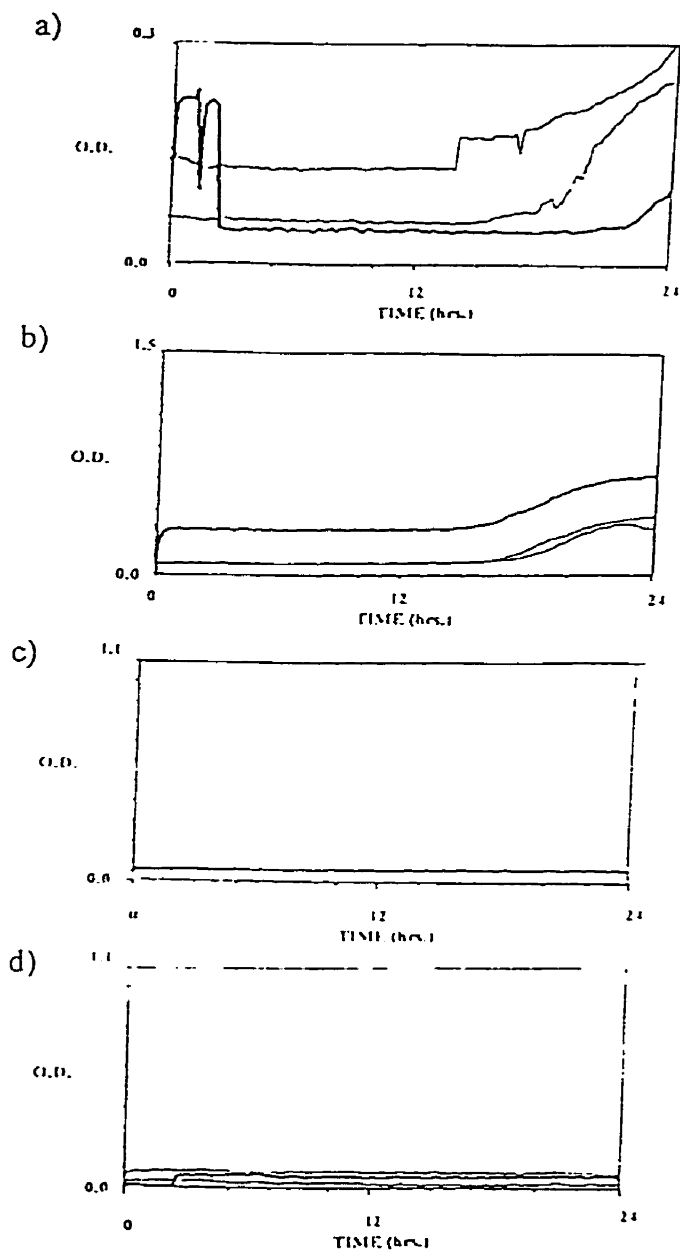



**Figure 22: Growth Curve of *Staphylococcus aureus* [R2] Exposed to Quaternary Ammonium Compound**

Growth of *S. aureus* [R2] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without sodium hypochlorite; b) the same tray that contained various dilutions of QAC. Growth was also monitored in c) 1:500; d) 1:64000 dilutions of QAC.

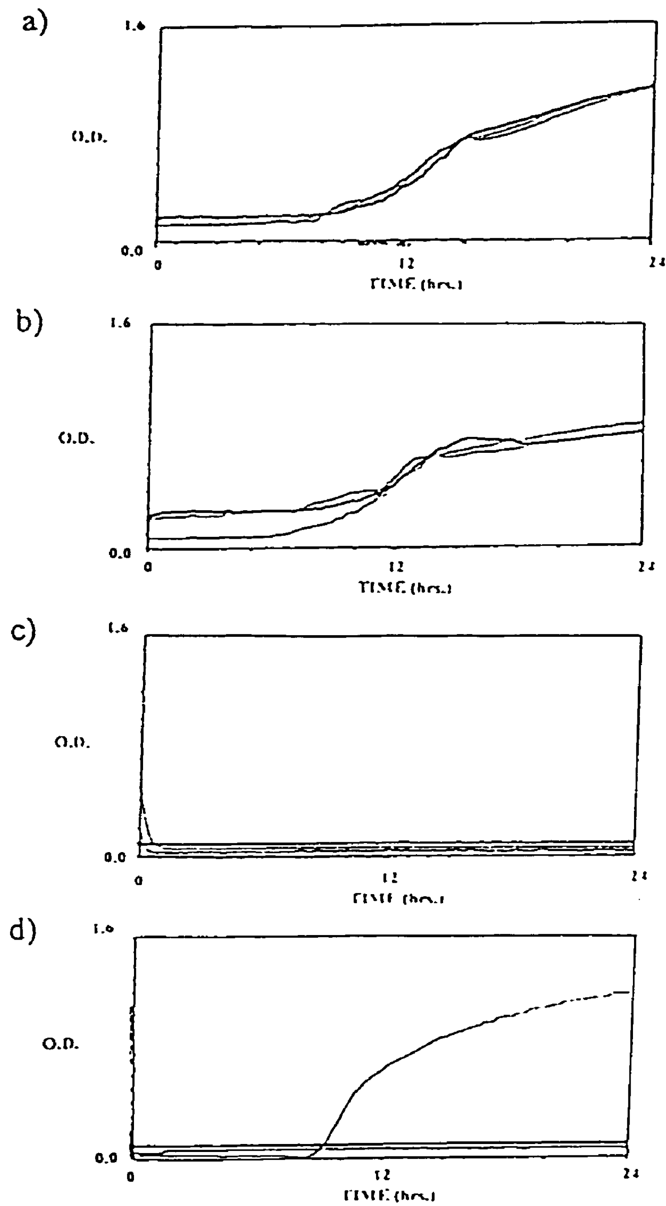



**Figure 23: Growth Curve of *Staphylococcus aureus* $^{R3}$ Exposed to Quaternary Ammonium Compound**

Growth of *S. aureus* $^{R3}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without QAC; b) the same tray that contained various dilutions of QAC. Growth was also monitored in c) 1:16000; d) 1:32000 dilutions of QAC.

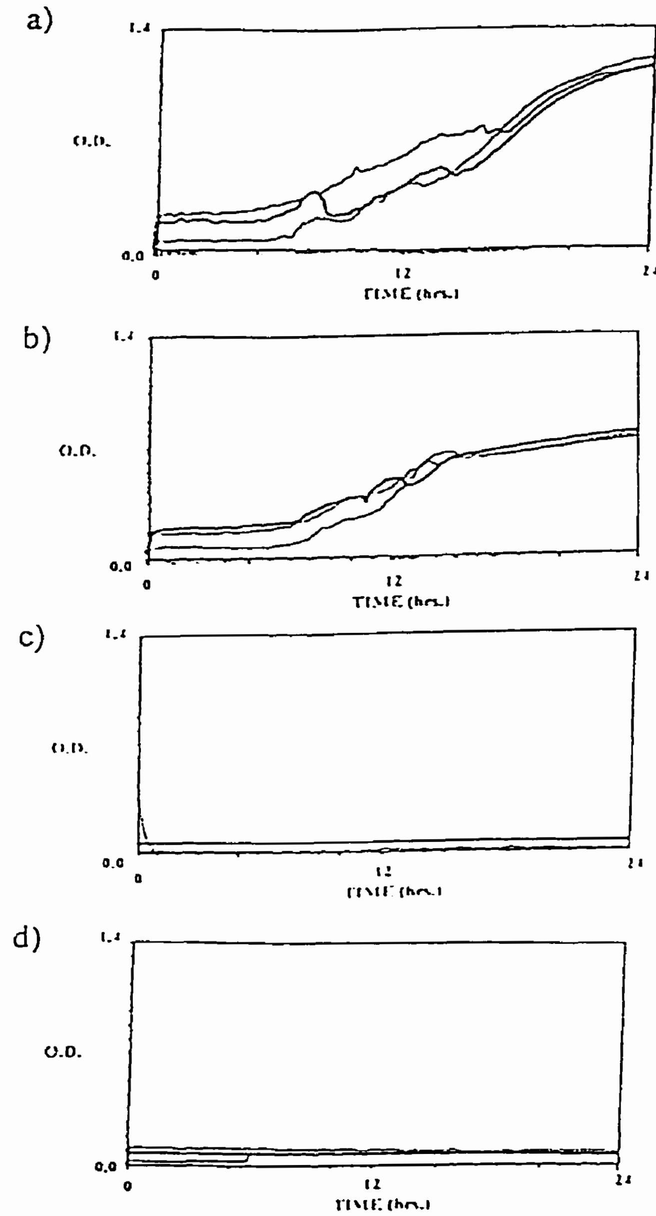



**Figure 24: Growth Curve of *Staphylococcus aureus* $^{S1}$ Exposed to Quaternary Ammonium Compound**

Growth of *S. aureus* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without QAC; b) the same tray that contained various dilutions of QAC. Growth was also monitored in c) 1:500; d) 1:64000 dilutions of QAC.

**DISINFECTANTS TESTED (Initial Concentration or Dilution)**

| **Test Organism** | G(1%) | SH(0.5%) | HP(3%) | P(1:250) | QAC(1:250) |
|---|---|---|---|---|---|
| *S. aureus* [S1] | 16 | 8 | >128 | 16 | >128 |
| *S. aureus* [R2] | 128 | 32 | >128 | >256* | >256* |
| *S. aureus* [R3] | 64 | 128 | >128 | 16 | 128 |

**Table 18: Bacterial Outgrowth of *Staphylococcus aureus* in the Suspension Test Assay**

Bacteria ($10^5$ cfu's/ml) were inoculated in microwell trays containing TSB + 10% FBS and serial dilutions of the initial concentration of each test disinfectant (G=glutaraldehyde; SH= sodium hypochlorite; HP= hydrogen peroxide; P= phenolic and QAC= quaternary ammonium compound). Bacterial growth was monitored by a multiple growth curve reader over a period of 24 hours at 37°C. The data in the table represents the reciprocal of the dilution of the initial concentration where bacterial outgrowth was first detected. Data was compiled from three replicates. Data that is denoted by > refers to no bacterial outgrowth detected in the lowest concentration of disinfectant tested. (*) The initial concentration of phenolic and quaternary ammonium compound used with *S. aureus* [R2] was a 1:500 dilution.

**b)** ***Enterococcus faecium***

There were two *E. faecium* strains tested in this part of the evaluation. The *E. faecium*$^{R2}$ strain is a multi-resistant (VRE) strain and the *E. faecium*$^{S1}$ strain is an ATCC strain that is not vancomycin resistant. When these strains were tested with glutaraldehyde, the two positive controls showed typical growth curves (Figs. 25 and 27). The *E. faecium*$^{R2}$ strain showed typical lag, exponential and stationary phases within the 24 hour observation period, in both the same and separate tray controls. The maximum optical density was approximately 0.7, which was consistent in both controls. The OD50 is ten hours with a lag phase duration of seven hours. The two positive control growth curves for the *E. faecium*$^{S1}$ strain were also similar to each other. Lag, exponential and stationary phases were evident within the 24 hour period. The maximum OD and OD50 for the internal and external growth controls for *E. faecium* $^{S1}$ were similar enough to allow interpretation of the data for the growth curves on the test. The comparison of OD50 and lag phase duration indicated that the *E. faecium*$^{S1}$ strain grows much slower and to a lower maximum turbidity in TSB compared to the *E. faecium* $^{R2}$ strain. The maximum OD and OD50 for both internal and external positive controls growth curves were similar enough to allow interpretation of the data for the growth curves on the test tray. When exposed to glutaraldehyde, outgrowth of the *E. faecium* $^{R2}$ (Fig.26) and *E. faecium*$^{S1}$ (Fig.28) strain was observed in a concentration of 0.125%.

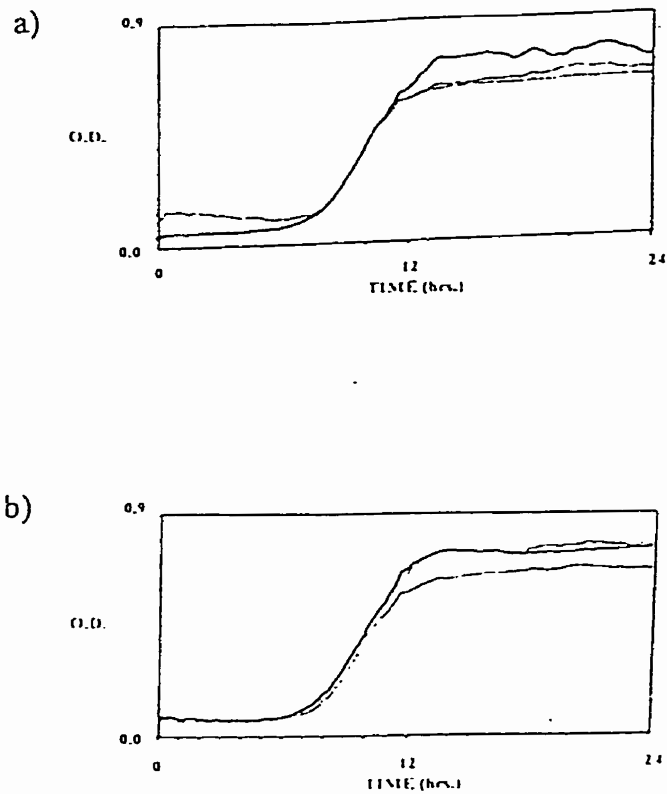



**Figure 25: Positive Control Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Glutaraldehyde**

Growth of *E. faecium* $^{R2}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

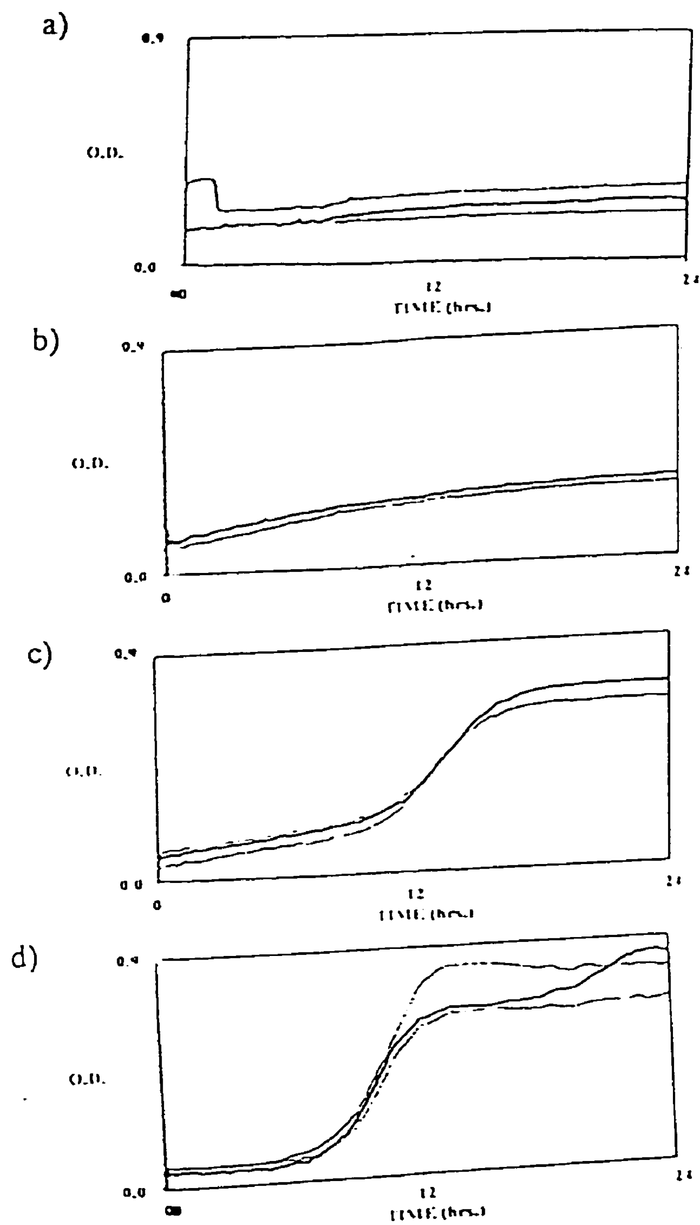



**Figure 26: Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *E. faecium* $^{R2}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.25%; c)0.125% and d) 0.008% glutaraldehyde concentrations.

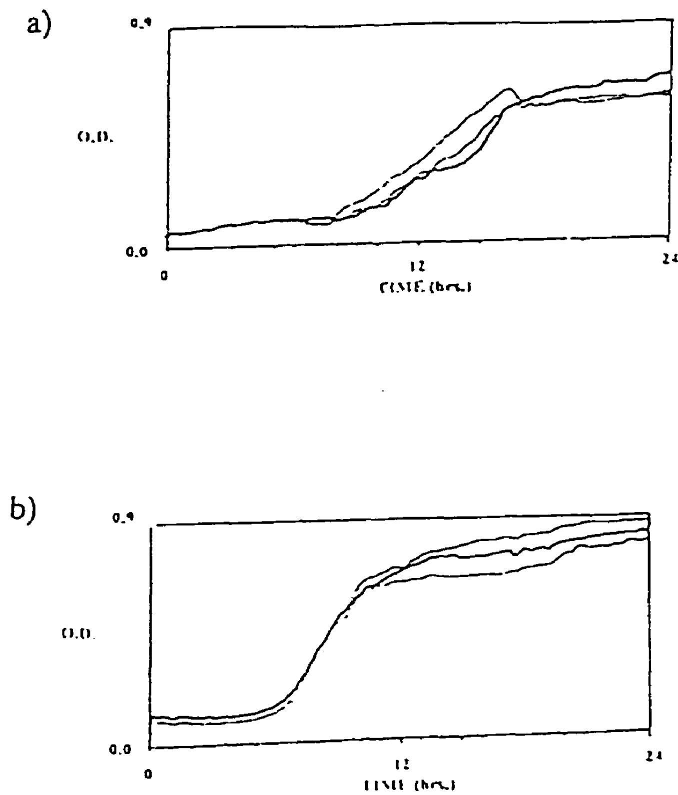



**Figure 27: Positive Control Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Glutaraldehyde**

Growth of *E. faecium* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

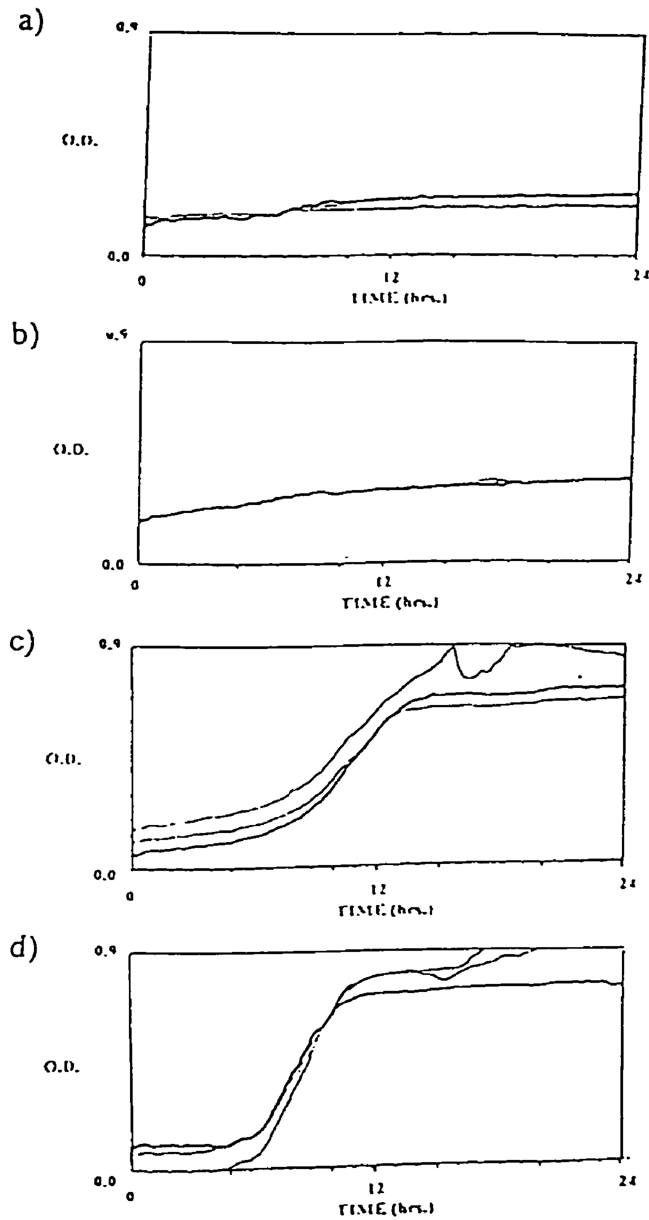


**Figure 28: Growth Curve of *Enterococcus faecium* S1 to Varied Concentrations of Glutaraldehyde**

Growth of *E. faecium* S1 was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.25%; c)0.125% and d) 0.008% glutaraldehyde concentrations.

Positive controls for exposure to sodium hypochlorite were much similar to those observed with glutaraldehyde. Both strains showed similar growth curves for the internal and external positive controls (Figs. 29 and 31). Both strains first showed outgrowth in 0.03% sodium hypochlorite (Figs. 31 and 32).

Three percent hydrogen peroxide was the initial concentration used in the next set of experiments. The positive controls for the *E. faecium*$^{R2}$ (Fig. 33) and *E. faecium*$^{S1}$ (Fig. 34) strains clearly showed lag, exponential and stationary phases of growth within 24 hours. The lag phases duration of the *E. faecium*$^{S1}$ strain was slightly higher in the same tray positive control compared with the control on the separate tray. In contrast, the *E. faecium*$^{R2}$ strain showed a longer lag phase in the control that was on the separate tray. Otherwise, the maximum OD was similar in both the internal and external control of each strain. Upon exposure to all concentrations of hydrogen peroxide, neither of the two test strains showed any growth within the 24 hour observation period.

Positive controls of the two *E. faecium* strains when exposed to the phenolic compound proved to be similar whether the control was on the same tray as the disinfectant or on a separate tray (Figs. 35 and 37). Lag phases were slightly longer in the controls on the same tray as the disinfectant compared to the separate tray control although these values only differed by a maximum of two hours. OD50 was also lower in the controls on the separate tray but these values also only differed by a couple of hours at the most. When exposed to the phenolic disinfectant, the lowest dilution in which growth did occur was 1:2000 in both test strains (Figs. 36 and 38). Maximum growth at this dilution was slightly lower than in the positive controls but in the highest dilution of 1:32000, maximum growth

was observed that was comparable to the maximum growth observed in the positive controls.

The positive controls upon exposure to QAC showed similarities to the growth curves of the phenolic disinfectant. Lag phase duration, maximum OD and OD50 values were similar in external and internal positive control growth curves. The lowest dilution in which we observed any growth when exposed to QAC was 1:16000 for both strains.

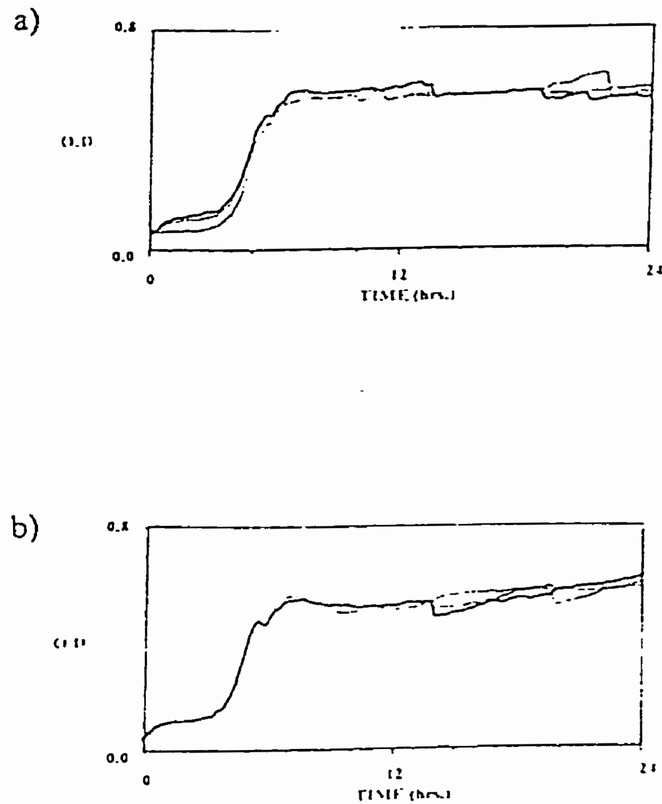



**Figure 29: Positive Control Growth Curve of *Enterococcus faecium* [R2] Exposed to Sodium Hypochlorite**

Growth of *E. faecium* [R2] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

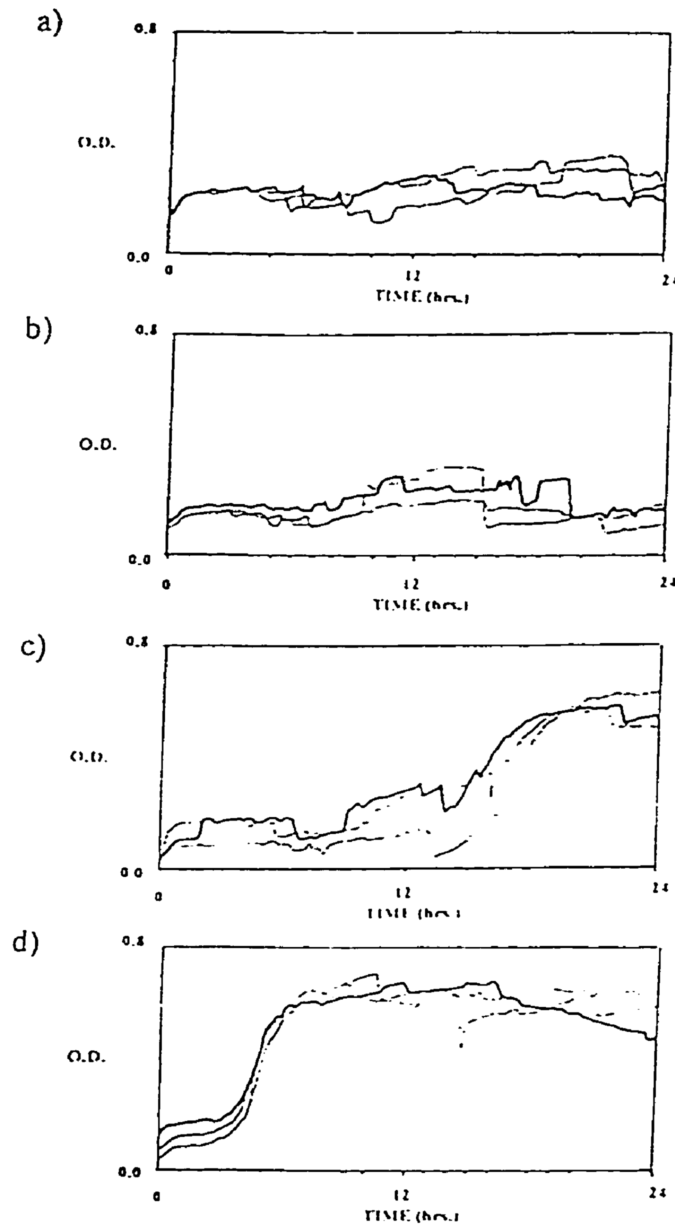



**Figure 30: Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *E. faecium* $^{R2}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.06%; c)0.03% and d) 0.004% sodium hypochlorite concentrations.

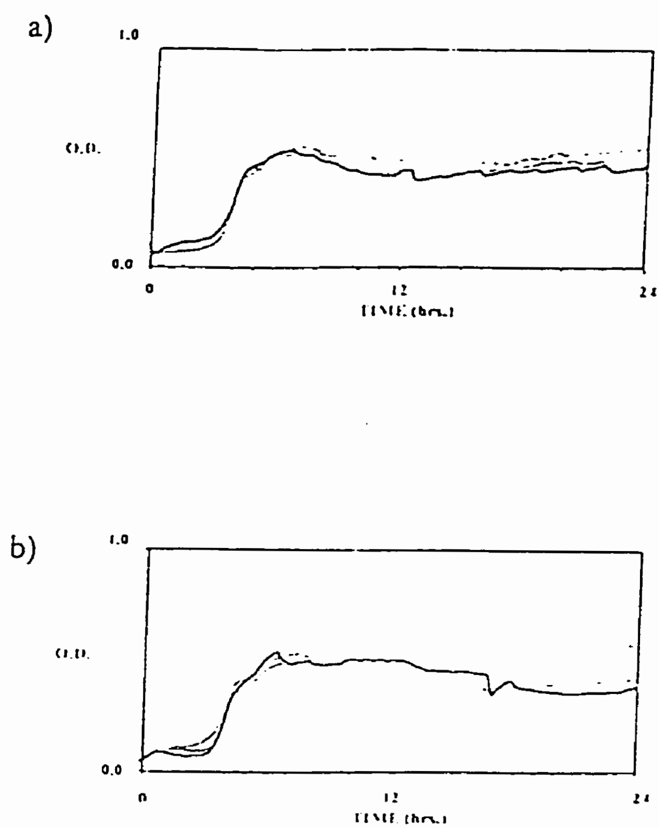



**Figure 31: Positive Control Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Sodium Hypochlorite**

Growth of *E. faecium* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

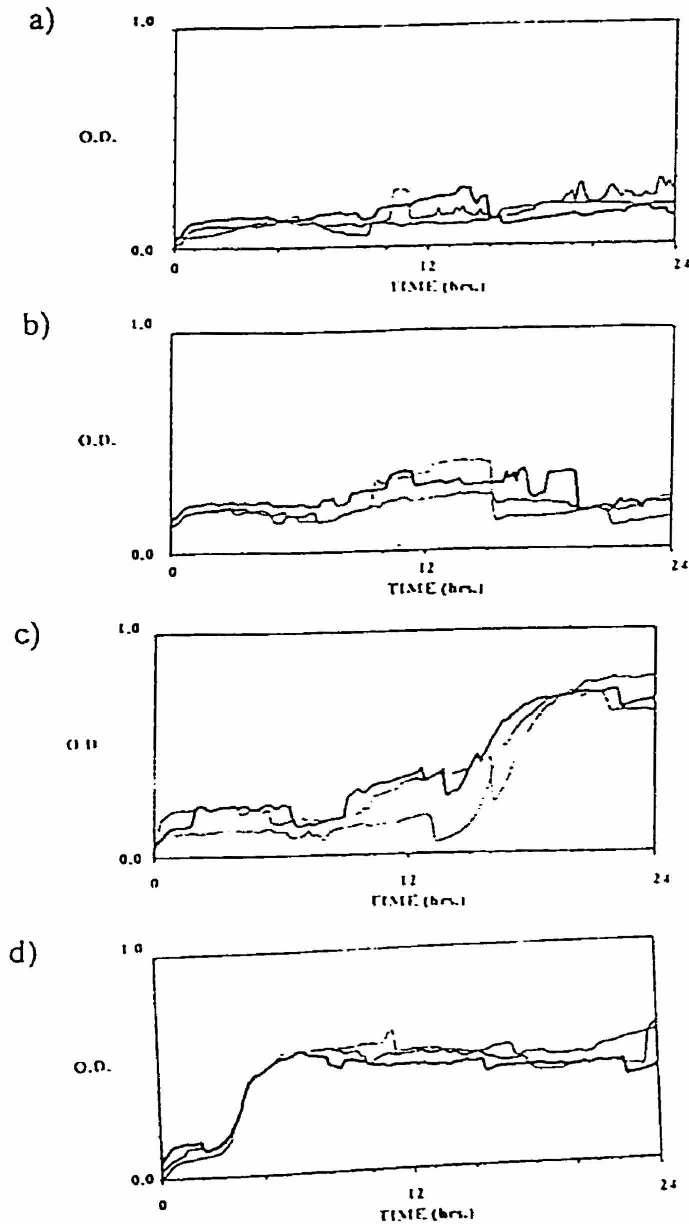



**Figure 32: Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *E. faecium* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.06%; c)0.03% and d) 0.004% sodium hypochlorite concentrations.

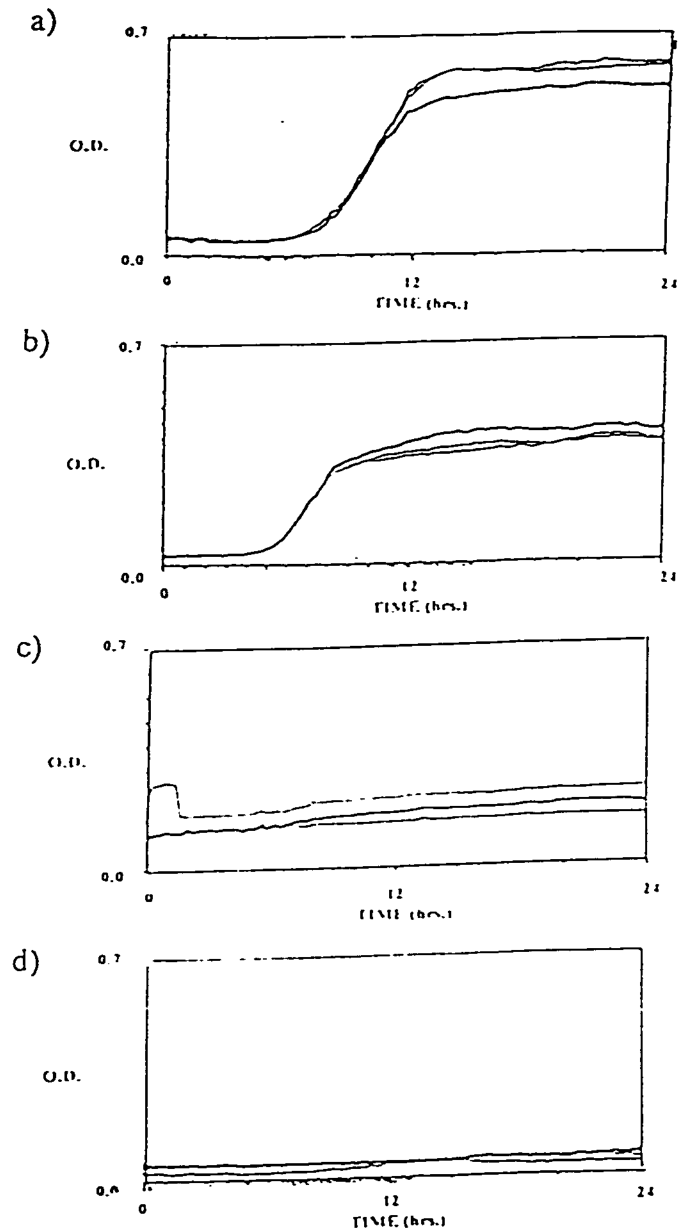



**Figure 33: Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Hydrogen Peroxide**

Growth of *E. faecium* $^{R2}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

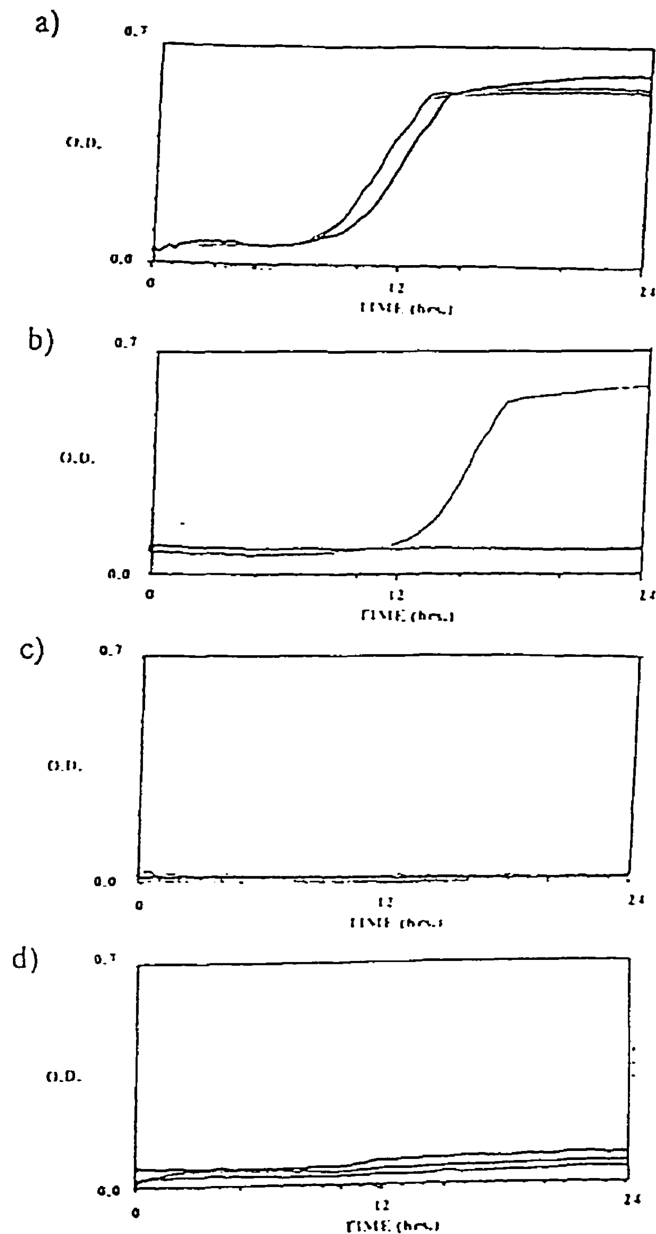
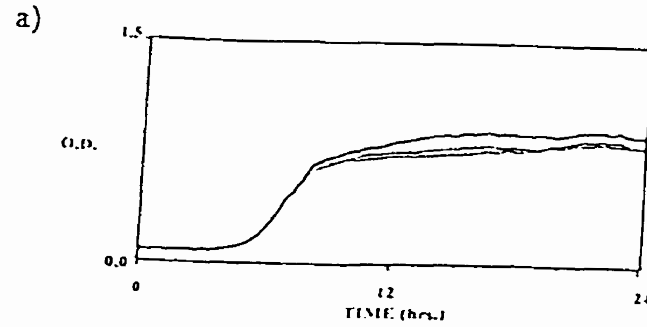



**Figure 34: Growth Curve of *Enterococcus faecium* [S1] Exposed to Hydrogen Peroxide**

Growth of *E. faecium* [S1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

a)




b)

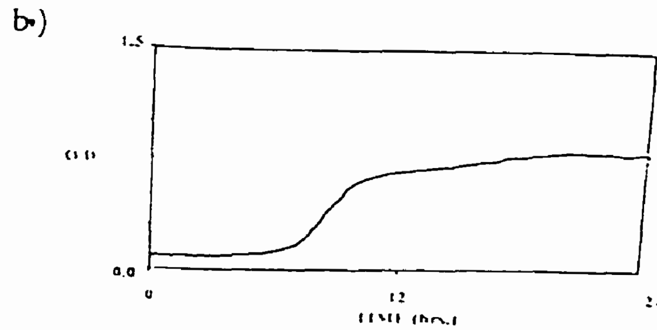



**Figure 35: Positive Control Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Phenolic Disinfectant**

Growth of *E. faecium* $^{R2}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

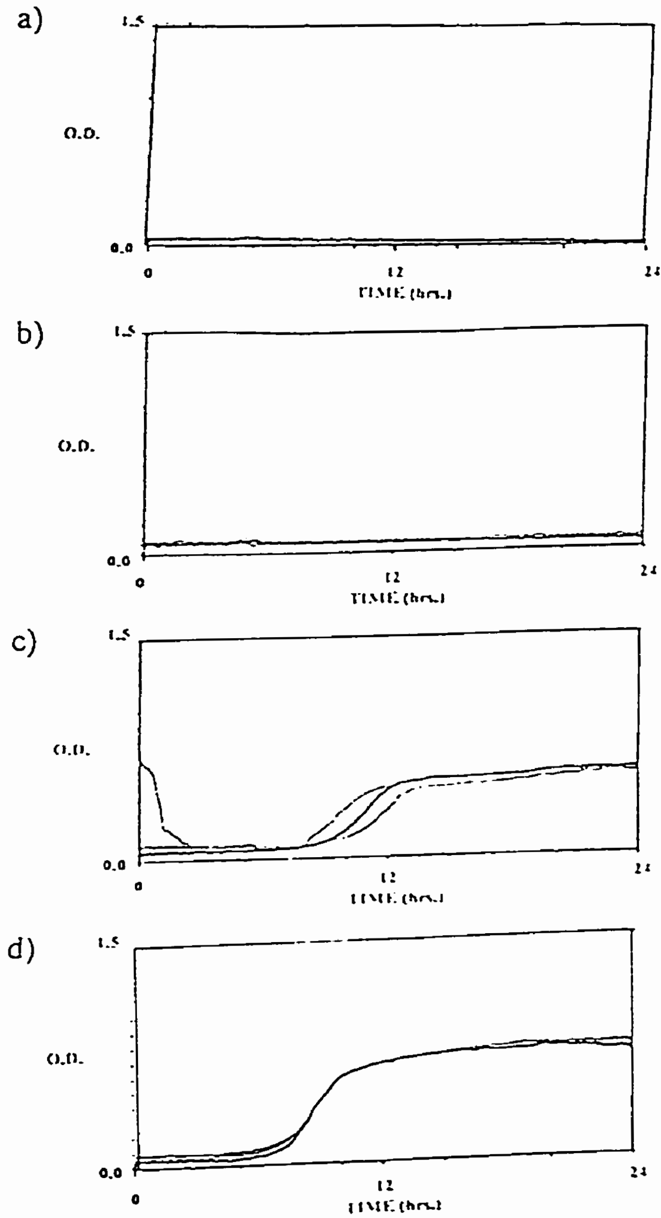
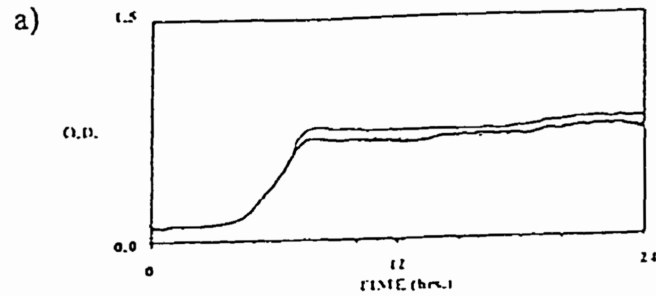



**Figure 36: Growth Curve of *Enterococcus faecium* R2 Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *E. faecium* R2 was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:1000; c)1:2000 and d) 1:32000 dilution of phenolic disinfectant

a)




b)

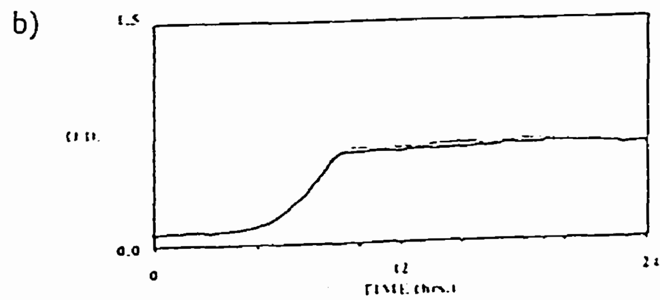



**Figure 37: Positive Control Growth Curve of *Enterococcus faecium* $^{S1}$Exposed to Phenolic Disinfectant**

Growth of *E. faecium* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

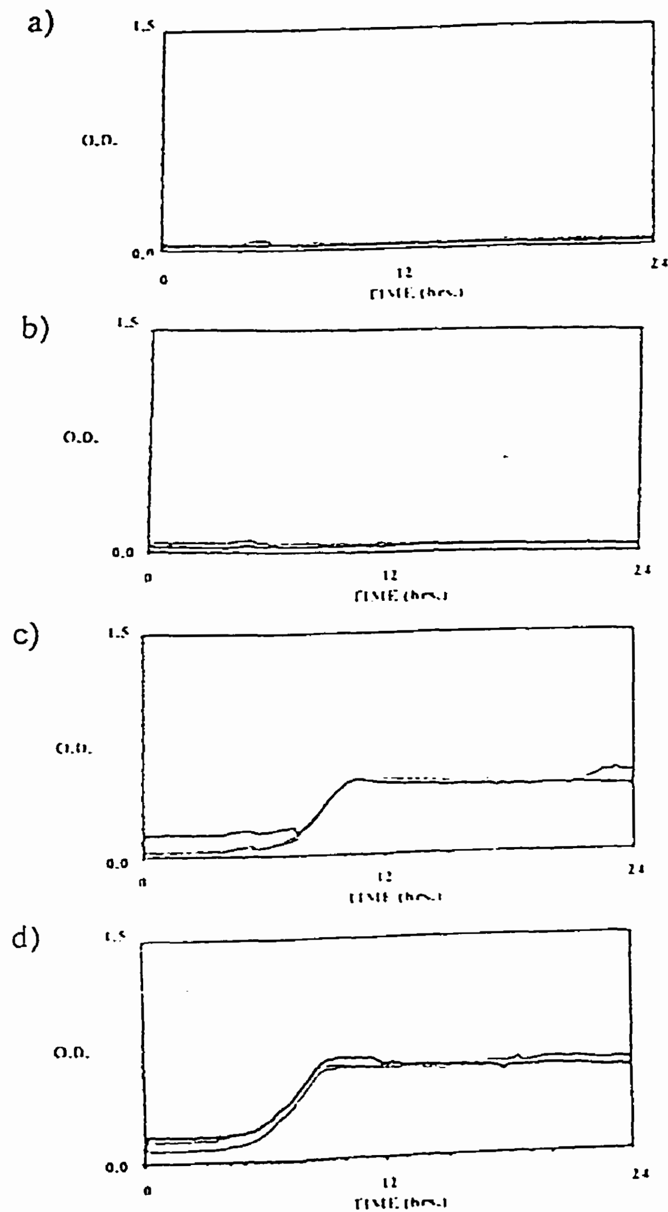



**Figure 38: Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *E. faecium* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:1000; c)1:2000 and d) 1:32000 dilution of phenolic disinfectant

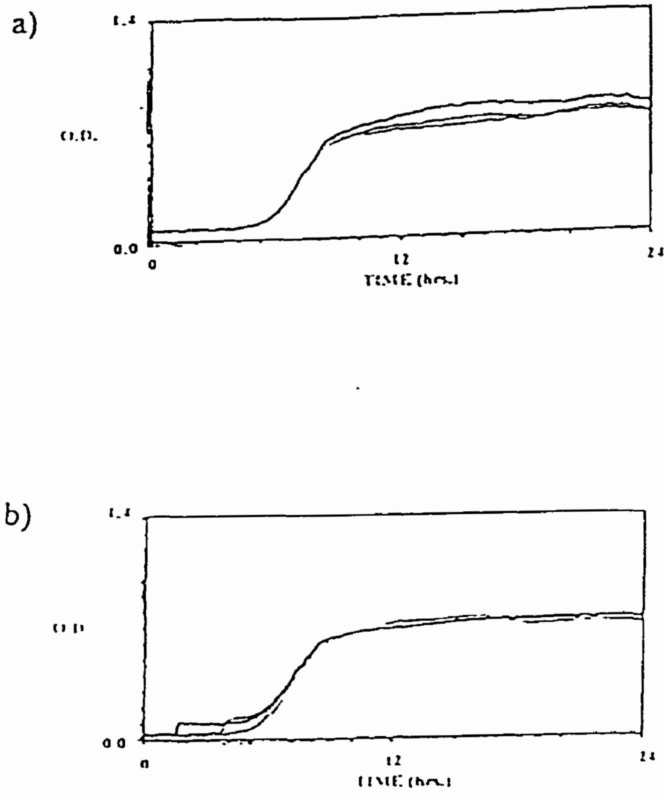



**Figure 39: Positive Control Growth Curve of *Enterococcus faecium* $^{R2}$ Exposed to Quaternary Ammonium Compound**

Growth of *E. faecium* $^{R2}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without QAC and b) the same tray that contained various dilutions of QAC.

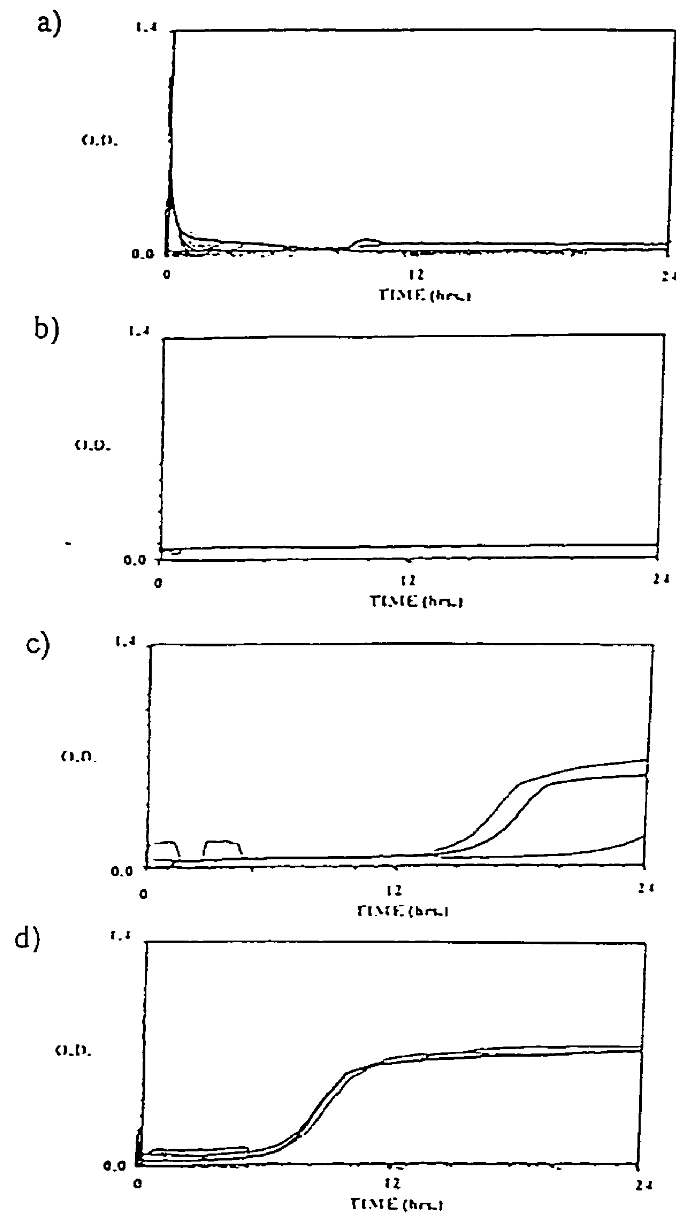



**Figure 40: Growth Curve of *Enterococcus faecium* R2 Exposed to Varied Dilutions of Quaternary Ammonium Compound**

Growth of *E. faecium* R2 was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of QAC were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:8000; c)1:16000 and d) 1:32000 dilution of QAC

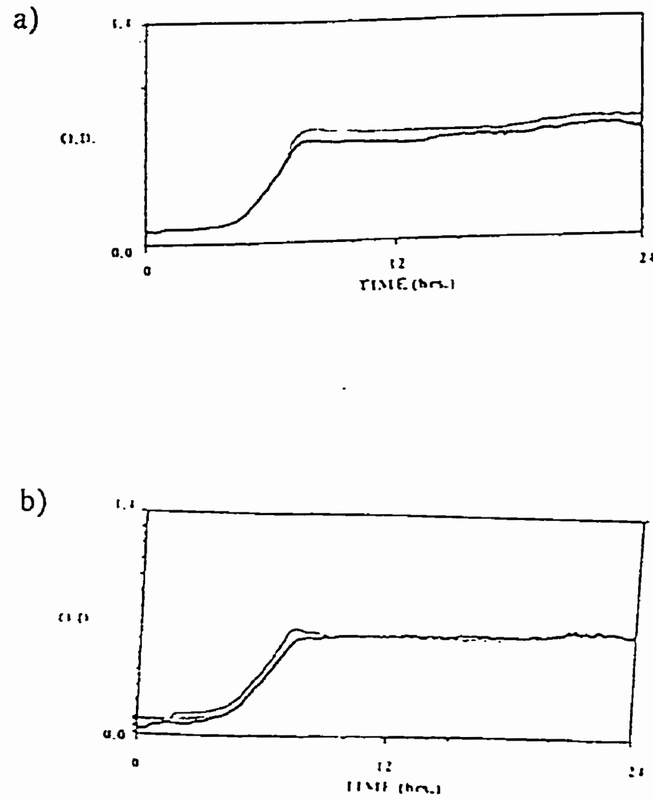



**Figure 41: Positive Control Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Quaternary Ammonium Compound**

Growth of *E. faecium* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without QAC and b) the same tray that contained various dilutions of QAC.

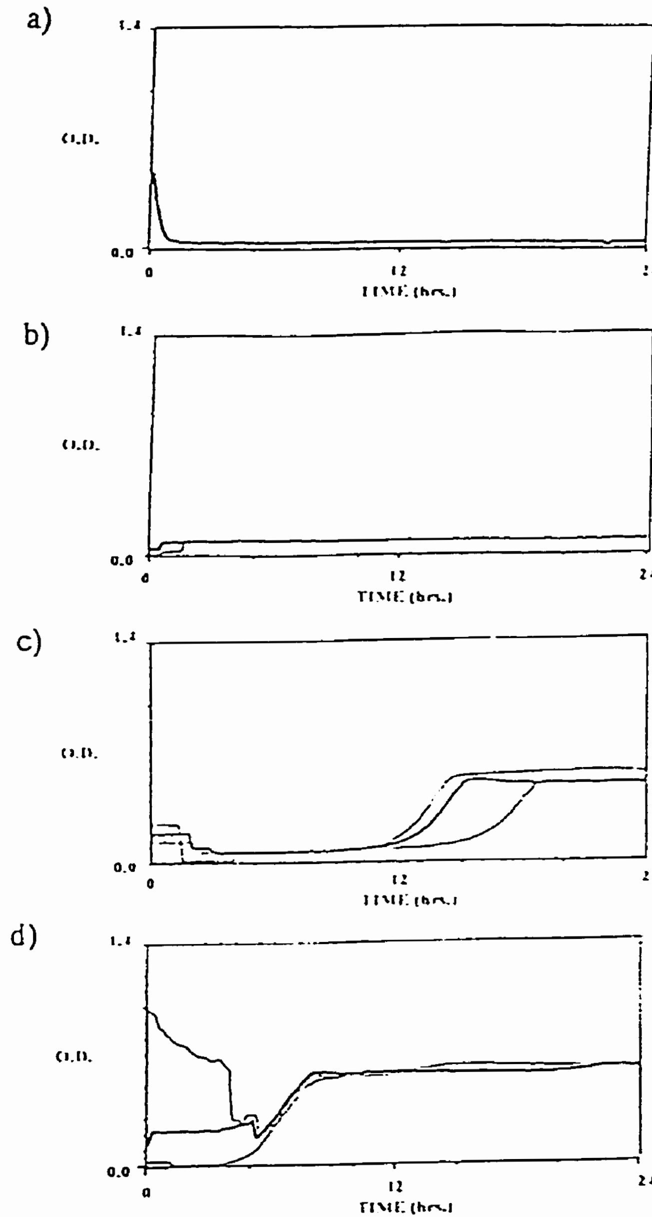



**Figure 42: Growth Curve of *Enterococcus faecium* $^{S1}$ Exposed to Varied Dilutions of Quaternary Ammonium Compound**

Growth of *E. faecium* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of QAC were tested but only selected growth curves have been shown including the organism in a) 1:250; b) 1:8000; c) 1:16000 and d) 1:32000 dilution of QAC

| Test Organism | DISINFECTANTS TESTED (Initial Concentration or Dilution) | | | | |
|---|---|---|---|---|---|
| | G(1%) | SH(0.5%) | HP(3%) | P(1:250) | QAC(1:250) |
| *E. faecium* S1 | 8 | 16 | >128 | 8 | 64 |
| *E. faecium* R2 | 16 | 16 | >128 | 8 | 64 |

**Table 19: Bacterial Outgrowth of *Enterococcus faecium* in the Suspension Test Assay**

Bacteria ($10^5$ cfu's/ml) were inoculated in microwell trays containing TSB + 10% FBS and serial dilutions of the initial concentration of each test disinfectant (G=glutaraldehyde; SH= sodium hypochlorite; HP= hydrogen peroxide; P= phenolic and QAC= quaternary ammonium compound). Bacterial growth was monitored by a multiple growth curve reader over a period of 24 hours at 37°C. The data in the table represents the reciprocal of the dilution of the initial concentration where bacterial outgrowth was first detected. Data was compiled from three replicates. Data that is denoted by > refers to no bacterial outgrowth detected in the lowest concentration of disinfectant tested.

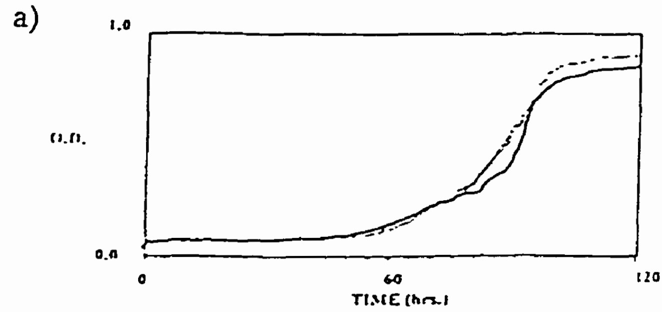
c) ***Mycobacterium chelonae***

Two test strains of *M. chelonae* included a glutaraldehyde resistant strain referred to as the *M. chelonae*$^{R1}$ strain and a multiple antibiotic susceptible ATCC strain called *M. chelonae*$^{S1}$.

The positive control situated on a separate tray was only performed once for each strain therefore this positive control is identical with all test disinfectants. The positive control situated on the same tray as the disinfectant was included with each disinfectant tested. The separate positive control for the *M. chelonae*$^{R1}$ (Fig. 43) strain exhibited typical lag, exponential and stationary phases. This strain showed a maximum OD of 0.9, an OD50 of 90 hours and a lag phase duration of approximately 60 hours. The ATCC strain (Fig. 45) exhibited slow growth characteristics even for the positive control on a separate tray. A lag phase and the initial stage of the exponential phase were observed within 120 hours however, the maximum OD achieved was only 0.4 a.u. The *M. chelonae*$^{S1}$ strain grows well on BA within 48 hours and since visual inspection of the growth in TSB over 120 hours showed good growth, the low maximum OD is not likely due to poor growth. The *M. chelonae*$^{S1}$ strain "pelleted" more readily at the bottom of the assay well compared to the *M. chelonae*$^{R1}$ strain. We believed the pelleting of this strain may account for its unusual growth curve. The *M. chelonae*$^{S1}$ strain's maximum OD was only 0.4 and the OD50 determined from the exponential phase that is visible on the growth curve is approximately 100 hours. The lag phase extends to approximately 70 hours.

The growth curve for both internal and external controls were similar and therefore the data from the test tray could be interpreted. Both strains were not able to grow in 1%

a)




b)

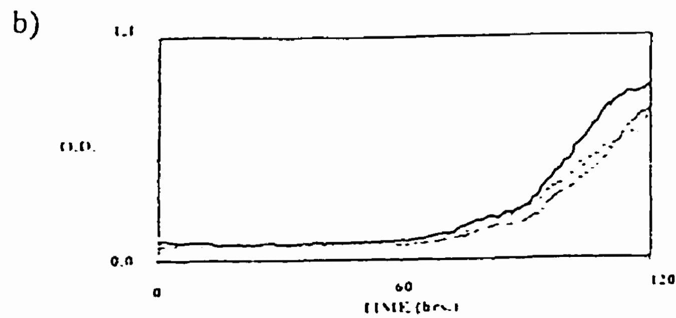



**Figure 43: Positive Control Growth Curve of *Mycobacterium chelonae* [R1] Exposed to Glutaraldehyde**

Growth of *M. chelonae* [R1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde.

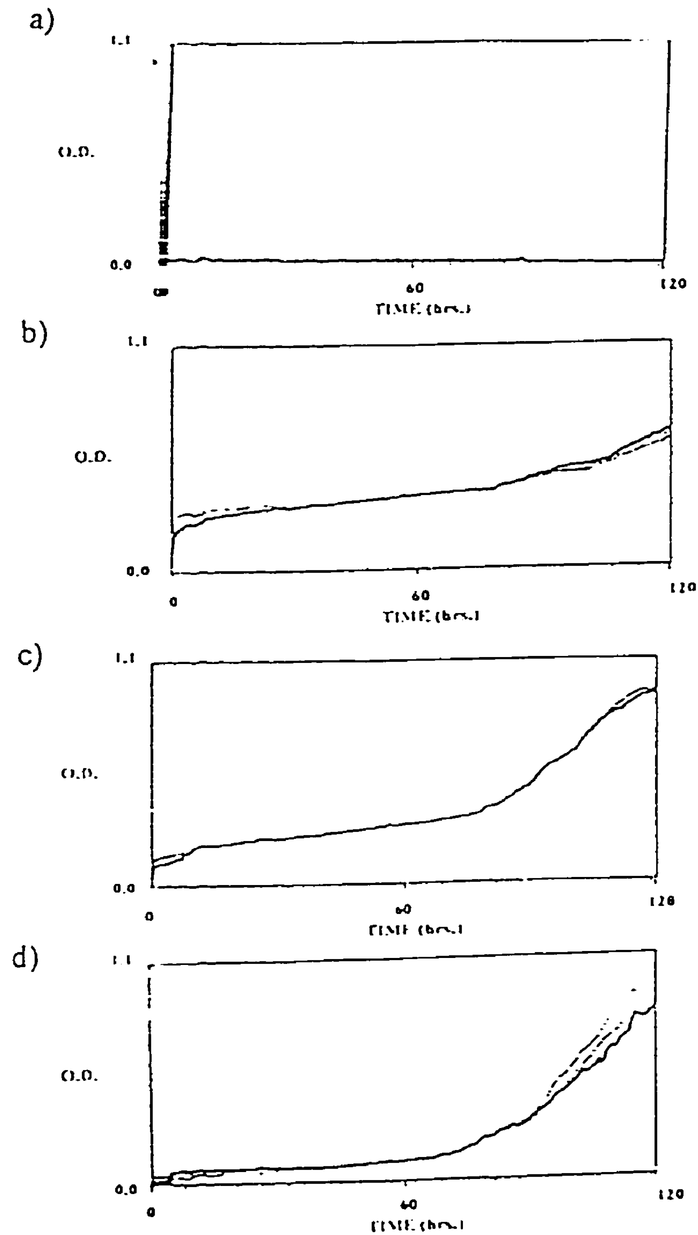
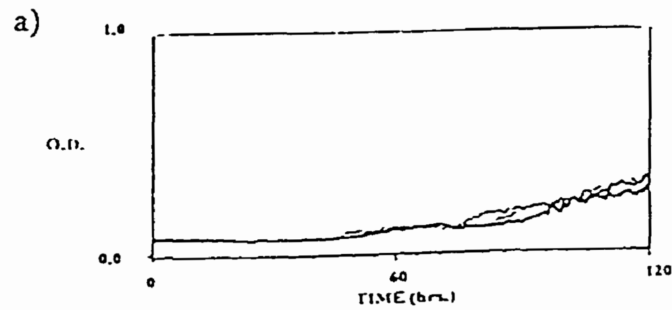



**Figure 44: Growth Curve of *Mycobacterium chelonae* $^{R1}$ Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *M. chelonae* $^{R1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.25%; c)0.125% and d) 0.008% concentrations of glutaraldehyde.

a)




b)

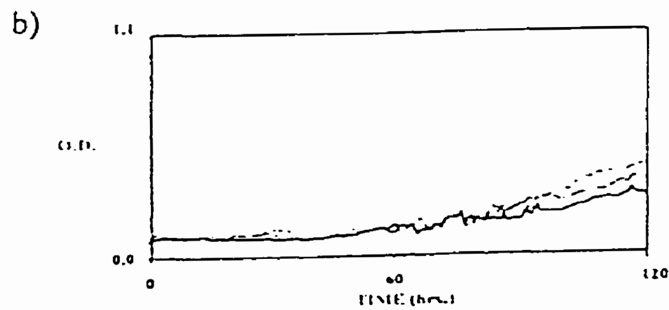
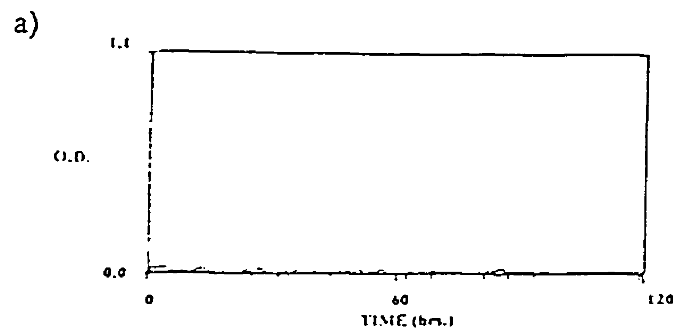



**Figure 45: Positive Control Growth Curve of *Mycobacterium chelonae* [S1] Exposed to Glutaraldehyde**

Growth of *M. chelonae*[S1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde.

a)




b)

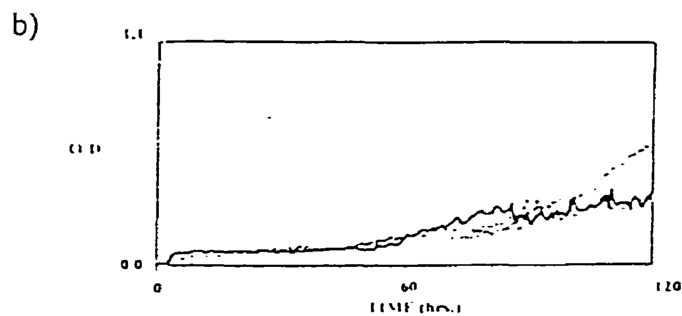



**Figure 46: Growth Curve of *Mycobacterium chelonae* S1 Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *M. chelonae* S2 was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1% and b)0.008% concentrations of glutaraldehyde

glutaraldehyde. *M. chelonae*$^{R1}$ strain first exhibited growth in 0.125% glutaraldehyde(Fig. 44) while the susceptible strain showed outgrowth in 0.008% glutaraldehyde (Fig. 46).

When exposed to sodium hypochlorite, both internal and external positive control growth curves for the *M. chelonae*$^{S1}$ strain were quite similar, while the internal positive control growth curve for the glutaraldehyde resistant strain exhibited an increased lag phase duration, a higher OD50 and lower maximum OD compared to the external positive control growth curve (Fig. 47 and Fig. 49). The differences in the positive control growth curves for the glutaraldehyde resistant strain were not significant enough to warrant a reduction in the initial concentration of sodium hypochlorite used. The highest concentration of sodium hypochlorite where growth did occur was 0.03% in both strains (Fig. 48 and Fig. 50).

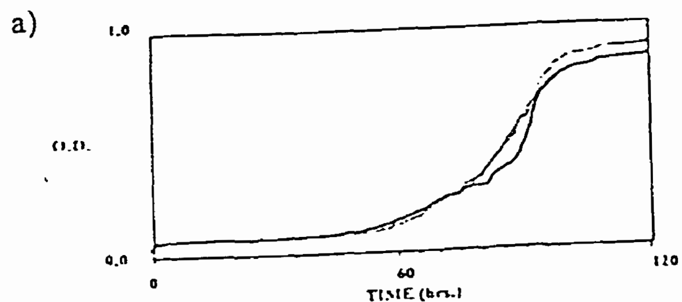
When exposed to hydrogen peroxide, the same tray positive control growth curves showed the same characteristics as the separate tray positive controls. The *M. chelonae*$^{R1}$ strain growth curve exhibited a lag similar to the separate tray positive control but a longer exponential phase was observed in the same tray control resulting in a shortened stationary phase visible within 120 hours (Fig. 51). The maximum OD was 0.9. The same tray positive control growth curve for the *M. chelonae*$^{S1}$ strain was very similar to the separate tray positive control except that the same tray positive control showed a higher maximum OD, approximately 0.5 (Fig. 52). No concentration of hydrogen peroxide tested could support the growth of any of the test strains.

The same tray positive controls when exposed to the phenolic compound were also similar to the separate tray positive controls. Maximum OD, OD50 and lag phase durations were the same in both controls for both test strains (Fig. 53 and Fig. 55). Upon exposure

to the phenolic compound, the *M. chelonae*$^{S1}$ strain showed growth in 1:32000 of the phenolic disinfectant (Fig. 55). The *M. chelonae*$^{R1}$ strain did show significant growth in the 1:8000 dilution (Fig. 54).

Same tray positive controls of the QAC exposed set showed growth curves similar to the separate tray positive controls (Fig. 56 and Fig. 58). The *M. chelonae*$^{S1}$ strain exhibited growth in 1:32000 dilution of QAC. *M. chelonae*$^{R1}$ strain showed growth in the 1:16000 dilution, the second most dilute concentration.

a)




b)

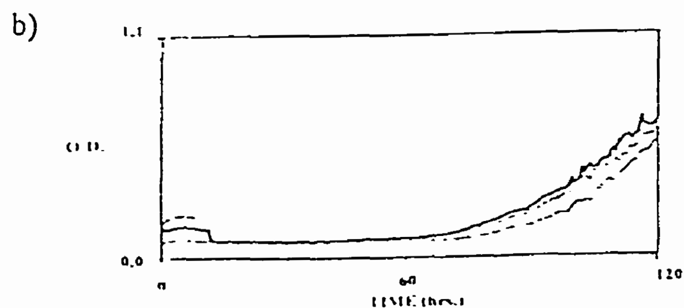



**Figure 47: Positive Control Growth Curve of *Mycobacterium chelonae* [R1] Exposed to Sodium Hypochlorite**

Growth *M. chelonae* [R1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

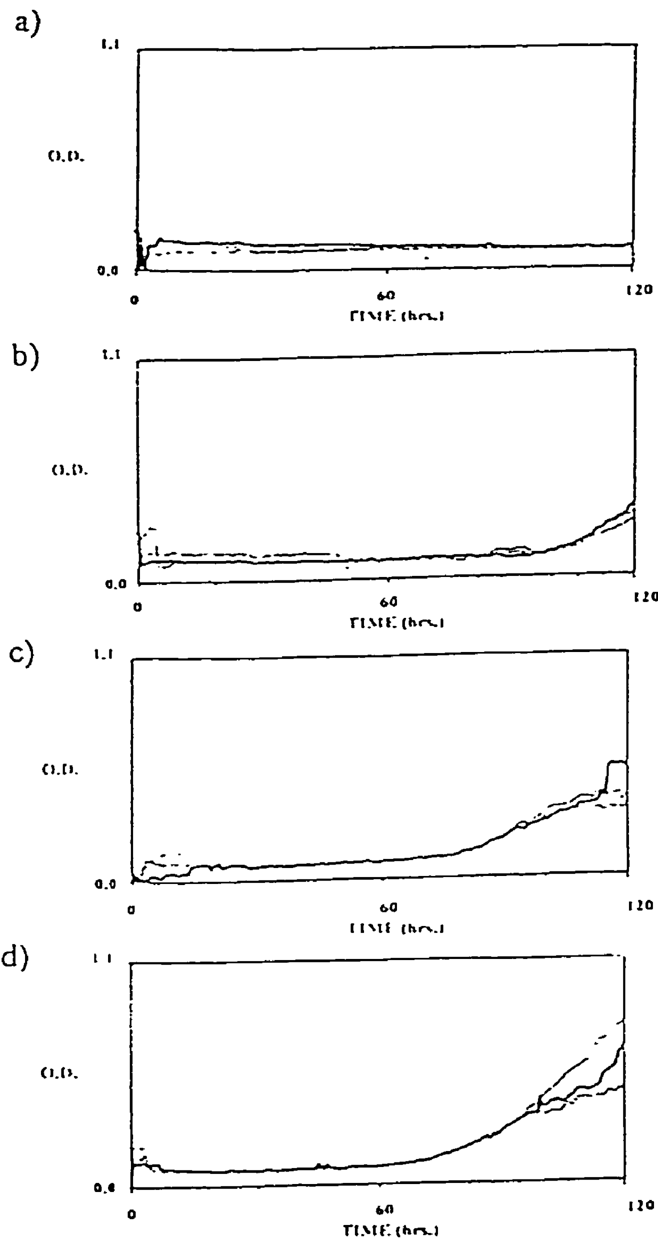
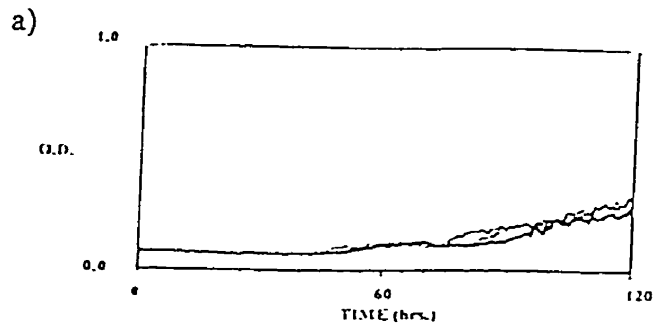



**Figure 48: Growth Curve of *Mycobacterium chelonae* $^{R1}$ Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *M. chelonae* $^{R1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.06%; c)0.03% and d) 0.004% concentrations of sodium hypochlorite.

a)




b)

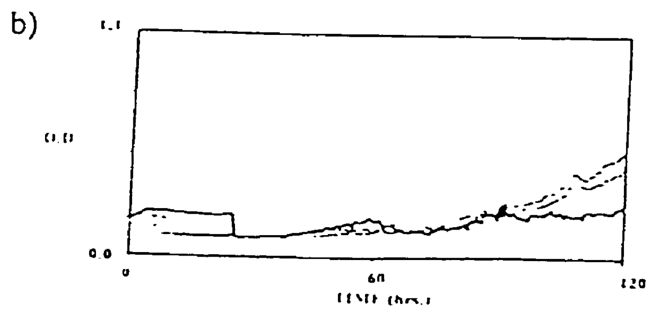



**Figure 49: Positive Control Growth Curve of *Mycobacterium chelonae* [S1] Exposed to Sodium Hypochlorite**

Growth of *M. chelonae* [S1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

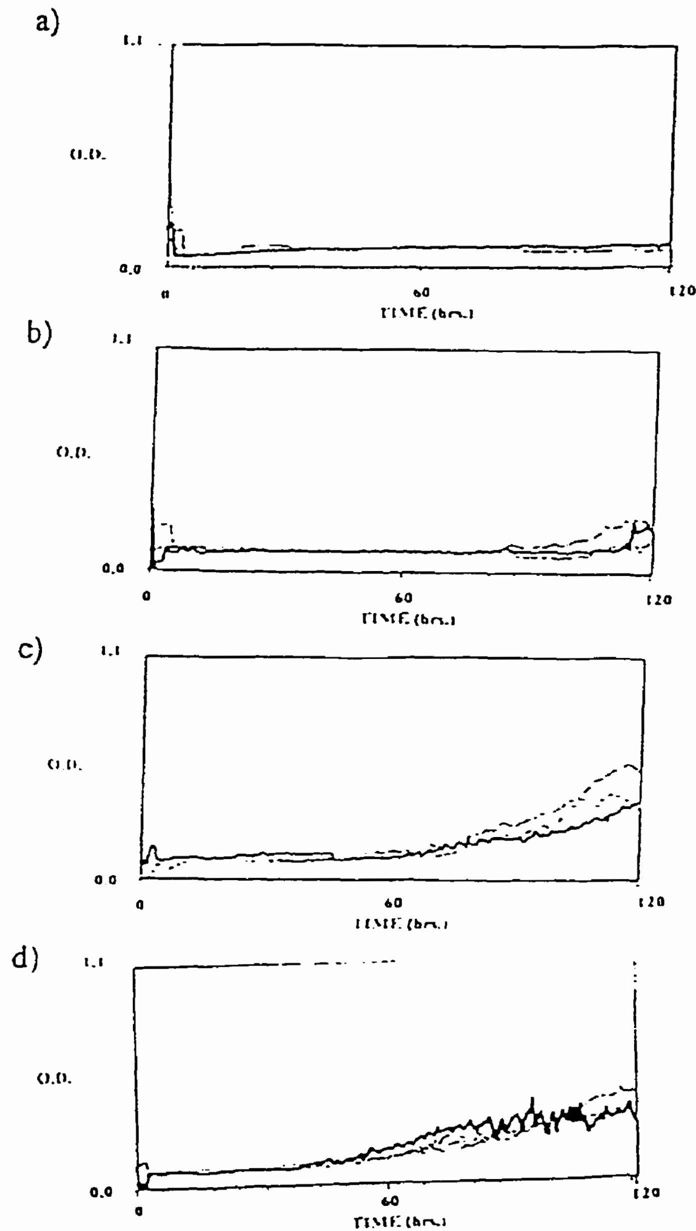



**Figure 50: Growth Curve of *Mycobacterium chelonae* $^{S1}$ Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *M. chelonae* $^{S2}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.06%; c)0.03% and d) 0.004% concentrations of sodium hypochlorite.

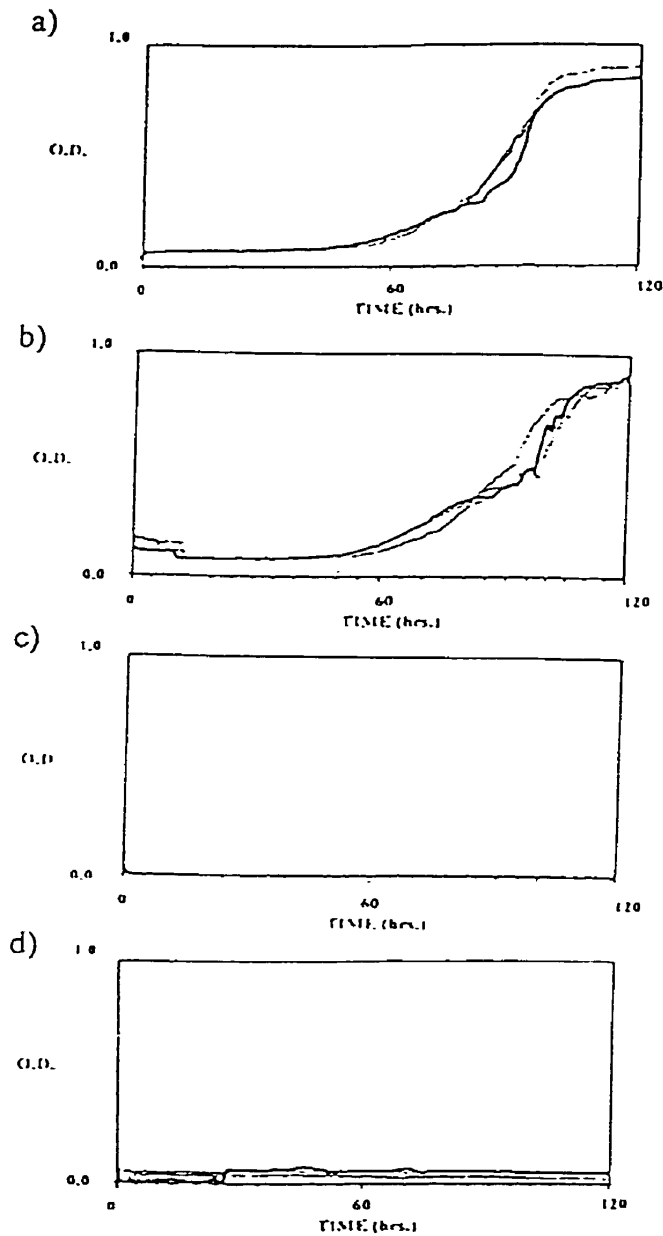



**Figure 51: Growth Curve of *Mycobacterium chelonae*[R1] Exposed to Hydrogen Peroxide**

Growth of *M. chelonae*[R1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

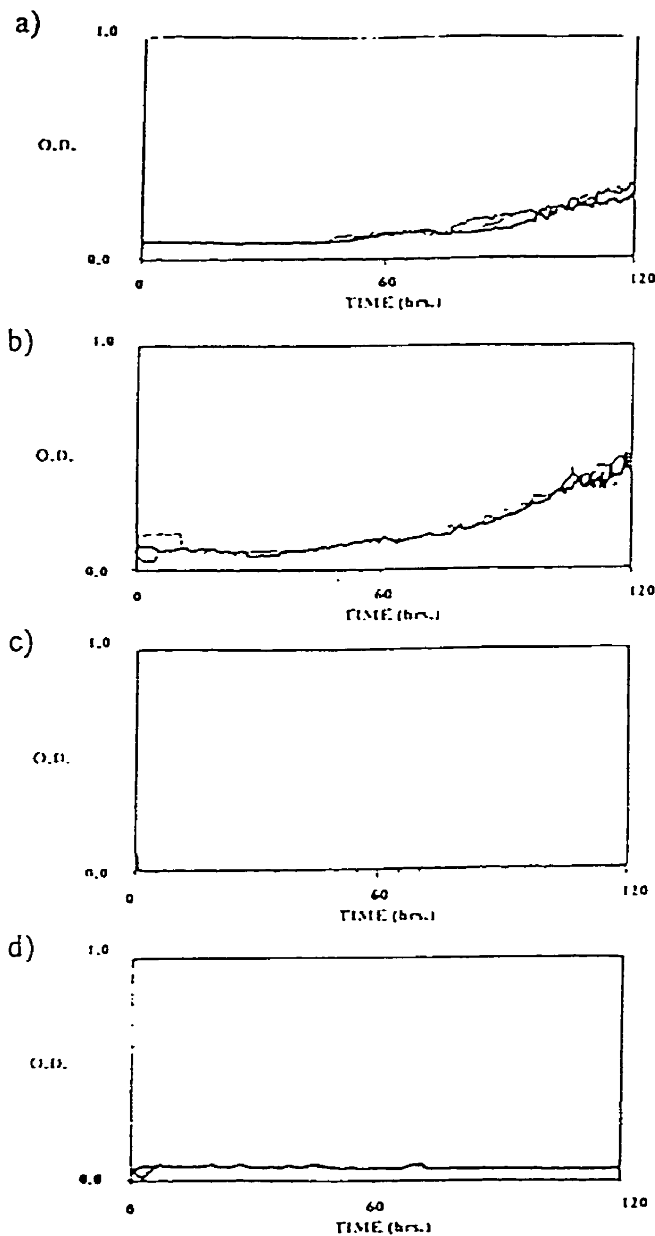
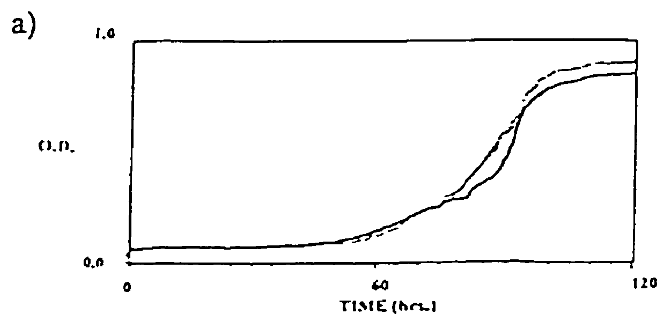



**Figure 52: Growth Curve of *Mycobacterium chelonae*[S1] Exposed to Hydrogen Peroxide**

Growth of *M. chelonae*[S1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide concentrations.

a)




b)

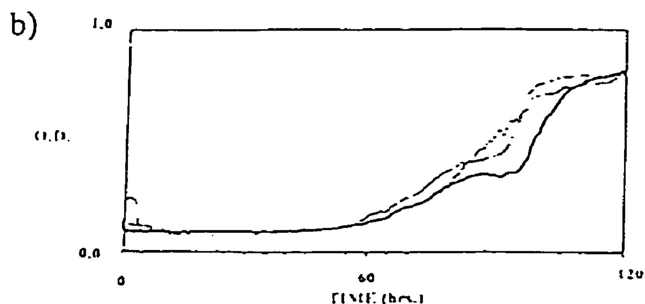



**Figure 53: Positive Control Growth Curve of *Mycobacterium chelonae* $^{R1}$ Exposed to Phenolic Disinfectant**

Growth of *M. chelonae*$^{R1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

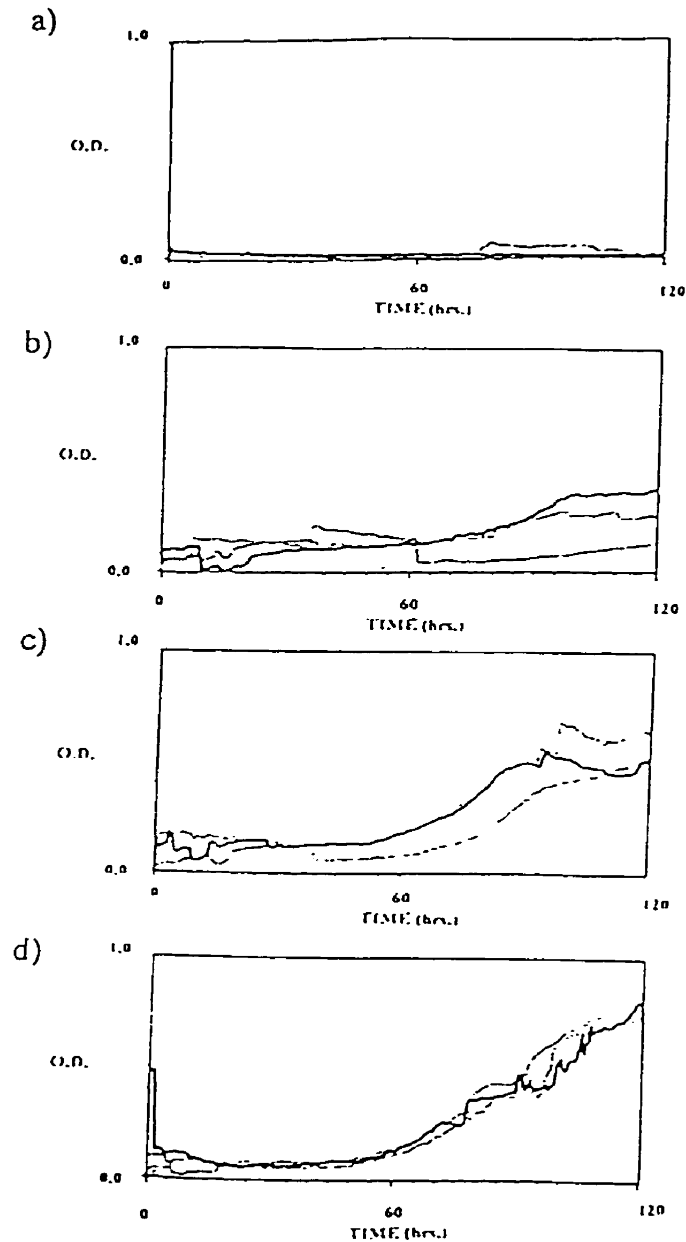



**Figure 54: Growth Curve of *Mycobacterium chelonae* R1 Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *M. chelonae* R1 was monitored using turbidity measurements over a 24 hour period. A series of eight dilution of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b) 1:4000; c) 1:8000 and d) 1:32000 dilutions of phenolic disinfectant.

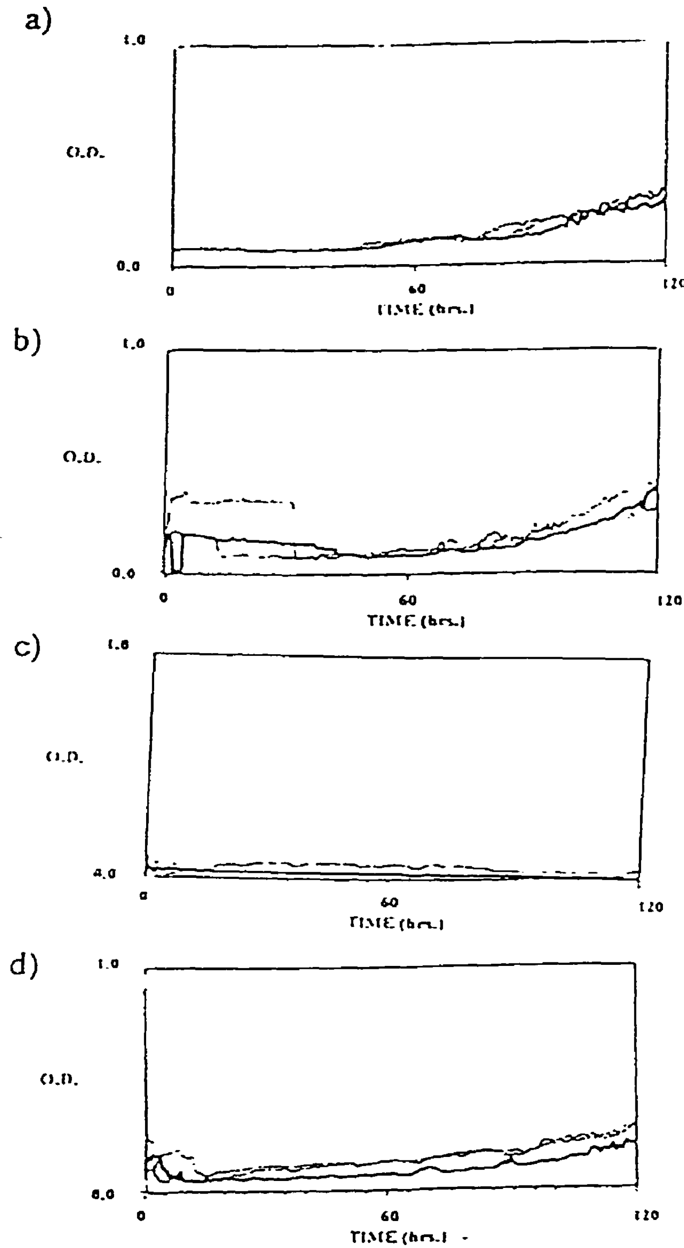
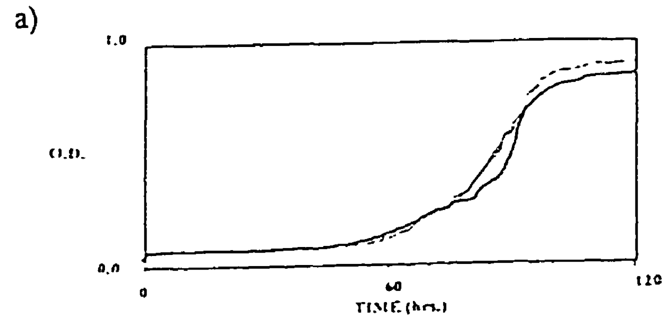



**Figure 55: Growth Curve of *Mycobacterium chelonae* [S1] Exposed to Phenolic Disinfectant**

Growth of *M. chelonae*[S1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without phenolic disinfectant; b) the same tray that contained various dilutions of QAC. Growth was also monitored in c) 1:250; d) 1:32000 dilutions of QAC.

a)



b)

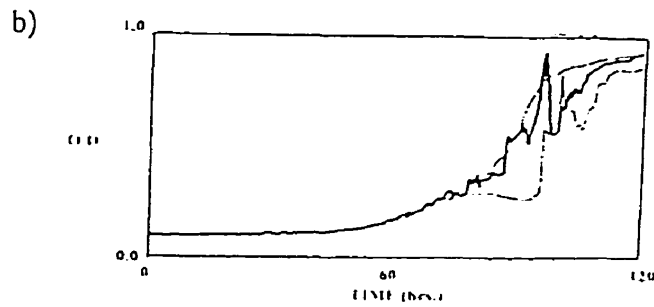


**Figure 56: Positive Control Growth Curve of *Mycobacterium chelonae* [R1] Exposed to Quaternary Ammonium Compound**

Growth of *M. chelonae*[R1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without QAC and b) the same tray that contained various dilutions of QAC.

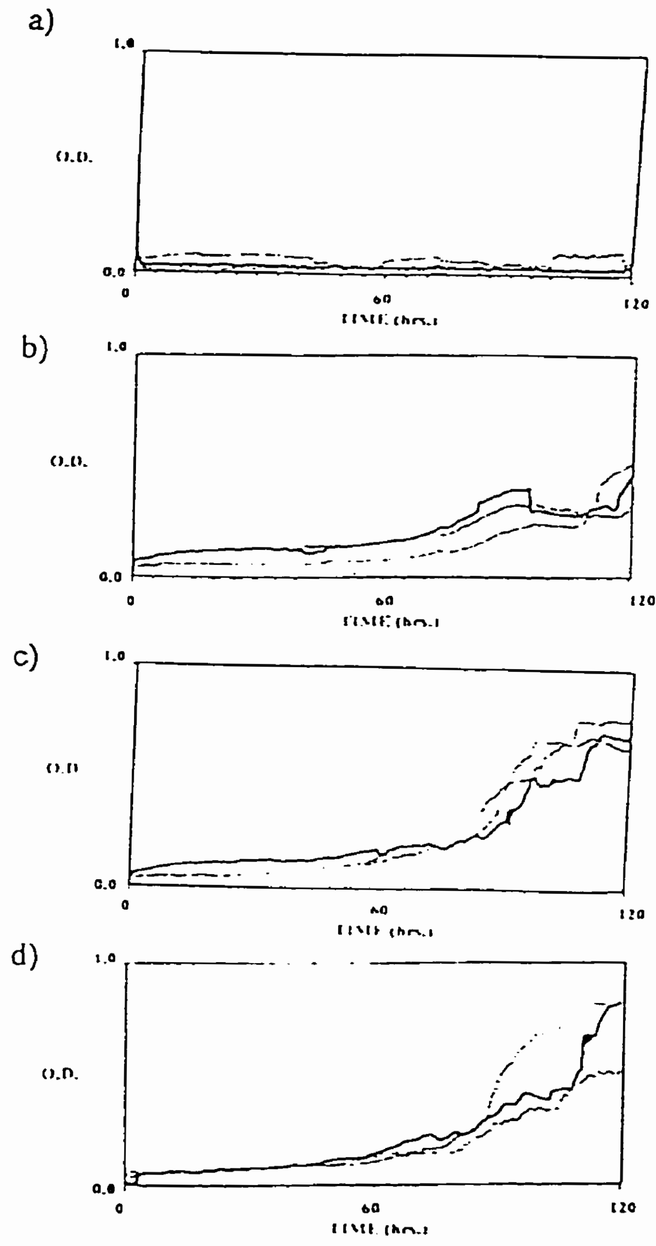



**Figure 57: Growth Curve of *Mycobacterium chelonae* R1 Exposed to Varied Dilutions of Quaternary Ammonium Compound**

Growth of *M. chelonae* R1 was monitored using turbidity measurements over a 24 hour period. A series of eight dilution of QAC were tested but only selected growth curves have been shown including the organism in a) 1:250; b) 1:8000; c) 1:16000 and d) 1:32000 dilution of QAC

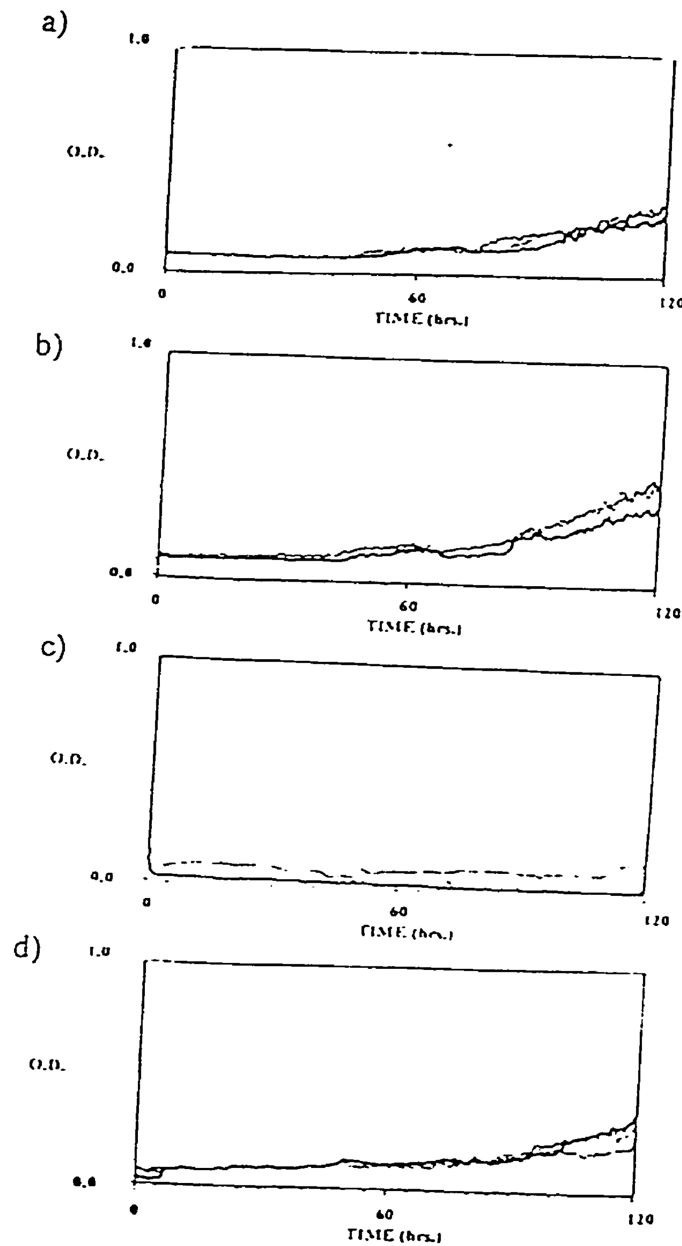



**Figure 58: Growth Curve of *Mycobacterium chelonae* [S1] Exposed to Quaternary Ammonium Compound**

Growth of *M. chelonae*[S1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without QAC; b) the same tray that contained various dilutions of QAC. Growth was also monitored in c) 1:250; d) 1:32000 dilutions of QAC.

**DISINFECTANTS TESTED (Initial Concentration or Dilution)**

| **Test Organism** | **G(1%)** | **SH(0.5%)** | **HP(3%)** | **P(1:250)** | **QAC(1:250)** |
|---|---|---|---|---|---|
| *M. chelonae* [S1] | 128 | 16 | >128 | 128 | 128 |
| *M. chelonae* [R1] | 8 | 16 | >128 | 32 | 64 |

**Table 20: Bacterial Outgrowth of *Mycobacterium chelonae* in the Suspension Test Assay**

Bacteria ($10^5$ cfu's/ml) were inoculated in microwell trays containing TSB + 10% FBS and serial dilutions of the initial concentration of each test disinfectant (G=glutaraldehyde; SH= sodium hypochlorite; HP= hydrogen peroxide; P= phenolic and QAC= quaternary ammonium compound). Bacterial growth was monitored by a multiple growth curve reader over a period of 120 hours at 20°C. The data in the table represents the reciprocal of the dilution of the initial concentration where bacterial outgrowth was first detected. Data was compiled from three replicates. Data that is denoted by > refers to no bacterial outgrowth detected in the lowest concentration of disinfectant tested.

**d)** ***Acinetobacter baumannii***

Two test strains of *A. baumannii* were used in the suspension test method. The multiple resistant *A. baumannii*$^{R1}$ strain and the multiple susceptible *A. baumannii*$^{S1}$ strain. Two positive growth curve controls were performed with each assay run including an internal (on the same tray as the disinfectant) and an external control (on a separate tray without any disinfectant).

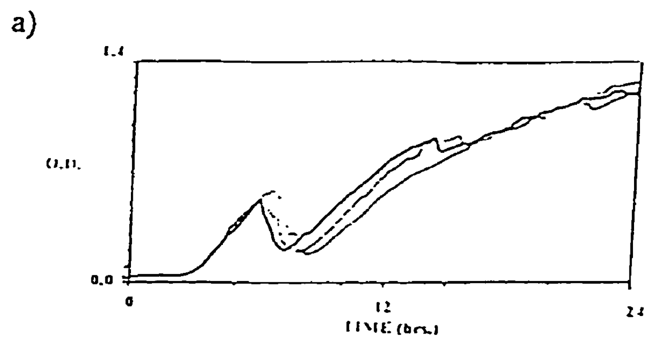
When exposed to glutaraldehyde, the two positive controls of each strain exhibited similar growth characteristics. *A. baumannii*$^{R1}$ (Fig. 59) strain displayed a maximum OD of 1.3 in both internal and external positive controls. The lag phase duration and OD50 were also the same in both controls. This strain also exhibits an unusual, strain-specific growth pattern.

*A. baumannii*$^{S1}$ (Fig. 61) does not exhibit this biphasic type of growth. Its growth is more typical of bacterial growth curves. The lag, exponential and stationary phases are evident in the 24 hour observation period. The lag phase was approximately 4 hours, the maximum OD is 1.5. a.u. and the OD50 was approximately 8 hours for both the internal and external growth curves.

When exposed to the highest concentration of 1% glutaraldehyde, neither strain shows any bacterial growth although both strains did show outgrowth in 0.06% glutaraldehyde (Figs. 60 and 62). Although both strains show maximum OD's comparable to the positive controls, it is obvious that the lag phase durations have increased.

The positive control growth curves of the sodium hypochlorite assay were similar to the curves of the glutaraldehyde exposed controls. The *A. baumannii*$^{R1}$ (Fig. 63) strain

a)




b)

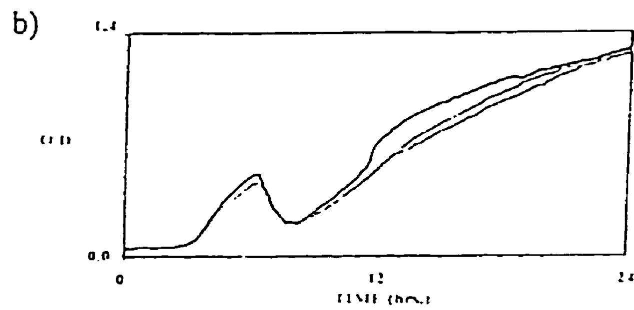



**Figure 59: Positive Control Growth Curve of *Acinetobacter baumannii* [R1] Exposed to Glutaraldehyde**

Growth of *A. baumannii* [R1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

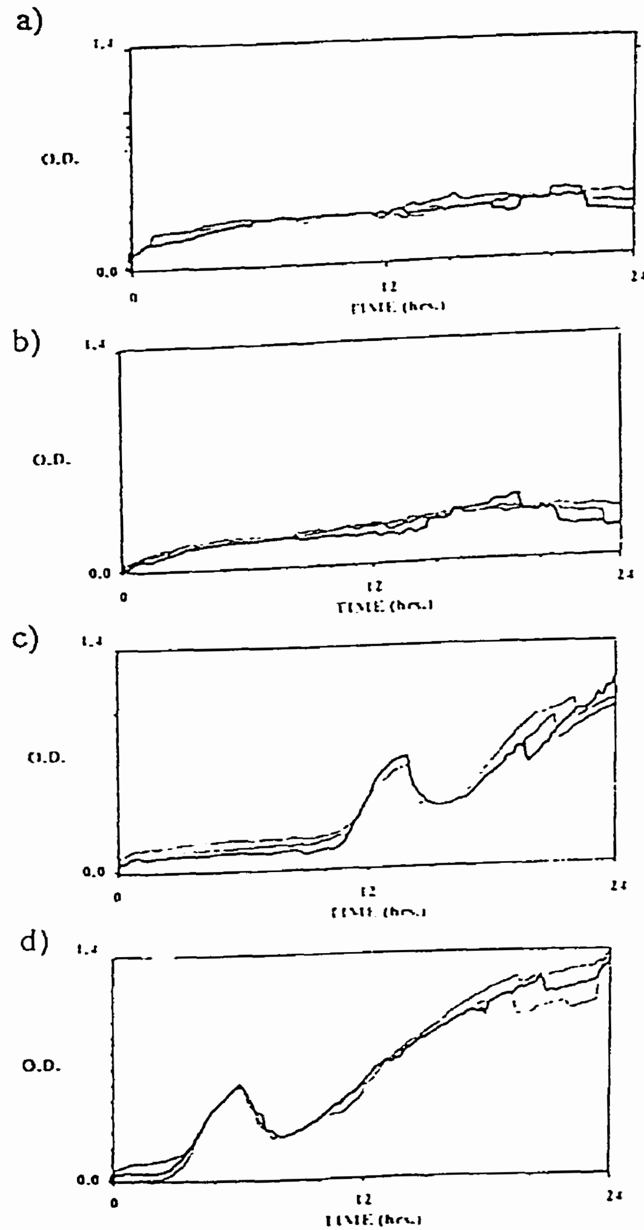



**Figure 60: Growth Curve of *Acinetobacter baumannii* $^{RI}$ Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *A. baumannii* $^{RI}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.125%; c)0.06% and d) 0.008% glutaraldehyde concentrations.

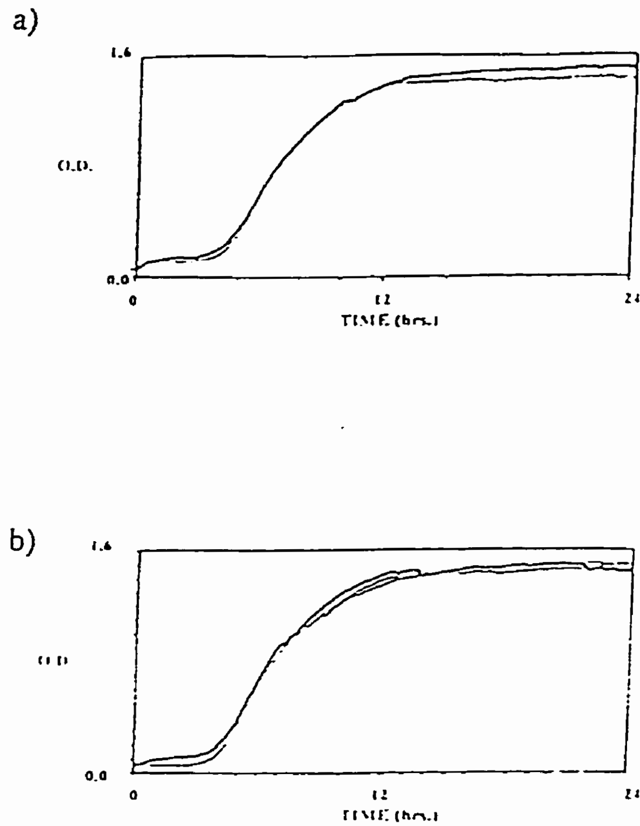



**Figure 61: Positive Control Growth Curve of *Acinetobacter baumannii*$^{S1}$ Exposed to Glutaraldehyde**

Growth of *A. baumannii* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without glutaraldehyde and b) the same tray that contained various concentrations of glutaraldehyde..

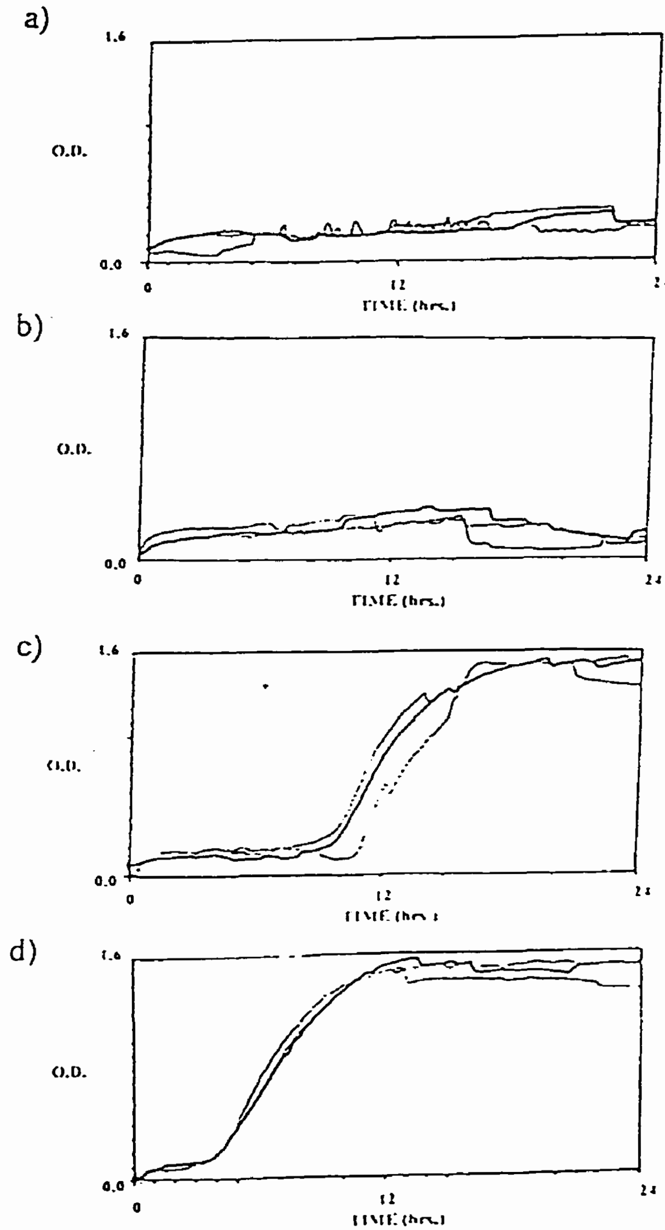



**Figure 62: Growth Curve of *Acinetobacter baumannii* $^{S1}$ Exposed to Varied Concentrations of Glutaraldehyde**

Growth of *A. baumannii* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of glutaraldehyde were tested but only selected growth curves have been shown including the organism in a) 1%; b)0.125%; c)0.06% and d) 0.008% glutaraldehyde concentrations.

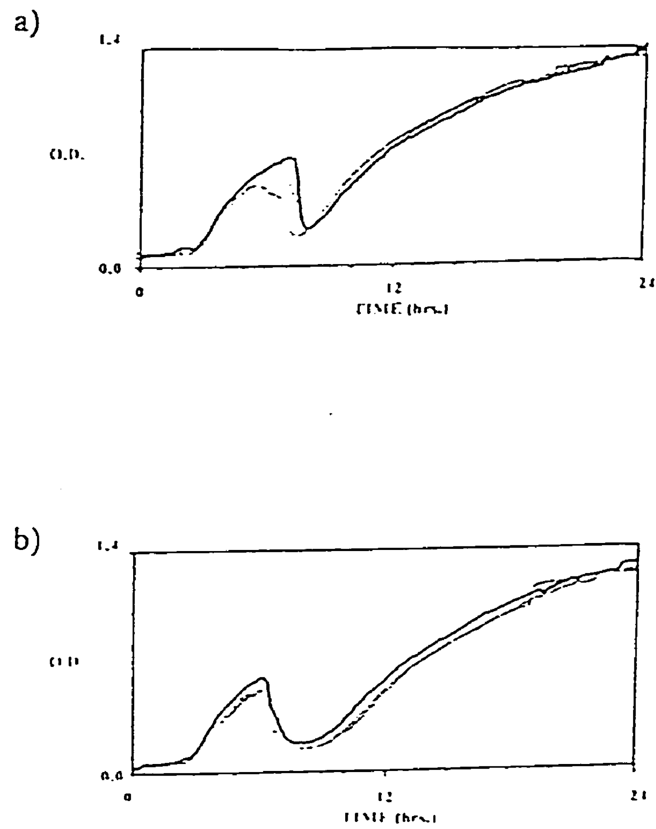



**Figure 63: Positive Control Growth Curve of *Acinetobacter baumannii* [R1] Exposed to Sodium Hypochlorite**

Growth of *A. baumannii* [R1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

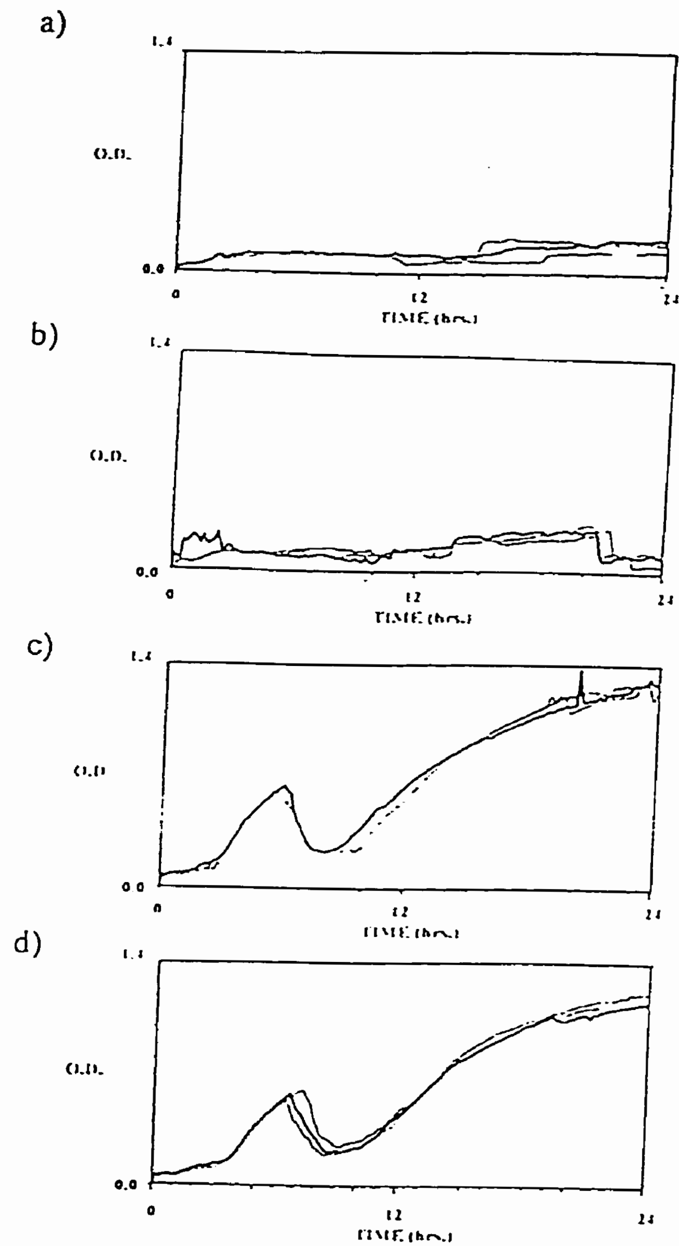



**Figure 64: Growth Curve of *Acinetobacter baumannii* [RI] Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *A. baumannii* [RI] was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.03%; c)0.016% and d) 0.004% sodium hypochlorite concentrations.

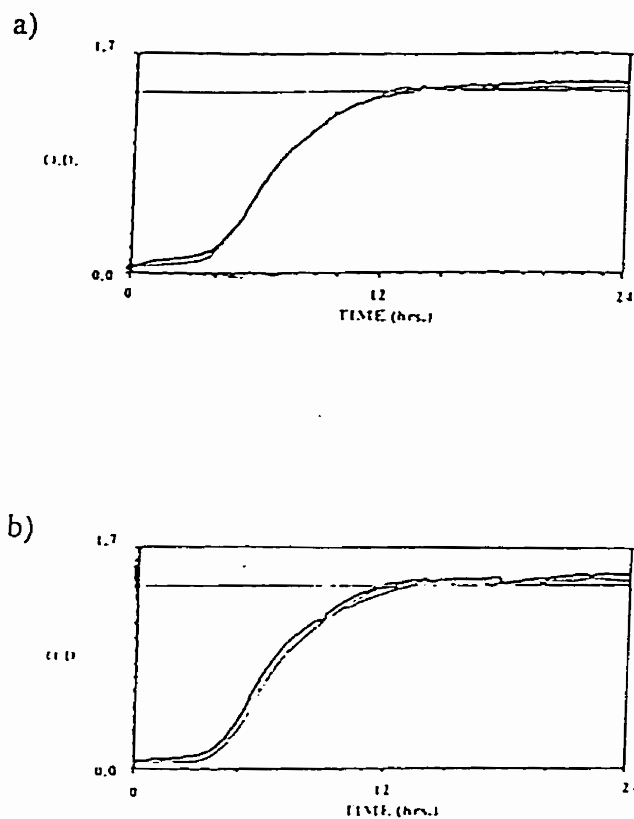



**Figure 65: Positive Control Growth Curve of *Acinetobacter baumannii* $^{S1}$ Exposed to Sodium Hypochlorite**

Growth of *A. baumannii* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without sodium hypochlorite and b) the same tray that contained various concentrations of sodium hypochlorite.

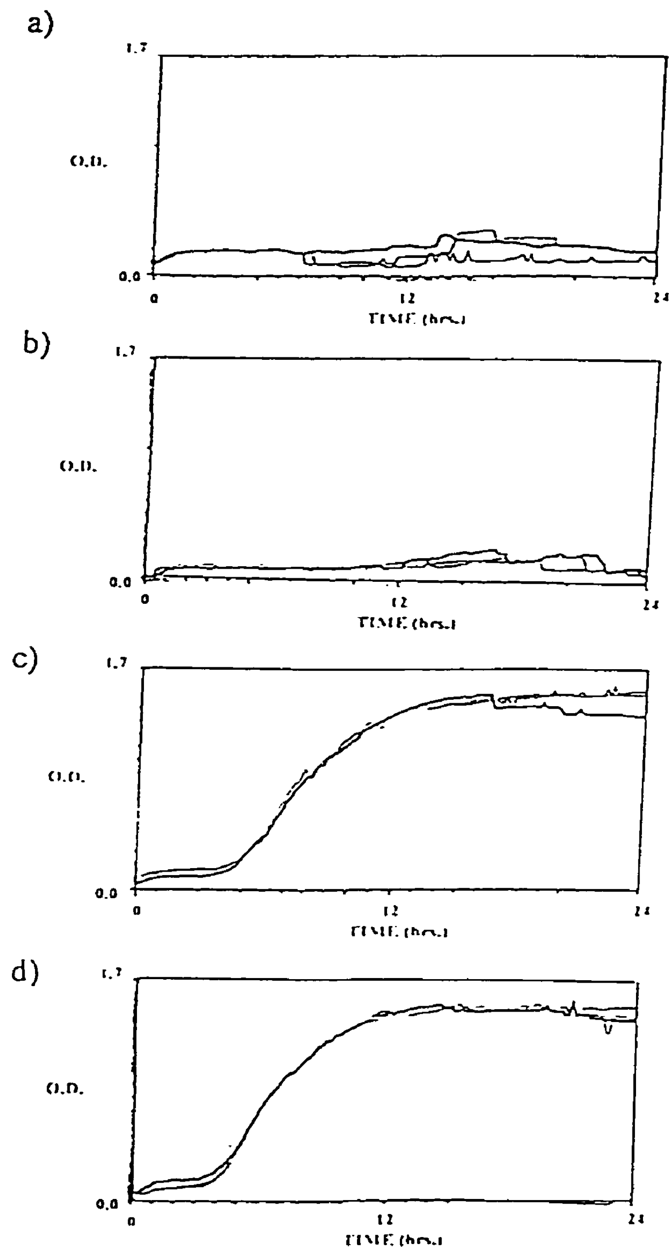



**Figure 66: Growth Curve of *Acinetobacter baumannii* S1 Exposed to Varied Concentrations of Sodium Hypochlorite**

Growth of *A. baumannii* S1 was monitored using turbidity measurements over a 24 hour period. A series of eight concentrations of sodium hypochlorite were tested but only selected growth curves have been shown including the organism in a) 0.5%; b)0.03%; c)0.016% and d) 0.004% sodium hypochlorite concentrations.

exhibited its characteristic biphasic growth pattern. There was no significant difference in growth patterns of the same tray or separate tray control. The same is true for *A. baumannii*$^{S1}$ (Fig. 65). The first concentration of sodium hypochlorite tested was 0.5%. Both of the test strains showed no growth at this concentration. Bacterial outgrowth did occur in 0.016% glutaraldehyde in each strain (Figs. 64 and 66).

The positive control growth curves of the *A. baumannii*$^{R1}$ strain corresponding to exposure to hydrogen peroxide did not exhibit the bi-phasic pattern of growth (Fig.67). The growth pattern was similar to the growth pattern observed in *A. baumannii*$^{S1}$ strain (Fig.68). The maximum OD, lag phase duration and OD50 were similar in both external and internal controls for both test strains. No concentration of hydrogen peroxide supported the growth of any of the test strains.

The positive controls for the *A. baumannii*$^{R1}$ strain, when exposed with the phenolic based disinfectant, did not show the bi-phasic phase pattern of growth (Fig.69). The maximum OD of the separate tray control was 1.3 and slightly decreased in the same tray control. All other values were equivalent in the two controls. A slight decrease in maximum OD's was also found with the same tray control of the *A. baumannii*$^{S1}$ strain (Fig. 71).

The first dilution of the phenolic disinfectant (1:250) supported no growth of any test strain. A dilution of 1:500 of the phenolic based compound did support the outgrowth of the two test strains (Figs.70 and 72). The growth patterns at this dilution were characterized by a decrease in maximum OD's, longer lag phase durations and increased OD50 values.

The two positive controls for QAC exposed assay were similar in lag phase duration

but the same tray controls tended to have slightly lower maximum OD's than their separate tray control counterparts. The lowest dilution that did support growth in both strains was 1:8000. The growth pattern of the *A. baumannii*$^{R1}$ strain in this dilution was significantly different from the positive controls. Only 1 replicate out of the three performed showed any growth. The growth of this replicate was characterized by an extremely long lag phase (13 hours) and only reached a maximum OD of 0.9. The differences in growth pattern of the *A. baumannii*$^{S1}$ strain in the 1:8000 dilution compared to the positive controls was not as obvious. The maximum OD decreased to 1.3 and the lag phase duration was increased to 6 hours.

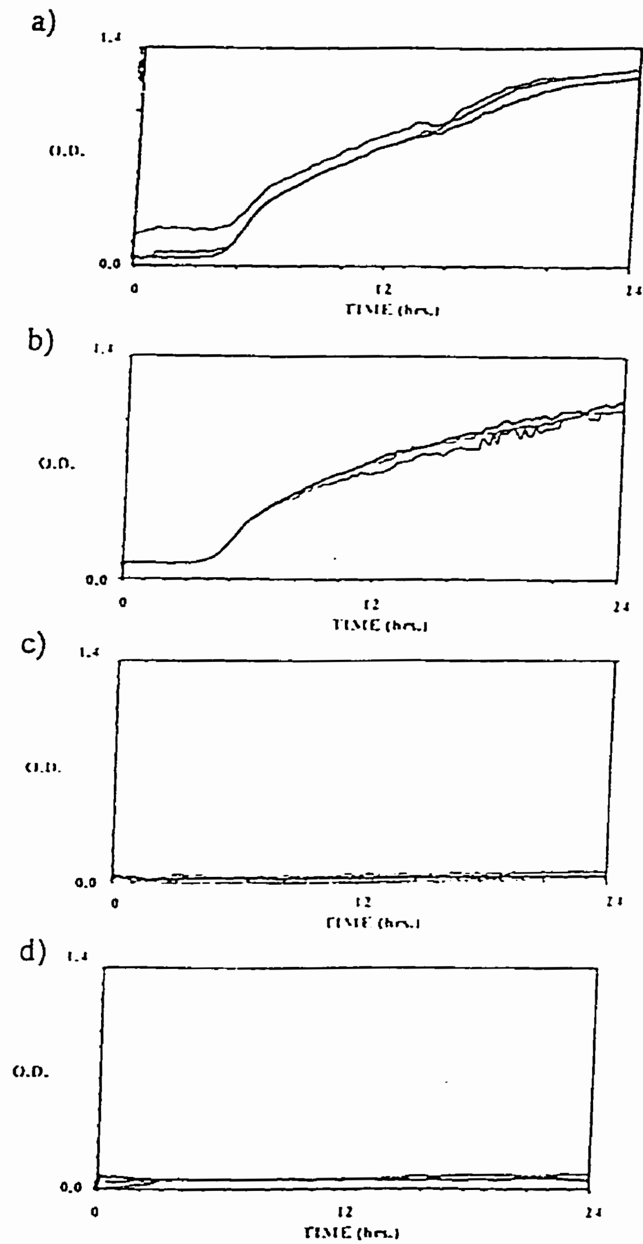



**Figure 67: Growth Curve of *Acinetobacter baumannii*$^{R1}$ Exposed to Hydrogen Peroxide**

Growth of *A. baumannii*$^{R1}$ was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

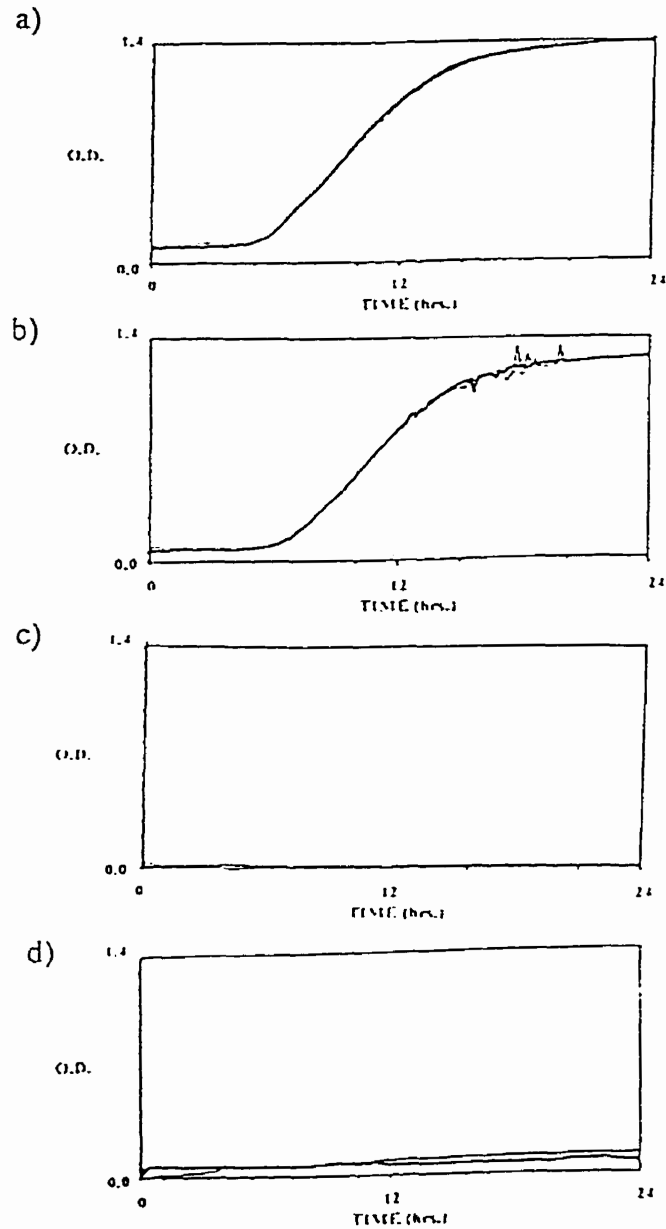
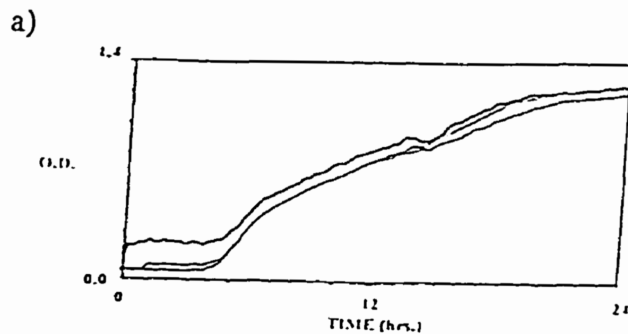



**Figure 68: Growth Curve of *Acinetobacter baumannii* [S1] Exposed to Hydrogen Peroxide**

Growth of *A. baumannii* [S1] was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without hydrogen peroxide; b) the same tray that contained various concentrations of hydrogen peroxide. Growth was also monitored in c) 3%; d) 0.02% hydrogen peroxide.

a)




b)

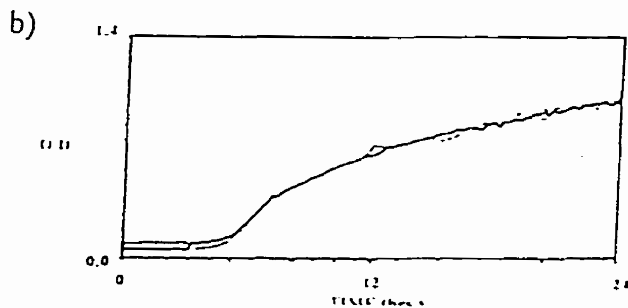



**Figure 69: Positive Control Growth Curve of *Acinetobacter baumannii* $^{R1}$ Exposed to Phenolic Disinfectant**

Growth of *A. baumannii* $^{R1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

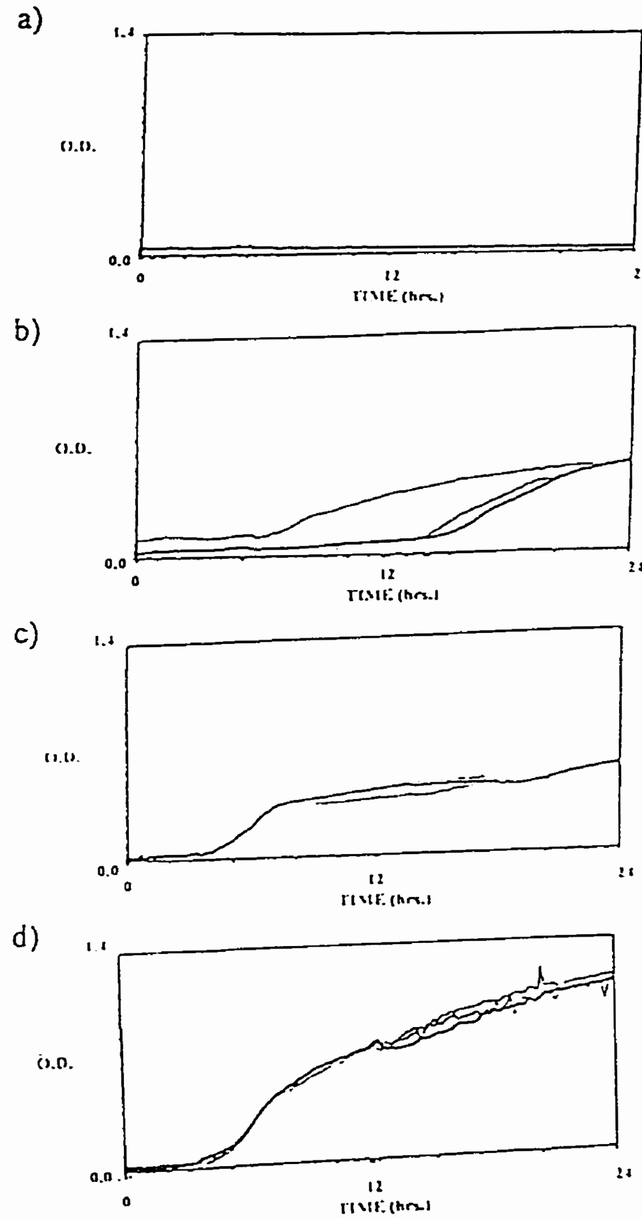



**Figure 70: Growth Curve of *Acinetobacter baumannii* [R1] Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *A. baumannii* [R1] was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:500; c)1:1000 and d) 1:32000 dilution of phenolic disinfectant.

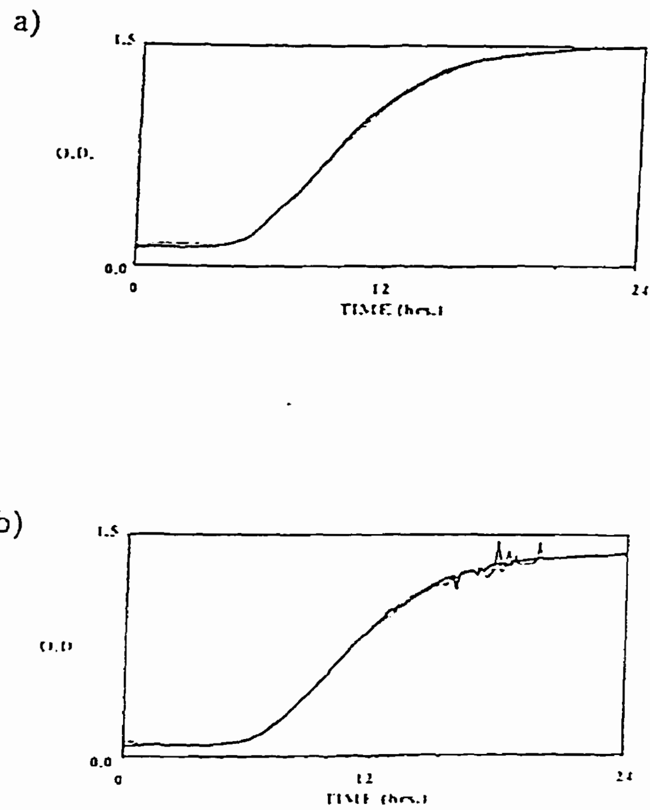



**Figure 71: Positive Control Growth Curve of *Acinetobacter baumannii* $^{S1}$ Exposed to Phenolic Disinfectant**

Growth of *A. baumannii* $^{S1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without phenolic disinfectant and b) the same tray that contained various concentrations of phenolic disinfectant.

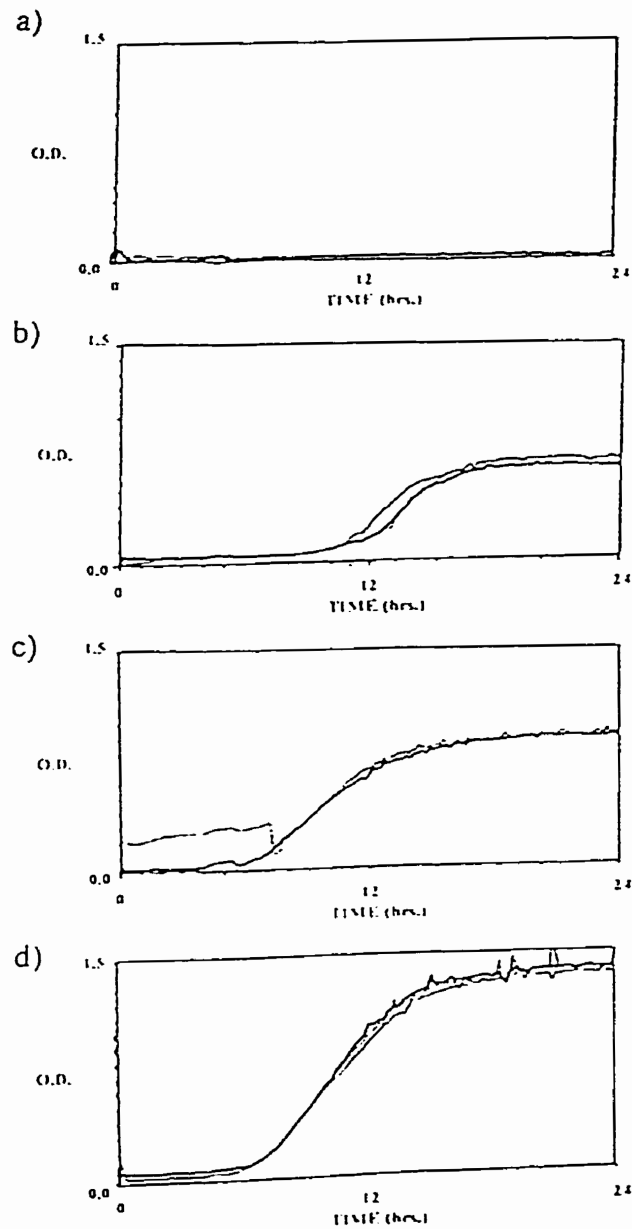



**Figure 72: Growth Curve of *Acinetobacter baumannii* $^{S1}$ Exposed to Varied Dilutions of Phenolic Disinfectant**

Growth of *A. baumannii* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of phenolic disinfectant were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:500; c)1:1000 and d) 1:32000 dilution of phenolic disinfectant

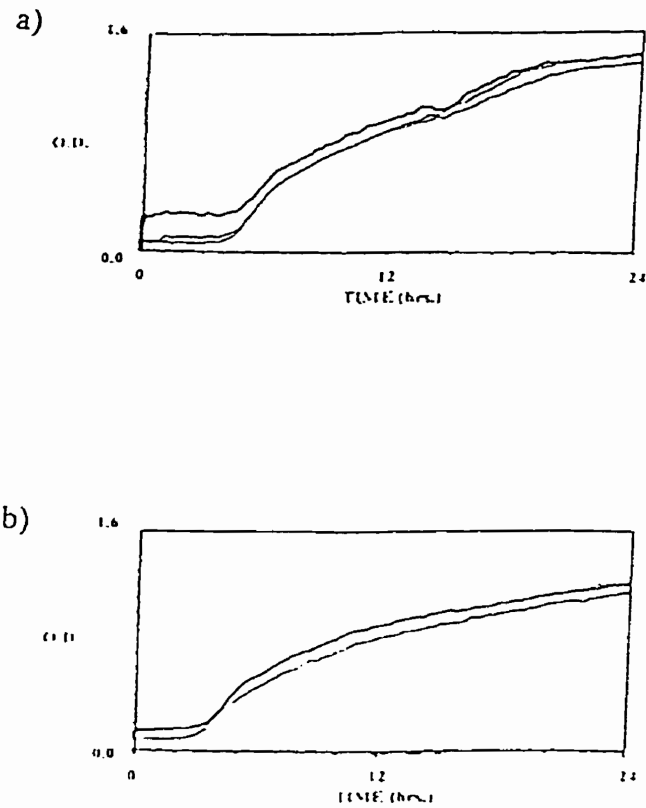



**Figure 73: Positive Control Growth Curve of *Acinetobacter baumannii* $^{R1}$ Exposed to Quaternary Ammonium Compound**

Growth of *A. baumannii* $^{R1}$ in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a) a separate tray without QAC and b) the same tray that contained various dilutions of QAC.

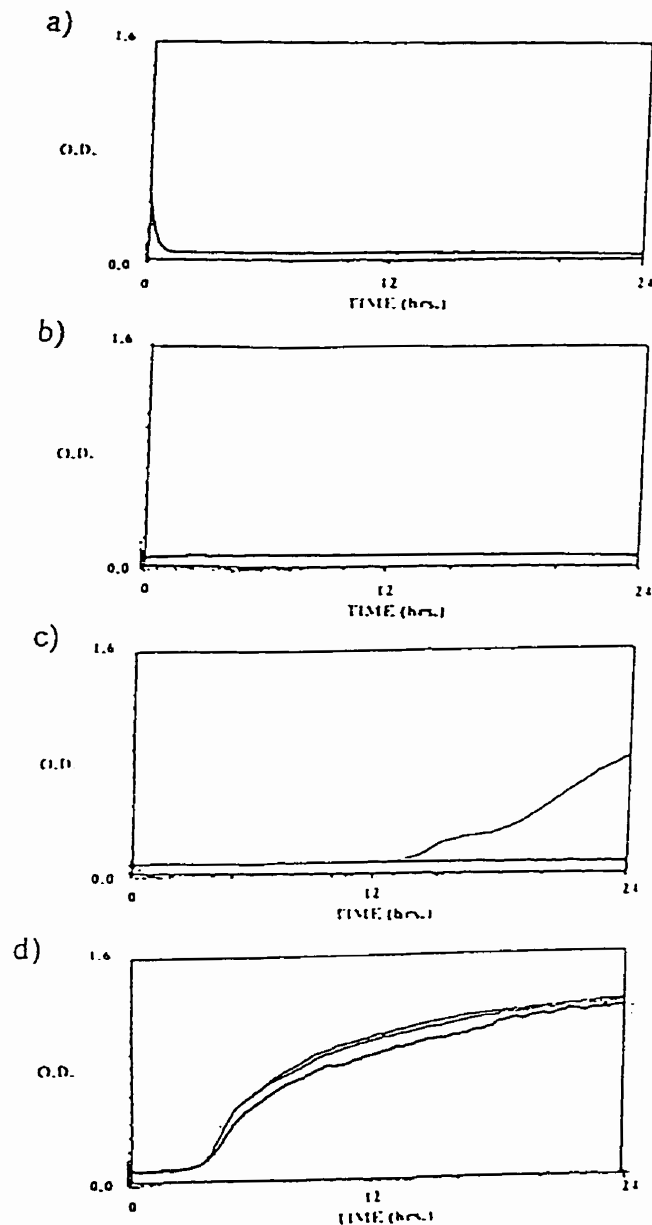



**Figure 74: Growth Curve of *Acinetobacter baumannii* RI Exposed to Varied Dilutions of Quaternary Ammonium Compound**

Growth of *A. baumannii* RI was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of QAC were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:4000; c)1:8000 and d) 1:32000 dilution of QAC

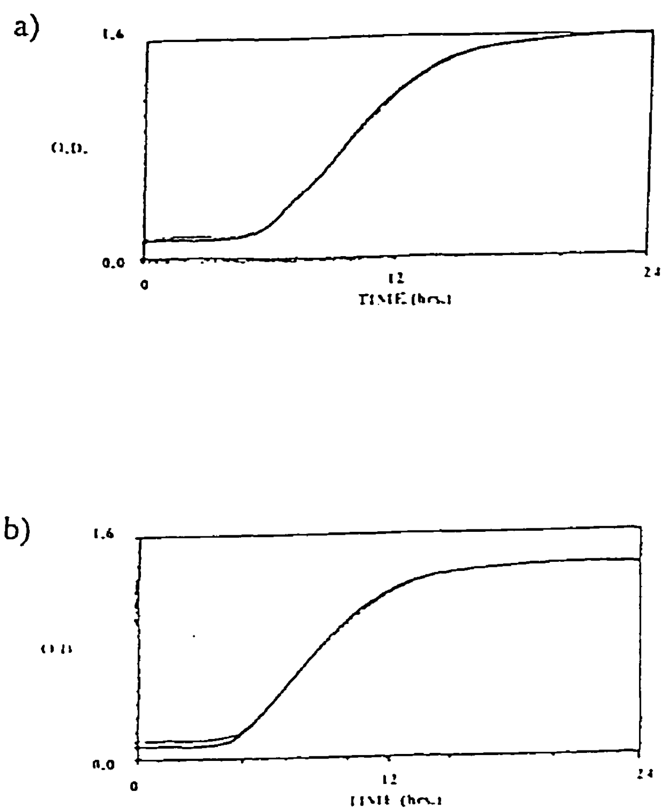



**Figure 75: Positive Control Growth Curve of *Acinetobacter baumannii* [S1] Exposed to Quaternary Ammonium Compound**

Growth of *A. baumannii* [S1] in each test well was monitored using turbidity measurements over a 24 hour period. Positive controls consisted of the test organism in a)a separate tray without QAC and b) the same tray that contained various dilutions of QAC.

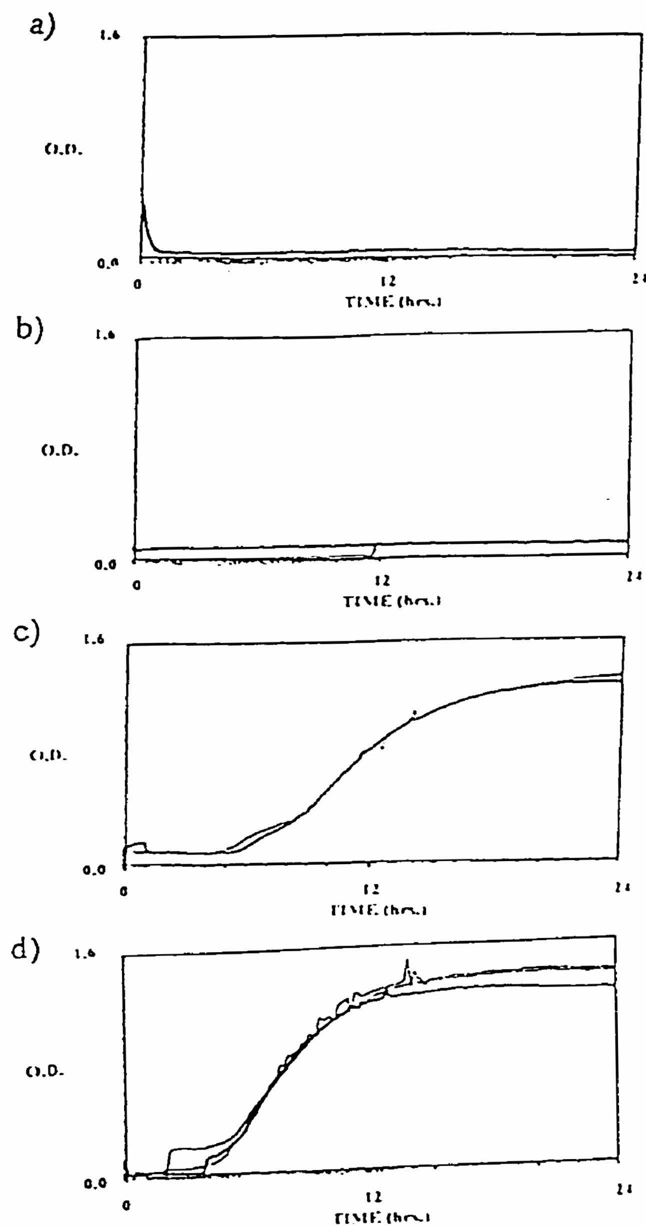



**Figure 76: Growth Curve of *Acinetobacter baumannii* $^{S1}$ Exposed to Varied Dilutions of Quaternary Ammonium Compound**

Growth of *A. baumannii* $^{S1}$ was monitored using turbidity measurements over a 24 hour period. A series of eight dilutions of QAC were tested but only selected growth curves have been shown including the organism in a) 1:250; b)1:4000; c)1:8000 and d) 1:32000 dilution of QAC

DISINFECTANTS TESTED (Initial Concentration or Dilution)

| Test Organism | G(1%) | SH(0.5%) | HP(3%) | P(1:250) | QAC(1:250) |
|---|---|---|---|---|---|
| *A. baumannii* [S1] | 16 | 32 | >128 | 2 | 32 |
| *A. baumannii* [R1] | 16 | 32 | >128 | 2 | 32 |

**Table 21: Bacterial Outgrowth of *Acinetobacter baumannii* in the Suspension Test Assay**

Bacteria ($10^5$ cfu's/ml) were inoculated in microwell trays containing TSB + 10% FBS and serial dilutions of the initial concentration of each test disinfectant (G=glutaraldehyde; SH= sodium hypochlorite; HP= hydrogen peroxide; P= phenolic and QAC= quaternary ammonium compound). Bacterial growth was monitored by a multiple growth curve reader over a period of 24 hours at 37°C. The data in the table represents the reciprocal of the dilution of the initial concentration where bacterial outgrowth was first detected. Data was compiled from three replicates. Data that is denoted by > refers to no bacterial outgrowth detected in the lowest concentration of disinfectant tested.

# DISCUSSION

## i) Introduction

The numbers of hospital-acquired infections have steadily increased in many countries (130). Although many antibacterial agents have been developed and introduced into clinical use over the past five decades, bacterial infections are still a serious cause of patient morbidity and mortality (64). This is largely due to the development more immunocompromised patients and multiple antibiotic resistance in these organisms. As a result, attention is being directed to the responses of various types of bacteria to chemical disinfectants, specifically, whether antibiotic resistant bacteria are cross-resistant to chemical disinfectants.

The objective of this research was to determine whether multiple antibiotic resistant bacteria are associated with higher resistance to chemical disinfectants when compared to the same multiple antibiotic susceptible organism. Many investigators have studied this possible association but have concluded that multiple antibiotic resistant organisms are not more resistant to chemical disinfectants than multiple antibiotic susceptible strains (4, 5, 8 and 129). Our research project served to expand the methods used by previous investigators by using a range of test bacteria that have been linked to hospital outbreaks and multiple antibiotic resistant organisms, the addition of an organic challenge and variations in disinfectant concentration and exposure time. This study was carried out in two distinct assays. The Surface Carrier Test employed a PVC lumen carrier that was inoculated with test bacteria that were suspended in an organic soil (ATS). The organic soil was formulated to simulate the residual components found on patient used medical devices. The organisms in the test soil were dried onto the lumen carrier which was exposed to the disinfectant and quantitative

measurements of residual, viable bacteria were taken. The Surface Carrier Test determined the efficacy of the test disinfectant to kill bacteria in a physical environment in which disinfectant contact with the bacteria was reduced (ie. it must penetrate from the top of the film of organisms to the bottom). The Suspension Test does not involve any physical "road blocks" because the disinfectant and bacteria were in constant contact. The bacteria were inoculated into a medium containing an organic challenge different from the ATS used in the Surface Carrier Test. Test disinfectants were added directly to the bacteria containing media. Bacterial growth was monitored using continuous spectrophotometric absorbance measurements. Although the Surface Carrier Test and the Suspension Test generated data that is distinct from each other, the results from each test were compiled and this information has given us new insight into the relationship between chemical disinfectants and multiple antibiotic resistant organisms.

**ii) Surface Disinfection**

It is well known that cleaning and/or disinfection is important in the health care setting (62). This is true for medical devices and environmental surfaces. Adequate cleaning and/or disinfection plays a role in the reduction of nosocomial infections. According to Spaulding's classification of patient care items and equipment (122), health care environmental surfaces fall into the category of non-critical items. Non-critical items have virtually no risk of transmitting infectious agents to patients or staff (106) therefore low-level disinfection of these items is often performed. Medical devices, on the other hand, require a more increased level of disinfection. Medical devices are usually divided into semi-critical and critical items. Semi-critical items come into contact with mucous membranes of the patient while critical

items enter sterilized areas of the body such as sterile tissues or the vascular system (106). Semi-critical items must at least be high-level disinfected and critical items must be sterilized.

Choosing an appropriate disinfectant for semi-critical and critical items is difficult and often confusing. The microbial activity of every commercially available disinfectant is indicated on each package. The manufacturer's label claims indicate the activity of each disinfectant, the main active ingredient, the use-concentration and the disinfectant exposure time to be used to achieve the level of disinfection noted. The difficulty and confusion arise from the many choices one has. There has also been an increase in the numbers of disinfectants claiming activity against multiple antibiotic resistant bacteria. This study was aimed at assessing the disinfectant efficacy of the test disinfectants against multiple antibiotic resistant bacteria in an environment that simulates the disinfection of surface of medical devices that are hard to clean and may harbour residual organisms and patient-secretions after cleaning (ie. flexible endoscopes). To simulate this environment, the multiple antibiotic test bacteria are inoculated onto a PVC lumen in the presence of a "worst-case" organic challenge.

The basis of disinfection is to drastically reduce the risk of contamination from one person to another. One factor involved in reducing this risk is the ability to remove the microorganisms from the surface that they are on. It is known that cleaning of medical devices prior to disinfection is very important in reducing the bacterial load (62). If the devices are not properly cleaned, high-level disinfection or sterilization can not be guaranteed (62) because a high bacterial load will interfere with the efficacy of the disinfectant. Because environmental surfaces are categorized as "non-critical", recent emphasis has been on physical "cleaning" rather than disinfection.

Removal of bacteria from medical devices is largely dependent upon the adherence of the bacteria to the surface. The harder it is to remove the bacteria, the more likely it will remain on the surface. If the adherent bacteria are not destroyed by the disinfection or sterilization procedure, the risk of spread of these residual bacteria to other patients is increased.

In the Surface Carrier Test, we observed adherence differences to the PVC lumen carrier in the range of test bacteria. We noted these differences when we subjected the test bacteria to our fluid control. The fluid control was performed to determine the level of bacteria that were left on the lumen carrier after it was subjected to the assay in the absence of disinfectant. Differences in adherence were observed between the Gram-negative organisms (ie. *Acinetobacter baumannii* and *Alcaligenes* species) and the Gram-positive organisms, (ie. *Staphylococcus aureus* and *Enterococcus faecium*). *Mycobacterium chelonae* also showed greater adherence to the PVC lumen carrier compared to the Gram-negative organisms evaluated.

The recoverable bioburden results showed that the Gram-negative organisms tended to be washed off the PVC lumen carrier more readily than the Gram-positive organisms (Tables 5, 6, 8 & 9). The difference in adherence between the two types of organisms was approximately 2 $\log_{10}$. Because of the "wash-off", the recoverable bioburden values for the Gram-negative organisms tested were so low that we could not obtain a positive control (no disinfectant) with $10^6$ residual organisms. As such, we could not assess whether a 6 $\log_{10}$ reduction could be achieved by the various disinfectants evaluated.

The greater adherence, after fluid exposure of Gram-positives to surfaces such as PVC

compared to Gram-negatives has not been previously reported. The difference in adherence abilities may be due to the distinct structure of the cell wall between the two types of organisms (ie. high peptidoglycan content of Gram-positive organisms compared to the lipid bilayer and LPS of Gram-negatives). The ability of the Gram-positive bacteria to remain on the carrier more readily may be a factor in its role in nosocomial infections. This is the reason why cleaning of the medical devices before disinfection is very important (62). If a medical device such as a flexible endoscope is not cleaned before disinfection and the fluid movement of the chemical disinfectant in the narrow lumened channel of a flexible endoscope does remove a substantial amount of bacteria, as observed in this study, the killing ability of the disinfectant on vegetative bacteria must be absolute to eliminate the risk of subsequent bacterial transmission to another patient. Unfortunately, we have also observed in the Surface Carrier Test, that high level disinfectants like glutaraldehyde are not completely effective(Table 5 & 7) against multiple antibiotic resistant *Staphylococcus aureus* and glutaraldehyde resistant *Mycobacterium chelonae*. For *M. chelonae*, there have been reports of infections caused by contaminated medical devices (1, 46, 63, 90106, 121, 136). When the increased adherence ability of Gram-positive bacteria is coupled with the ability of these organisms to survive on dry surfaces (142), the risk of transmission within the hospital environment would appear to be enhanced.

Variances in adherence were also observed among the *A. baumannii* strains (Table 8). The multiple antibiotic resistant strains showed decreased adherence levels when compared to the susceptible strain. This is interesting in the context of increasing reports of non-antibiotic resistant *A. baumannii* playing a prominent role in nosocomial infections and

hospital outbreaks (12, 13, 26, 28, 35, 42, 133, 141).

Although the main objective of this study was to determine the disinfectant resistance levels of multiple antibiotic resistant bacteria compared to their susceptible counterparts, the efficacy of the test disinfectants against the test bacteria could also be assessed. Sodium hypochlorite, at a use-concentration of 0.5% was strikingly and completely effective (no detectable, residual bacteria) against the whole range of test bacteria in the Surface Carrier Test. Sodium hypochlorite has been widely used for hard surface disinfection (78) and is sporicidal at high concentrations. The results show that even at 0.5%, sodium hypochlorite was very effective in killing both multiple antibiotic resistant and susceptible bacteria. The only limiting factor attributed to this disinfectant is it's corrosive nature on metals therefore metallic environmental surfaces and medical devices should not be disinfected with sodium hypochlorite.

Another finding related to the Surface Carrier Test was the inability of 0.04% hydrogen peroxide to totally kill the majority of the bacteria evaluated. Hydrogen peroxide is a widely used biocide for disinfection, sterilization and antisepsis (78) and is considered a high level disinfectant at higher concentrations (10%-30%) and longer contact times (17). In this study, we determined the activity of both a 3% and 0.04% hydrogen peroxide solution, which are the use-dilutions recommended by the manufacturer. With the exception of *Alcaligenes*, the residual bacteria left after exposure to 0.04% hydrogen peroxide was 1-4 $logs_{10}$ depending on the recoverable bioburden values. In our study, hydrogen peroxide had greater activity against Gram-positive than Gram-negative bacteria. However, the presence of catalase or other peroxidases in Gram-positive microorganisms could increase their

tolerance to lower concentrations of hydrogen peroxide (78). Hydrogen peroxide can be chemically broken down into water ($H_20$) and oxygen ($0_2$) by the enzymatic activity of catalase which thereby reduces the antimicrobial activity of this disinfectant. The range of Gram-positive test bacteria gave us the opportunity to determine if any one organism could overcome the effects of 0.04% hydrogen peroxide better than the others. *Staphyloccocus aureus* is a Gram-positive, catalase positive organism while *Enterococcus faecium* is a Gram-positive, catalase negative organism. We expected that *Staphylococcus aureus* would exhibit slightly higher tolerance for hydrogen peroxide compared to *Enterococcus faecium* because it contains catalase. The results of the experiment showed that neither organism was effectively killed by 0.04% hydrogen peroxide than the other. This observation suggested that at this low concentration, hydrogen peroxide was ineffective at killing Gram-positive bacteria whether or not they possessed the enzyme, catalase. However, 3% hydrogen peroxide, survival of *Staphylococcus aureus* was evident while survival of *Enterococcus faecium* in the higher concentration did not exist. These data suggested that the use of 0.04% hydrogen peroxide to disinfectant environmental surfaces or medical devices contaminated with multiple antibiotic resistant or susceptible bacteria would not be effective. A higher percentage ($\geq$ 3%) of hydrogen peroxide should be used for disinfecting these surfaces.

The main objective of this study, again, was to determine whether multiple antibiotic resistant organisms were more resistant to chemical disinfectants than antibiotic susceptible strains. In all organisms and disinfectants tested, no differences were observed. The conclusion based on the Surface Carrier Test was that multiple antibiotic resistant organisms were not more resistant to chemical disinfectants than antibiotic susceptible bacteria despite

the presence of an organic (ATS) and physical (surface) challenge.

**iii) Suspension Testing**

The reuse of chemical germicides for decontamination of medical devices such as flexible endoscopes is routinely performed in most health care facilities (124). Due to reuse, accumulation of organic material, dilution of the disinfectant and changes in pH of the disinfectant are known to occur. Although manufacturers indicate the recommended period of use for a certain disinfectant, these recommendations are not always adhered to resulting in suboptimal efficacy of the disinfectant. Factors that are known to affect disinfectant efficacy are concentration of disinfectant, contact time, temperature, pH, organic load and aging of the disinfectant (124). All of these factors may occur in the reuse of the disinfectant, therefore, evaluating disinfectants under some or all of these conditions is valuable. The study of disinfectant efficacy under reuse conditions has not been extensive but of the few studies that have been performed, failure to inactivate of *Mycobacterium* species has been observed (124).

The Suspension Test performed in this study was intended to evaluate killing efficacy in the presence of an organic challenge. Suspension testing is important because reused chemical disinfectants are often diluted to the recommended use-dilution and stored until next use. Reuse periods of up to 28 days are commonly recommended for glutaraldehyde products (124). During this time, bacterial growth may occur in the disinfectants and result in contamination of the objects that are being disinfected. The Suspension Test examined the killing abilities of test disinfectants on bacterial suspensions in concentrations lower than manufacturers' recommendations and over prolonged exposure times to evaluate organism

survival under conditions that simulated in-use conditions. The Suspension Test also provided us with more information regarding the ability of multiple antibiotic resistant bacteria to outgrow the effects of disinfectants at lower concentrations or over longer contact times.

In the Surface Carrier Test, 0.5% sodium hypochlorite possessed the best killing ability over the entire range of test bacteria. In the Suspension Test, we observed that hydrogen peroxide, at a concentration of 3%, was the most effective against the widest range of test bacteria. The test dilutions for hydrogen peroxide ranged from 1% to 0.004%. Even at the lowest concentration, 0.004%, no bacterial outgrowth was observed. This was an interesting observation because the conclusions made in the Surface Carrier Test were somewhat different. In the Surface Carrier Test, 3% hydrogen peroxide provided better killing ability than 0.04% but residual bacteria was still observed. The lower (0.04%) concentration was ineffective against all bacteria in the Surface Carrier Test. Meanwhile, the Suspension Test results show no growth of any test bacteria in 0.004% hydrogen peroxide, which is a further 1:10 dilution beyond the lowest concentration used in the Surface Carrier Test. These results could be explained by three factors that differentiated the Surface Carrier Test from the Suspension Test. The first factor is the absence of any physical challenge in the Suspension Test. The penetration needed in the Surface Carrier Test may have compromised the disinfectant efficacy at both the 3% and 0.04% concentrations. In the Suspension Test, bacteria and disinfectant were constantly in contact. The actual exposure of the disinfectant to the bacteria in the Suspension Test may have been much greater than in the Surface Carrier Test. The presence of air bubbles that may have restricted contact between disinfectant and bacteria and the inherent properties of the PVC surface such as microscopic crevices may have

shielded the bacteria from the disinfectant. Any or all of these factors could account for the differences in results.

The second factor that may have been responsible for the differences in survival is the nature of the organic challenge. In the Surface Carrier Test, the ATS contained whole sheep blood. In the Suspension Test, a modification of the artificial test soil was used because whole sheep blood interfered with the spectrophotometric readings involved in the Suspension Test. Therefore, the organic challenge was provided by the addition of 10% FBS to the reaction media. The red blood cells in the ATS contained the enzyme, catalase which breaks down hydrogen peroxide into water and oxygen (17, 78). FBS does not contain red blood cells and therefore has no catalase to break down even low concentrations of hydrogen peroxide in the Suspension Test media. This difference may account for the effectiveness of 0.004% hydrogen peroxide in preventing bacterial growth in the Suspension Test.

The third factor that could account for the differences seen with hydrogen peroxide is that growth was only monitored by spectrophotometric methods. A sample of the test media plated onto nutrient media and incubated after the assay was complete was not performed. This additional test would have assessed whether viable bacteria were present that were non-replicating because the spectrophotometric measurements would only have captured bacterial growth due to replication of the organism.

Most disinfectants are formulated for optimal, broad-spectrum antimicrobial activity at high concentrations. With reused disinfectants, concentration may be compromised due to repetitive uses. The inability to detect the effects of dilution on the activity of disinfectants in-use may lead to unrecognized reduced efficacy. There is also the possibility that over-

dilution of these disinfectants could lead to the survival of bacteria (103). The Suspension Test data for glutaraldehyde supported the importance of routine monitoring of the minimal effective concentration for disinfectants that are re-used (124).

Glutaraldehyde formulations of $\geq$2% are the most widely used high level disinfectants for reprocessing flexible endoscopes (90). For most formulation of 2% glutaraldehyde used in the reprocessing of flexible endoscopes, the manufacturer recommends a reuse period of 14 days. As observed in the Suspension Test, the dilution of 2% glutaraldehyde could compromise its efficacy. The dilution of 2% glutaraldehyde greatly affects its efficacy compared to the other test disinfectants (Tables 13, 14, 15, 16). The highest concentration of glutaraldehyde that supported the outgrowth of *Enterococcus faecium* was 0.125% solution which is a 1:16 dilution of 2% glutaraldehyde. Glutaraldehyde seemed to be the most sensitive to dilution. This observation emphasized that, for glutaraldehyde, as well as other high level disinfectants, the minimum effective concentration (MEC) of disinfectants should be tested and that reuse conditions must also be adhered to otherwise even vegetative antibiotic susceptible microbial strains may survive.

*Acinetobacter baumannii* showed the most resistance to the phenolic and QAC compounds (Table 16). These results differed from the observations of the Surface Carrier Test (Table 8). As little as a 1:2 dilution of the manufacturer's recommended use-dilution of the phenolic compound could greatly reduce the disinfectant efficacy. Phenolic compounds that are made up and not used immediately are usually stored until needed. Inadvertent dilution or the slight decrease in activity of the active ingredients could compromise the killing abilities of this disinfectant.

The resistance of *Acinetobacter baumannii* in the phenolic and QAC was observed for both multiple antibiotic resistant strains and the multiple antibiotic susceptible strains. This supports the possible association of the nosocomial importance of this species of bacteria (12, 13, 26, 28, 35, 42, 133, 141) with the organism's observed resistance to these disinfectants.

In regard to whether multiple antibiotic resistant bacteria were more resistant to the test disinfectants than multiple antibiotic susceptible strains, the Suspension Test data demonstrated that multiple antibiotic resistant strains were not more resistant to the disinfectants evaluated.

**iv) In-Use Issues**

In the literature, importance is stressed regarding the adequate contact time of a disinfectant to achieve the level of disinfection needed. (9, 16, 17, 29, 48, 78, 90, 103, 104, 106, 121, 124). Although manufacturers' state the exposure times of each disinfectant, these times are not always adhered to. A previous survey on the disinfection of flexible endoscopes has indicated an average disinfectant contact time of 10-20 minutes with 2% glutaraldehyde with contact that may be as short as 4 minutes (124). Decreased contact time may have a detrimental effect on disinfectant efficacy and cause errors in reprocessing. The study of actual disinfectant contact times has not been thoroughly assessed in the past but it has become increasingly important to study the realistic in-use disinfectant contact times to determine the efficacy of disinfectants under "in-use" conditions.

In the Surface Carrier Test and in our evaluations, bacteria were exposed to the test disinfectants for a contact time that was based on the manufacturer's minimum recommendation that was listed on their product. The times we chose either exceeded

(sodium hypochlorite and hydrogen peroxide) or met (glutaraldehyde, phenolic and QAC ) these values. The contact times used for the Modified Surface Carrier Test were based on observations of the disinfectant contact times used by housekeeping staff while they cleaned patient rooms.

The survey of several housekeeping staff while they cleaned patient rooms revealed that even with the same staff member, the disinfectant exposure time varies. This variation arose from the fact that each room that was cleaned and/or disinfected was quite different. Even if the staff member was conscientious about their cleaning routine, circumstances beyond their control forced them to modify their routine to accommodate the situation.

The greatest factor influencing the contact time of the disinfectant was whether the patients' rooms were occupied, either with a patient or the patient and visitors. Unoccupied rooms were less confining and the housekeeping staff found that they could clean the room at their own pace. When the patient's room was occupied, housekeeping staff tended to try to be less intrusive and tried to be in the room for the least amount of time. This could potentially lead to sub-optimal cleaning and disinfection resulting in disinfectant contact times that were reduced. Another factor that may have reduced the disinfectant contact times was the workload of the staff member. The more rooms the staff member must clean in a specific amount of time, the more likely he/she would spend less time on each room.

The cleaning/disinfecting observations led us to evaluate two alternative contact times for the Modified Surface Carrier Test. These contact times were chosen based on the most common, longest and shortest disinfectant contact time (Table 10) which were 1 and 10 minutes.

The Modified Surface Carrier Test results showed a slight increase in survival of both *E. faecium* strains when tested against 0.04% hydrogen peroxide but no apparent changes with gluataraldehyde, sodium hypochlorite and 3% hydrogen peroxide (Table 11) with a contact time of 10 minutes. A contact time of 1 minute showed increased survival in both strains to all the disinfectants except 2% glutaraldehyde. The previous data for the Surface Carrier Test and Suspension Test results indicated us that the efficacy of 2% glutaraldehyde did not largely depend on its contact time but rather on variations in concentration. Whereas, substantial reductions in concentration of 3% hydrogen peroxide seemed not to have a great effect while changes in the contact time resulted in greater bacterial survival.

These observations stress the fact that the disinfection guidelines, specifically concentration and contact times, should be adhered to carefully. Every disinfectant is variable in its efficacy if any or all of these conditions are changed. These variations, as seen in the Modified Surface Carrier Test, may create an increased risk of bacterial spread and nosocomial infections.

**v) Summary**

The objective of this study was to determine whether multiple antibiotic resistant bacteria were more resistant to chemical disinfectants than multiple antibiotic susceptible strains. Previous studies have proven that multiple antibiotic resistant bacteria were not more resistant to disinfectants (4, 5, 8, 129). Our hypothesis was that multiple antibiotic resistant bacteria would show an increased resistance to chemical disinfectants compared to the susceptible strain. This was hypothesized because of several critical factors that previous studies did not evaluate.

In this study, the range of multiple antibiotic resistant bacteria was much greater and included species that exhibited mechanisms of resistance not previously studied in this context. These non-specific antibiotic resistance mechanisms included efflux pumps and decreased cell wall permeability. We wanted to study these types of mechanisms and their possible association with disinfectant resistance. Surface and suspension test methods were included and for both of these methods, an organic challenge was included to mimic the conditions found in patient-used medical devices. Because the previously published studies did not investigate these factors, we hypothesized that we might find previously unrecognized correlation between antibiotic resistance and disinfectant resistance. However, the data from both the Surface Carrier, Suspension and Modified Surface Carrier test indicated that multiple antibiotic resistant bacteria are just as resistant or susceptible to chemical disinfectants as multiple antibiotic susceptible bacteria. These data further support McDonnell and Russell's (78) observation that true resistance to disinfectants is difficult for microorganisms to develop because the concentration of the disinfectant's active component is so high (ie. in the mg/ml range) whereas for antibiotics, the active components are in a lower magnitude of concentration (ie. ug/ml). In other words, sheer excess concentration of disinfectants overwhelms most bacteria. Despite this observation, caution is advised because organisms such as glutaraldehyde-resistant *Mycobacterium chelonae* (46) have developed substantial resistance for high levels of glutaraldehyde indicating that we can not ignore the issue of disinfectant resistance despite its rarity.

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# APPENDIX

## In-Use Cleaning/Disinfecting Procedures Questionaire

Date______________________

Housekeeping Staff__________________________

Ward/Floor___________________

Type of Room__________________

Start_________________

Finish_________________

---

1.Cleaners/Disinfectants:
________________________
________________________
________________________
________________________

2. Concentration/Dilution:
One Conc.____________________
Additional Conc.________________
________________________________
________________________________

3. Frequency__________________________________________________________

| Object | Present | Routine | Contact | Dis./Clean. | Method | Comments |
|---|---|---|---|---|---|---|
| Patient Rm<br>Floor | | | | | | |
| Walls | | | | | | |

| Bed | | | | | | |
|---|---|---|---|---|---|---|

| Object | Present | Routine | Contact | Dis./Clean. | Methods | Comments |
|---|---|---|---|---|---|---|
| Patient Rm Bedrail | | | | | | |
| C. Button | | | | | | |
| Bed Table | | | | | | |
| Doorknob | | | | | | |
| Blinds | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Curtains | | | | | | |
| Medical Equipment | | | | | | |
| Closet | | | | | | |
| Commode | | | | | | |

| Object | Present | Routine | Contact | Dis./Clean. | Methods | Comments |
|---|---|---|---|---|---|---|
| Patient Rm<br><br>Other | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Other | | | | | | | |
| Other | | | | | | | |
| Bathroom Sink | | | | | | | |
| Walls | | | | | | | |
| Toilet | | | | | | | |
| Floor | | | | | | | |
| Furniture | | | | | | | |

| Other | | | | | | |
| --- | --- | --- | --- | --- | --- | --- |

Réutilisable pendant 14 jours dans un seau ou un plateau
Reusable for 14 days in a manual bucket/tray system

Solution désinfectante et stérilisante
Sterilizing and Disinfecting Solution
Rapid-Acting • Non-Staining • Non-Rusting

METRICIDE

Glutaral Disinfectant Solution
Activated Dialdehyde Solution
Solution de dialdéhyde activée

DANGER. Corrosive/Corrosif

Contents/Contenu 946 ml
DIN 01961995

DIRECTIONS FOR USE:

MC-1405

EXPIRATION DATE

FOR SALE IN CANADA ONLY / POUR VENTE AU CANADA UNIQUEMENT

14691A EXP

FOR WHITENING, STAIN REMOVAL, DEODORIZING AND DISINFECTING

POUR BLANCHIR, ÉLIMINER LES TACHES, DÉSODORISER ET DÉSINFECTER

10 min.

10 min.

**CAUTION:** Avoid contact with skin and eyes. Do not mix with a toilet bowl cleaner, rust remover, household ammonia or acids. Never directly mix full strength bleach with any other household product. Use only as directed. Store tightly capped in an upright position. Keep out of reach of children.

**FIRST AID:** Contains Sodium Hypochlorite. If splashed in eyes or on skin, flush thoroughly with water. If swallowed, give one to two glasses of milk or warm water. Call a physician or poison control centre immediately. Keep patient warm.

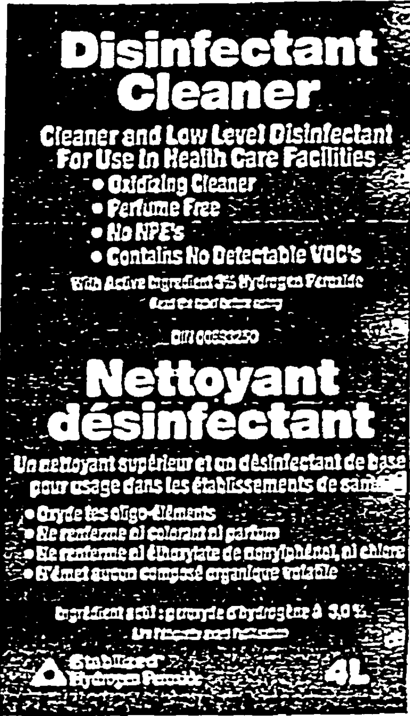
GUARANTEE: 5.25% SODIUM HYPOCHLORITE WHEN PACKED

GARANTIE: 5.25% D'HYPOCHLORITE DE SODIUM À L'EMPAQUETAGE

Questions? 1-800-268-6757

5 80440

HYDROX




ENVIRONMENTAL BENEFIT

Hydrogen Peroxide breaks down to water and oxygen

**GENERAL PURPOSE CLEANING**

Light [illegible] Cleaning – 1:250 (14 mL per 4 L [illegible] water) Daily floor cleaning and damp dusting. Mop and allow to dry, leaves surfaces streak free.

Heavily Contaminated Soils – (1:70) (56 mL per 4 L of water) For [illegible] Contact areas such as Over-Bed Tables, Call Buttons and for Showers, Bathtubs, Sinks, Taps, Counter Tops, Toilet Bowls & Urinals. Damp wipe and allow to air dry. [illegible] spray wipe application, dilute 14 mL per 1 L of water. Use foam sprayer.

*For all cleaning tasks, follow infection control protocol to prevent spread of resistant micro-organisms.*

**CLEANING & DISINFECTING SURFACES OF BLOOD AND BODY FLUIDS**

*Gloves should be worn.*

1) Remove excess blood and fluids with [illegible] material.

2) Clean contaminated area. Apply HYDROX diluted 56 mL to 4 L of water, soak 30 seconds, wipe dry.

3) Disinfect contaminated area. Apply HYDROX undiluted and allow to remain wet for 10 minutes. Wipe surface dry or rinse.

**DISINFECTANT USE**

Disinfection of Non-critical Equipment *(such as stethoscopes, bedpans, [illegible] poles, EKG/EEG leads, wheelchairs, any other instrument only coming in contact with intact skin and requiring low level disinfection)* Pre-clean items prior to disinfection with solution of HYDROX diluted 56 mL per 4 L warm water. Place pre-cleaned items in undiluted HYDROX to disinfect. Allow to soak 10 minutes. Remove and rinse with clean water. Change solution when visibly soiled or daily.

Disinfection of Environmental Surfaces *(such as floors, walls, table tops, counters, etc.)* Apply HYDROX undiluted to pre-cleaned surfaces. Allow to stand 10 minutes. Rinse with clean water.

**PRECAUTIONS AND FIRST AID**

Caution: Undiluted product can cause eye irritation. Avoid eye and [illegible] contact with undiluted product. If eye contact occurs, flush immediately with clean water for 10 minutes. If irritation persists, contact a physician. In case of skin contact thoroughly rinse with water.

Read M.S.D.S. before using product.

Lot #11183
Expiry Date: Sept/00

# LpH®se

## GENERAL DESCRIPTION

LpHse is a research developed, non-alkaline, phenolic germicidal detergent designed to be effective in hard (400 ppm as $CaCO_3$) or soft water and in the presence of 5% organic soil. The use-dilution of this product is specifically formulated to clean, disinfect and deodorize in a simple one step process. Dilute with tap water as dispensed. LpHse may be used on hard, non-porous environmental surfaces such as floors, walls, counters, tools, carts and other equipment in hospitals, nursing homes, clinics, pharmaceutical manufacturing facilities, and animal research facilities.

*Note: This product is not to be used as a sterilant or high level disinfectant on any surface or instrument that (1) is introduced directly into the human body, either into or in contact with the bloodstream or normally sterile areas of the body, or (2) contacts intact mucous membranes but which does not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the body.*

| FEATURES | BENEFITS |
|---|---|
| Advanced phenolic formula | Broad spectrum, tuberculocidal and virucidal including HIV (AIDS) |
| Contains a non-alkaline detergent | Responsible for excellent cleaning results |
| Hard water (400 ppm as $CaCO_3$) effective | Disinfection assured in most tap waters |
| 5% serum effective | Cleaning and disinfection accomplished in one-step |

## GERMICIDAL PROPERTIES

The official test for determining the germicidal efficacy of a one-step cleaner disinfectant is the Use-Dilution Method as stated in the A O A C Methods of Analysis. LpHse concentrate diluted 1:256 in 400 ppm (as $CaCO_3$) hard water is effective against the following microorganisms in the presence of 5% blood serum, 10 minutes at 20°C.

330-300-6466
Rev 8/97

CALGON VESTAL Division
PO Box 147
St Louis, Missouri 63166-0147 • USA
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Infection Prevention and Contamination Control...Worldwide

CALGON VESTAL Division
TECHNICAL INFORMATION

TECHNICAL DATA

# COVERAGE PLUS®

## GENERAL DESCRIPTION

Coverage Plus is a one-step cleaner, disinfectant and deodorizer consisting of four quaternary ammonium compound chains formulated with a biodegradable detergent system. The use dilution has broad spectrum activity against bacteria and viruses, including HIV (AIDS). Coverage Plus is fungicidal and inhibits the growth of mold and mildew. This product is effective in the presence of 5% serum and in water up to 400 ppm hardness (as $CaCO_3$).

Coverage Plus may be used for the cleaning and disinfecting of most hard surfaces such as floors, walls, woodwork, bathroom fixtures, equipment and furniture. Coverage Plus is intended for use in institutions such as hospitals, nursing homes, schools, medical and dental offices and other public areas.

*Note: This product is not to be used as a sterilant or high level disinfectant on any surface or instrument that (1) is introduce directly into the human body, either into or in contact with the blood stream or normally sterile areas of the body, or (2) contacts intact mucous membranes but which does not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the body.*

| FEATURES | BENEFITS |
| --- | --- |
| Hard water effective | Disinfection assured with tap water of up to 400 ppm hardness as $CaCO_3$ |
| 5% Serum effective | Cleaning and disinfection accomplished in one-step |
| Concentrated | Cost effective |
| Broad Spectrum activity including HIV-1 (AIDS) | Assures effective disinfection of hard non-porous surfaces |
| Advanced detergent system | Provides superior cleaning ability |

## GERMICIDAL PROPERTIES

The official test for determining the germicidal efficacy of a one-step cleaner disinfectant is the use-dilution method as stated in the A O A C Methods of Analysis. Coverage Plus diluted 1:256 in

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## DIRECTIONS FOR USE

Cleaning and Disinfecting Washable Hard Non-Porous Surfaces:
Add one-half ounce (15cc) to each measured gallon of water used. Always add Coverage Plus to premeasured water. Gently mix for a uniform solution. Apply solution with a cloth, sponge, mop or brush using normal cleaning methods. Thoroughly wet all surfaces to be cleaned, then remove excess solution with a wrung-out applicator. Treated surfaces should remain wet for ten minutes. For heavily soiled areas, a preliminary cleaning is required. Prepare a fresh solution daily or when use-solution becomes visibly dirty. Coverage Plus is a complete product. Do not add other chemicals. Use only as directed. If frozen, thaw and remix before use.

## STORAGE

Prohibition: Do not contaminate water, food or feed by storage or disposal. Do not store on side. Avoid creasing or impacting of side walls.

## SERVICE

- Sales
  Service is one of the most important assurances for consistent quality performance of your operation. Your representative will tailor a service program for your facility that will provide effective and trouble-free operations.

- Technical
  CALGON VESTAL is pleased to provide a completely staffed and equipped Technical Service Laboratory capable of performing needed tests and providing both telephone and on-site assistance when needed. Your representative will provide you with more details on how this service can benefit your particular situation.

## PRECAUTIONS

Information concerning human and environmental exposure may be reviewed on the Material Safety Data Sheet for the product. For additional information regarding incidents involving human and environmental exposure, call 1-314-535-1395 and ask for the Regulatory Affairs Group of CALGON VESTAL Division.

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